# Food Additives and Packaging

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# Food Additives and Packaging

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# Foreword

The ACS Symposium Series was first published in 1974 to provide a mechanism for publishing symposia quickly in book form. The purpose of the series is to publish timely, comprehensive books developed from the ACS sponsored symposia based on current scientific research. Occasionally, books are developed from symposia sponsored by other organizations when the topic is of keen interest to the chemistry audience.

Before agreeing to publish a book, the proposed table of contents is reviewed for appropriate and comprehensive coverage and for interest to the audience. Some papers may be excluded to better focus the book; others may be added to provide comprehensiveness. When appropriate, overview or introductory chapters are added. Drafts of chapters are peer-reviewed prior to final acceptance or rejection, and manuscripts are prepared in camera-ready format.

As a rule, only original research papers and original review papers are included in the volumes. Verbatim reproductions of previous published papers are not accepted.

**ACS Books Department**

# Preface

This ACS Symposium Series book evolved from the ACS symposium "Food Additives and Packaging," sponsored by the Division of Agricultural and Food Chemistry (AGFD) at the 245th ACS National Meeting & Exposition in New Orleans, LA, April 7–11, 2013. There had not been such a large meeting of scientists, regulators, and legal representatives of the industry in this field or an ACS Symposium related to this topic for at least 10 years. The theme of the Spring 2013 ACS Meeting, "Food and Energy," was perfect.

The purpose in organizing this book was to broaden the readers' understanding of the rules and regulations governing the use of food additives and food packaging materials in the U.S. and globally. Furthermore, the book investigates novel materials and applications related to food additives and food packaging materials and explores concerns, issues, and current events in the field. The book particularly highlights global regulations, research, development, applications, and evaluation of food additives and food packaging materials. These areas are dynamic, constantly changing, and expected to attract the interest of a broad and diverse readership.

When consumers think of food additives, they usually think of chemicals directly added to food, like preservatives, food colorings and flavorings, and sugar substitutes. However, the term "food additive" is legally defined to cover any substances which may become components of food. The term "food contact substances" (FCS) is used in the U.S. to include components of packaging materials, and materials used in the processing, handling, and storage of food.

This Symposium Series book is divided into two parts: Part I on *Global Regulations* and Part II on *Research and Development, Applications, and Evaluations*. This book contains 19 timely chapters written by global authorities in the fields of food additives, food processing aids, and food packaging materials, which are designed to attract the interest of a broad, diverse readership.

Part I of this book highlights how food additives and packaging materials are classified and regulated in different parts of the world and addresses some of the scientific, legal, and practical issues related to these regulations from the perspective representatives. Part I contains monographs on general aspects of regulatory processes in various countries (U.S., EU, Thailand and Japan) and specific aspects, such as GRAS substances, color additives, enzymes, flavorings, safety assessments, and the National Environmental Policy Act (NEPA).

Part II presents some current topics related to the research, development, applications, and evaluation of food additives and food packaging materials. Part II contains monographs on applying regulatory knowledge for packaging compliance and evaluating food packaging for pre-packaged irradiated food, and

on various emerging technologies, such as a control release packaging system and high pressure processing that can improve the appearance, texture, taste, or shelf-life of food; it also includes monographs that discuss other aspects, such as bisphenol A, PET packaging materials, nanomaterials, and biomaterials.

The audience for this book includes food scientists, packaging scientists, researchers, regulators, government officials, regulatory staff in the food industry and personnel at law offices interested in obtaining updates on food additive and food packaging material regulations, as well as the emerging research and development, applications, and evaluation. It will also interest food technologists and engineers designing food packages, polymer chemists, and polymer engineers developing new packaging materials.

We would like to thank the authors of the chapters and Ashlie Carlson, Arlene Furman, Tim Marney, and Bob Hauserman from the ACS Books department for their incredible patience when we missed nearly every deadline. It has been a pleasure and joy to work with you all.

We also thank Allan Bailey from the Division of Food Contact Notification, U.S. FDA, for his invaluable input to this book.

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# Editors' Biographies

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Dr. Vanee Komolprasert has been a consumer safety officer for over nine years at the Office of Food Additive Safety, U.S. Food and Drug Administration, College Park, MD. She manages the multidisciplinary review of pre-market notification submissions for the safe use of new food contact substances and food packaging. She also provides professional service to representatives of the regulated food packaging and food processing industry. Before joining OFAS, she was a research scientist for 14 years at FDA in Chicago, IL, investigating several high-priority projects on food packaging safety. She has published 30 peer-reviewed articles and book chapters. She has a Ph.D. in agricultural engineering, a Professional Engineer (P.E.) license in chemical engineering, an M.S. in packaging, and a B.S. in food technology.

## Dr. Petra Turowski

Dr. Petra Turowski has been a chemist, consumer safety officer, and team leader at the U.S. Food and Drug Administration over a period of 14 years, working in the Center for Food Safety and Applied Nutrition's Offices of Food Additive Safety; Nutrition, Labeling, and Dietary Supplements; and Premarket Approval. She serves as team leader for the Division of Food Contact Notification's Committee for Global Harmonization and Data Sharing – Europe. She has worked on or overseen hundreds of submissions and petitions from the industry, including Food Contact Notifications, Pre-notification Consultations, and New Dietary Ingredient Applications. Before joining FDA, Petra was an assistant professor of chemistry at Smith College and an adjunct assistant professor in the molecular and cellular biology program at the University of Massachusetts at Amherst for seven years. She was a Teaching Fellow at Amherst College, has written many publications, received numerous awards and fellowships, and holds Ph.D. and S.B. degrees from the Massachusetts Institute of Technology and the University of California at Berkeley, respectively.

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## Chapter 1

# Global Regulation of Food Additives

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This chapter compares and contrasts the common elements of the major national and international processes for the safety assessment and regulation of food additives. The chapter describes the general approach to safety assessment and the limited ways in which the major regulatory processes differ. The chapter focuses on the U.S. and E.U. regulatory approaches but also discusses the processes used by the FAO/WHO Joint Expert Committee of Food Additives and the Codex Committee on Food Additives.

## Background

Food additive regulation is an area of prominence since the beginning of U.S. food safety laws over 100 years ago. Although the U.S. Pure Food and Drugs Act of 1906 did not provide for premarket approval of these ingredients it did acknowledge concerns for chemicals in food and established the beginnings of FDA's regulation of food colors. The regulation of food additives globally has been led for many decades by the three most robust and distinct safety assessment programs currently in place as well as their predecessor organizations. This "big three" include the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the European Union Scientific Committee on Food; more recently the European Food Safety Authority (EFSA) and the United States Food and Drug Administration (FDA).

However, the efforts of the Codex Alimentarius Commission's (Codex) Working Group on the General Standard for Food Additives (GSFA) over the past two decades as well as recent incidents regarding the safety of food ingredients worldwide as well as trade considerations have increased awareness amongst other nations of the need for consistent regulation in this area. As a result many

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more nations have begun to move toward a more robust premarket clearance and listing program for food additives. In most cases the assessment process in nations with less developed regulatory programs relies in one way or another on the previous reviews by JECFA and/or EFSA and/or FDA. However, this is not universally true and fully independent requirements for premarket review and listing certainly do exist, (e.g., in Canada, Australia and New Zealand, Japan and in China). Still even these more fully developed systems as a well as most evolving systems follow and remain highly compatible with the three most robust programs mentioned above. As a result, this chapter discusses the 'big three" in some detail as a basis for understanding all efforts worldwide. Thus, this is not an in depth treatment of any review system but is meant as an overview of the major features, points of similarity and points of difference.

Although many evolving systems follow the three longstanding programs they are also beginning to take into account certain cultural differences which may or may not be completely compatible with the other major standards. It may well be that the GSFA will over time constitute a point of unification among all these national standards.

## United States Regulation of Food Additives/Ingredients

The US program is easily the most complex of existing national programs. In large part this complexity derives from how U.S. law defines a "food additive" (*1*). This definition is unique from other national definitions and from the definition used by EFSA, Codex and the GSFA in some significant ways. First of all, the U.S. definition specifically includes so-called "indirect additives" and excludes so-called "color additives." As a result, the U.S. is unique in regulating components of food contact materials as "indirect" food additives and in regulating food colors separately from other food ingredients/additives. The U.S. regulatory framework also exempts from the food additive definition those substances authorized for use by FDA or the United States Department of Agriculture prior to September 6, 1958. The U.S. regulatory framework also stands apart from most other food additive regulatory frameworks by including processing aids under the food additive provisions of the law. Most other systems apply only a general safety standard to processing aids and to most food contact substances as well.

However, the biggest difference by far between the U.S. framework and other existing regulatory frameworks for food additives is the existence of the unique exemption from premarket approval requirements for uses of food ingredients that are generally recognized as safe by qualified experts. This provision was introduced into the U.S. law when FDA was granted premarket approval authority as a common sense measure to avoid requiring substances whose use in food was already widely recognized as safe to undergo extensive safety testing or to use limited Federal resources for premarket review. The so-called GRAS exemption permits safety to be based on either common use in food prior to 1958 or on scientific procedures similar to other food ingredients. As a practical matter, this means that GRAS uses of food substances do not require premarket authorization

by FDA or listing by FDA. The latter point, that there is no one government list of acceptable substances has always been a source of confusion and consternation with the U.S. food industry, consumers, and other international regulators.

FDA has promulgated regulations describing the requirements for a determination of GRAS status in 21 CFR 170.30. This regulation establishes that GRAS status must be based on the same quality and quantity of information as needed to establish the safety of a food additive. In addition, the data establishing safety of the use must be generally available and generally accepted. General availability has typically been interpreted to mean that at least the most critical data necessary to establish safety be published in peer review literature. By comparison, data that merely corroborates safety are not required to be published or even to be publically available. General acceptance of the data supporting safety has typically meant that there is no significant scientific disagreement regarding safety or regarding the data supporting safety. If either of these elements of general recognition (general availability and general acceptance) are missing then general recognition is not achieved and the use of the food ingredient would need to undergo premarket review by FDA as a food additive. Regulation as a food additive involves the conduct by FDA of a full safety assessment. If FDA judges the use of the additive to be safe then FDA is required to promulgate a regulation in 21 CFR permitting the intended use of the additive and including any specifications and limitations necessary to ensure safe use of the additive (*2*).

## Safety Assessment of Food Additives

The USFDA's approach to the safety assessment of food additives and ingredients is typical of the most sophisticated processes employed nationally, regionally, and by international standard setting bodies. The U.S. process typically consists of three distinct activities including estimation of dietary consumption, assessment of likely toxicity and a risk management decision regarding safety. In addition, when the use of an additive is such that it would constitute a macro ingredient in the diet, FDA will also consider whether the use of the additive has significant impacts on nutrition and whether any such impacts affect the safety of the intended use.

Estimates of dietary consumption of additives can be performed in a number of ways depending upon the specific circumstances (*3*). For additives with uses in a limited number or foods, it may be appropriate to base dietary consumption estimates on average food intakes for the specific population or on multiples of that average value as a simplified approach (*4*). However, the most rigorous dietary consumption estimates typically involve the use of probabilistic modeling employing the results of dietary intake surveys and typical and maximum use levels of the additive in particular foods. In these cases standard practice is to estimate a so-called "eaters only" exposure taking into account data only for those members of the population which have reported consumption of the food into which the additive is incorporated. In this way, the estimate is not diluted by data on persons who are not expected to consume the additive because they do not consume the food to which the additive is added. FDA typically bases a safety

assessment on a 90th percentile consumption level for the additive but can also use higher percentile consumption values for special circumstances including consumption by sensitive subpopulations.

FDA's assessment of the available toxicological data regarding a food additive addresses two broad questions 1) whether there is adequate toxicity data available to address any toxicological concerns regarding the safe use of the food additive and 2) whether those data actually demonstrate the safety of the estimated dietary exposure. The process of addressing the first question involves the consideration of relevant testing recommendations including FDA's Redbook (*5*) in relation to the characteristics of the food additive itself. FDA's process, like other more robust processes does not incorporate a rigid requirement for specific types of toxicity testing. Rather, FDA's Redbook and the general approach to assessing the adequacy of toxicity data to support the safety of food additives can include considerations regarding common consumption of the additive or similar substances and other information regarding the expected metabolic fate of the additive. In addition, once it is determined that a particular type of study is necessary to support safety of the use, this first part of FDA's review includes a further assessment of each required study in relation to relevant guidance, regulations, and standard practice in the design and conduct of the study. Typically, studies are reviewed with respect to Redbook or other guidelines (e.g., OECD or EPA) for adequacy of design and regarding whether they were conducted in compliance with good laboratory practice (GLP) regulations (*6*). FDA may also nominate the most critical studies and the laboratory conducting those studies for a GLP inspection.

The second portion of FDA's toxicity assessment of a food additive use is the actual evaluation of the toxicity testing data and the development of a point of departure for the safety assessment. With the exception of studies only available from the published literature, FDA reviewers typically receive and examine the raw data in toxicity studies to arrive at their own conclusions regarding the results including any adverse effects, no adverse effect levels, low effect levels, and other positive or negative indications for toxicological concern. Even though a data set may have been initially judged as adequate, the detailed review of the data can result in questions that must be assessed in additional studies or in repeated studies designed to address inadequacies in the original data set.

In most cases, the review of relevant toxicological data will result in establishing a no adverse effect level (NOAEL) on which to base the decision regarding the safety of the additive. In the past, FDA's practice has been to establish an acceptable daily intake (ADI) applying an appropriate safety factor (uncertainty factor) to the NOAEL. More recently, ADI's have been estimated less frequently and the safety decision is ordinarily based on the margin between the estimated dietary exposure and the NOAEL. This latter practice more closely mirrors the practice in other major safety review programs. The risk management decision with respect to permitting or not permitting the use of the food additive is then based on whether the estimated dietary exposure is below the ADI or whether the estimated dietary exposure is sufficiently lower than the NOAEL. For those cases where the likely dietary exposure is so low that data from which a NOAEL could be derived is not ordinarily necessary to establish safety, it is

common to reference the dietary exposure at which additional data might be recommended as a substitute for the ADI for the additive if short term testing does not suggest a need for testing on which a NOAEL might be based. In such cases, the risk management decision follows the same model as those decisions relying on longer term toxicity testing data.

## EFSA Regulation of Food Additives

EFSA's framework for the review of food additives does not differ too significantly from the U.S. process with respect to the data typically required or the review of that data. Variations in food consumption patterns and supporting data between EU nations can result in higher estimated consumer exposures depending on the use of the food additive. However, overall, significant differences do not exist between the EU and US processes of safety review and approval of particular food additive uses. As has been previously discussed, most national frameworks and the EFSA regional framework include food colors within the definition of food additives but exclude processing aids in contrast to the US framework. By contrast to the US, all "food additives" in the EU must be on an EFSA listing authorizing their use. Food ingredients, that is, substances commonly used in the preparation of food such as sugar, salt, etc., are exempt from this premarket approval process. Even though processing aids are exempt from premarket review by EFSA, manufacturers are still required to determine that their use is safe under the same general standard as food additives. Fundamentally, this treatment of processing aids does not differ significantly from FDA's implementation of the GRAS provisions in US food additive law although it is clearly more limited in scope. One other significant difference between the EU and US processes is that EFSA has a specific mandate for cyclic reconsideration of the safety of additives. Like the US, the EU requires listing of the food additive in the appropriate regulation once the safety assessment is complete. In the EU, the addition of additives to these regulations is handled by the European Commission. Currently EU food additive uses are governed in regulations EU 1330/2008 (Common Procedures); EU 1333/2008 (Approved Food Additives); EU 231/2012 (Specifications and Limitations); EU 1332/2008 (Approved Food Enzymes); EU 1334/2008 (Approved Food Flavors).

## International Review and Standard Setting Bodies

Since the mid-1990s, the CCFA has worked to develop the GSFA integrating food additive standards with the Codex Alimentarius food standards and providing a clear guideline for the uses of food additives which have been reviewed by JECFA and found to be safe. JECFA was established under the auspices of the Codex Alimentarius Commission (CAC) to conduct risk assessments for the use of food additives in food. JECFA brings together experts from around the globe to participate in the assessment of food additives. In assessing safety, JECFA follows the same general approach assessment of dietary exposure and likely toxicity as described for both the USFDA and EFSA programs above

as outlined in the EHC 240: Principles and methods for the risk assessment of chemicals in food. For those additives found by JECFA to be safe for their intended use JECFA will generally propose specifications regarding identity purity and use of food additives for adoption by the CCFA. CCFA meets as a group of delegations of member states as well as delegations for other interested organizations which provide technical support to the GSFA working group and to the CCFA. The CCFA annually reviews the work of JECFA and may adopt food additive provisions in the GSFA as appropriate. As of the 46th meeting of the CCFA, over 3000 food additive provisions have been incorporated into the GSFA and well over 2000 provisions associated with additives in use remain to be incorporated into the GSFA. In addition to adopting such existing provisions, the CCFA may also request further information and further review by JECFA for those specifications not adopted for newly proposed food additive uses or for review of food additives for which significant new safety data or new safety questions have come to light.

## Conclusions

Most if not all national authorities around the world have frameworks that fall into one of three categories: 1) Basic safety standards which food and food ingredients must meet with or without some specific food additive measures; 2) adoption formally or informally of international standards such as those established by the CCFA in the GSFA; 3) some explicit premarket review and listing requirement. Developing countries in particular may not have the resources to actively review the safety of all the food ingredients in use in their countries. This situation makes the work of the CCFA on the GSFA even more critical as the GSFA is the most obvious solution for those countries which may have much more serious public health issues which are a priority for the use of precious public health resources. Prior to the current work on the GSFA commencing in the mid-1990's, many such countries simply recognized legal use of a food additive in the U.S., the E.U. or another country with a premarket review requirement as evidence that the food additive meets a general safety requirement. Today, more and more countries, including those in the developing world are actively participating in the activities of the CCFA in development of the GSFA and seeking to use that process to address the public health need for food additive safety. Additionally, this wider acceptance of the GSFA supports harmonization of food additive standards for the purposes of international trade and thereby supports both developing and developed countries. Finally, there are a number of other countries with long standing premarket review requirements for food additives, including for example Australia, Canada, China, Japan Korea and New Zealand. This latter group characteristically will follow review and listing procedures along the lines and using similar scientific principles to JECFA, the U.S., and EFSA resulting in relatively homogenous reviews and standards. As a result we see that there is far more similarity than dissimilarity to food additive regulation throughout the world.

## References


1. A food additive is…"any substance the use of which results or may be reasonably expected to result, directly or indirectly, in its becoming a component of or otherwise affecting the characteristics of any food,…including any substance intended for use in producing manufacturing, processing, preparing, transporting or holding food; including any source of radiation intended for such use, if such substance is not generally recognized, among experts qualified by scientific training…to be safe under the conditions of its intended use…"
2. Beginning in October 1999, FDA began to authorize food contact substances including indirect food additives through the food contact notification process. This notification process does not result in a regulation in 21 CFR. FDA does list information on "effective" (i.e., those that complete the authorization process) notifications for food contact substances on its internet site. http://www.fda.gov/Food/IngredientsPackagingLabeling/PackagingFCS/Notifications/ucm116567.htm. Additionally, FDA has operated an exemption process for listing indirect food additives in 21 CFR since 1995. See Code of Federal Regulations, Title 21, Section 170.39 (Threshold of regulation for substances used in food-contact articles) (2013).
3. *Guidance for Industry: Estimating Dietary Intake of Substances in Food*, August 2006. Accessed March 2014 at http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/IngredientsAdditivesGRASPackaging/ucm074725.htm.
4. For foods with a sufficiently broad consumption within a population, it is well-known and accepted that 90th percentile and 95$^{th}$ percentile consumption in the population may be modeled at two and three times the average consumption, respectively.
5. *Guidance for Industry and Other Stakeholders: Toxicological Principles for the Safety Assessment of Food Ingredients* July 2007. Accessed March 2014. Available at http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/IngredientsAdditivesGRASPackaging/ucm2006826.htm.
6. Code of Federal Regulations, Title 21, Part 58: *Good Laboratory Practice for Nonclinical Laboratory Studies* (2013).

## Chapter 2

# Regulation of Food Additives in Japan

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In Japan, the Ministry of Health, Labour and Welfare (MHLW) introduced a designation system for food additives when they implemented the Food Sanitation Law. Currently, all food additives are classified into four groups: designated food additives; existing food additives; natural flavoring; and food/food ingredients used as additives. In the process of designating food additives, the safety and effectiveness of the food additives are scientifically confirmed and MHLW must seek advice from the Food Safety Commission Japan (FSCJ) concerning their potential health effects. The FSCJ conducts a scientific health risk assessment and establishes an acceptable daily intake (ADI) for each additive. All designated additives and some existing food additives are currently regulated by certain specifications and/or standards, including those concerning the chemical and physical characteristics of the additive, as well as its manufacturing, storage, and use. These specifications and standards, along with the specifications for labeling and storage, are published in an official compilation entitled, "Japan's Specifications and Standards for Food Additives". To ensure that the daily intake of food additives is below the standard for use level, we estimate the daily intake of food additives using the market basket method every year.

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## The Basis for Food Safety Legislation in Japan

Japanese food safety legislation is based on the 2003 "Food Safety Basic Law" and the 1947 "Food Sanitation Law", which were enacted to protect public health. The national government has the duty to formulate and enforce comprehensive measures for ensuring food safety.

The overall objective of the Food Safety Basic Law is to mandate measures for ensuring food safety. It defines the basic framework for ensuring food safety and the responsibilities of the national and local governments and food industry members, identifies the role of the consumer, and sets the basic policies for formulating specific measures based on risk analysis.

It is essential to assess, using every applicable standard within our authority, how the ingestion of various foods influences human health. The Food Safety Commission Japan (FSCJ) was established to conduct these assessments in 2003.

The framework for risk analysis is shown in Figure 1. The FSCJ assesses the risks of compounds and other substances in food, and establishes an ADI. The Ministry of Health, Labor and Welfare (MHLW) and the Ministry of Agriculture, Forestry and Fisheries (MAFF) have both established ADI values and other standards for risk management under their respective Food Sanitation Law and Agricultural Chemicals Regulation Law. The National Institute of Health Sciences (NIHS), which belongs to the MHLW, provides advice and develops testing methods with respect to regulation for the MHLW. All the groups communicate risk in the form of scientific advice.

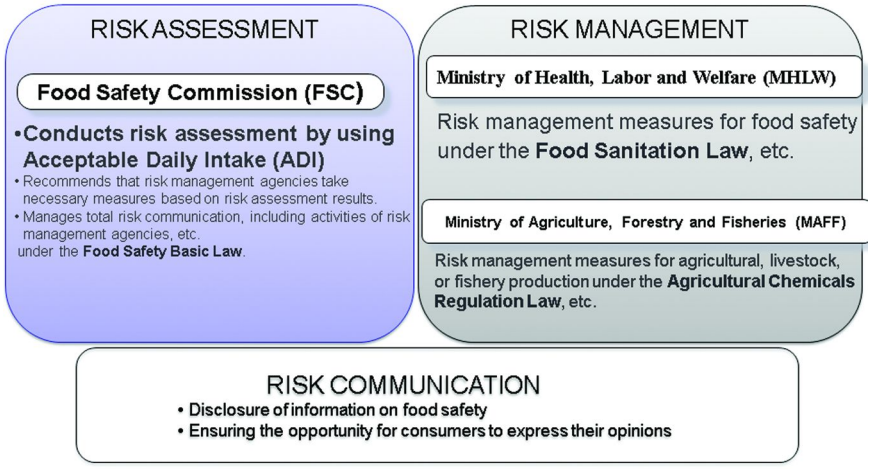



*Figure 1. Framework for risk analysis.*

The Food Sanitation Law governs foods and food additives as well as items that come in contact with food, such as utensils, packaging, toys for infants, and detergents. It also covers testing and inspection of domestic food facilities, import notifications, instructions for testing and monitoring, administrative dispositions, and penalties for non-compliance.

## The Regulation of Food Additives in Japan

### Designation of Food Additives

The MHLW introduced a designation system of food additives when they implemented the Food Sanitation Law in 1947. Under this system, only additives designated as safe by the MHLW are permitted in foods. Since 1947, all food additives have been regulated by this law. However, this designation system only applied to chemically synthesized additives. Therefore, the Food Sanitation Law was amended in 1995 to include non-synthetic additives, so-called "natural" food additives. Currently, all types of additives, synthetic and non-synthetic (natural) are equally subject to the designation system.

All additives are classified into four main groups, as shown in Figure 2. First, the designated food additives; as of April 10, 2014, 439 food additives have been designated in Japan, including 18 chemical groups of flavoring agents (*1*).

Second, the natural food additives that were already being marketed or used on the date of the amendment (in 1995) appear on the "List of Existing Food Additives" (*2*). Existing additives are being sequentially reviewed for safety by the MHLW. Currently, 365 natural food additives are listed. If the listed food additive is confirmed to have some toxicity or is no longer marketed, the food additive would be withdrawn from the list.

The third group consists of approximately 600 natural flavoring agents derived from plant or animal sources (*3*), such as vanilla and crab.

The fourth group contains food additives generally provided for eating or drinking as foods and which are used as food additives (*4*). This group currently includes approximately 100 substances, such as strawberry juice and agar.

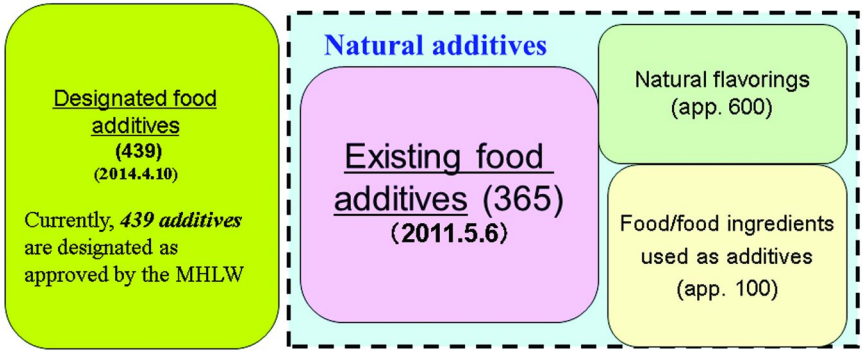



*Figure 2. Type of food additives in Japan.*

### Process of Food Additive Designation

The process of food additive designation is shown in Figure 3, and is briefly described as follows. When an application for food additive designation is submitted to the MHLW, it seeks advice from the FSCJ concerning potential health effects. All necessary documents are submitted to the FSCJ at the time

of the request. The commission conducts a scientific health risk assessment and establishes an ADI. After the MHLW receives the FSCJ's report and recommendation, the Pharmaceutical Affairs and Food Sanitation Council (PAFSC) discuss the adequacy of the draft standards. International evaluations are factored into the assessment during council discussions. If the discussion concludes that the additive is safe and effective, it is approved for use.

The NIHS has developed analytical methods for monitoring unauthorized food additives in processed foods (*5*, *6*).

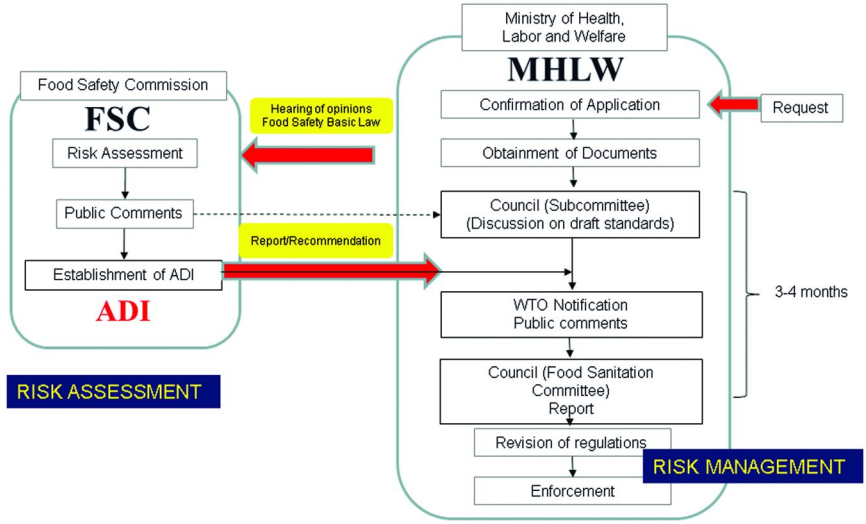



*Figure 3. Process of food additive designation.*

## Guidelines for Designation of Food Additives

Documents accompanying an application must comply with the "guidelines for designation of food additives", which are notified by MHLW. Moreover, the safety and effectiveness of an additive must be scientifically confirmed.

The purpose of the guidelines is as follows. The guidelines are designed to outline the procedures required for a food additive application, pursuant to Article 6 of the Food Sanitation Law, and for establishment of use standards for food additives, pursuant to Article 7 of the Food Sanitation Law.

The guidelines detail the necessary accompanying documentation for these applications, such as safety evaluation results, and the recommended methods for safety studies that are required to complete the documentation.

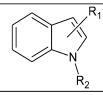
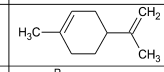
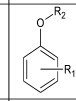
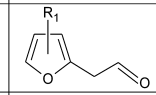
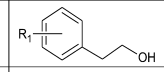
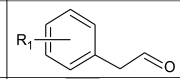
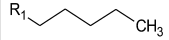
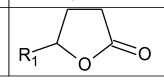
The principles of the guidelines are as follows. Food additives must be effective and present no hazard to human health. Additionally, their use must be of benefit(s) to consumers. The safety of food additives must be proven or confirmed in the intended use methods. In addition, it must be proven or confirmed that the

use of the food additive conforms to one or more of the following designated purposes: (1) to preserve nutritional quality, (2) to provide the necessary ingredient or component for food manufactured for special consumer groups, (3) to enhance shelf-life or stability, and (4) to assist in the manufacture, processing, or preparation of a food product.

The scope of food additives in Japan differs from that defined by the Codex Alimentarius Commission (CAC). Dietary supplements such as vitamins, minerals and amino acids, and flavoring and processing agents, which are not defined by the CAC as food additives, are all categorized as such in Japan.

In Japan, only food additives that are designated by the MHLW as flavorings are allowed to be used as flavoring chemicals. Currently, 129 substances are designated according to their individual chemical names, and the others are classified into 18 chemical groups (see Table 1). For the designated 129 substances, the specifications for each of them are individually established. Meanwhile, each of the 18 groups contains substances that are similar in chemical structure, and a list of these substances has been established and it is being updated by the MHLW. Currently, the list contains approximately 3000 substances.

**Table 1. Eighteen Chemical Groups of Flavoring Substances**

| | | | | | |
|---|---|---|---|---|---|
| 1. | Isothiocyanates* | R1-NCS | 10. | Thioethers* | R1-S-R2, R1-SSS-R2 |
| 2. | Indoles and Its Derivatives |  | 11. | Thiols (Thioalcohols)* | R1-SH |
| 3. | Ethers | R1-O-R2 | 12. | Terpene Hydrocarbons |  |
| 4. | Esters | R1-COOR2 | 13. | Phenol Ethers* |  |
| 5. | Ketones |  | 14. | Phenols* |  |
| 6. | Fatty Acids | R1-COOH | 15. | Furfurals and Its Derivatives* |  |
| 7. | Aliphatic Higher Alcohols |  | 16. | Aromatic Alcohols |  |
| 8. | Aliphatic Higher Aldehydes* |  | 17. | Aromatic Aldehydes* |  |
| 9. | Aliphatic Higher Hydrocarbons* |  | 18. | Lactones* |  |
| * except those generally recognized as highly toxic | | | | | |

The documentation required for designation of a food additive should contain:

I A summary
II The origin or details of development and overseas conditions of use
III Physicochemical characteristics and specifications (1. Name, 2. Structural and molecular formula, 3. Molecular formula and weight, 4. Assay, 5. Manufacturing methods, 6. Description, 7. Identification tests, 8. Specific properties, 9. Purity tests, 10. Loss on drying, 11. Loss on ignition, or water, 11. Residues on ignition, 12. Method of assay, 13. Stability, 14. Analytical method for the food additives in processed foods, 15. Principles to establish the proposed specifications),
IV Effectiveness
V Safety evaluation
VI Proposed use standards

The recommended safety evaluation studies are shown in Table 2. A variety of toxicity, reproductive, mutagenic, antigenic, and pharmacokinetic studies are required.

**Table 2. Recommended Safety Evaluation Studies**

| | |
|---|---|
| 1. | 28-day toxicity study |
| 2. | 90-day toxicity study |
| 3. | One-year toxicity study |
| 4. | Reproduction study |
| 5. | Teratogenicity study |
| 6. | Carcinogenicity study |
| 7. | Combined one-year toxicity/carcinogenicity study |
| 8. | Antigenicity study |
| 9. | Mutagenicity study |
| 10. | General pharmacological study |
| 11. | Metabolism and pharmacokinetic study |

**International General-Purpose Food Additives**

Separate from the designation process, the MHLW has decided to begin evaluating certain food additives with the intent of authorization even in the absence of an application. These food additives must meet the following two criteria: 1) Substances for which safety assessments have been completed by

the JECFA (Joint FAO/WHO Expert Committee on Food Additives) and whose safety has been confirmed within a certain level; 2) Substances that are widely used in the US and the EU countries, and in which the need is considered to be high.

**Table 3. Food Additives except Flavoring Agents of International General-Purpose Food Additives**

| | ***Name*** | ***Major purposes*** | | ***Name*** | ***Major purposes*** |
|---|---|---|---|---|---|
| 1. | Polysorbate20 | Emulsifier | 24. | Magnesium hydroxide | Acidity regulator |
| 2. | Polysorbate60 | | 25. | Sodium Stearoyl-2-lactylate | Emulsifier, stabilizer |
| 3. | Polysorbate65 | | 26. | Potassium lactate | Flavor enhancer, acid, acidity regulator, preservative, antioxidant synergist |
| 4. | Polysorbate80 | | 27. | PVP(Polyvinylpyrrolidone) | Bodying agent, stabilizer, clarifying gent, tableting adjuvant |
| 5. | Calcium stearate | Anticaking agent, emulsifier | 28. | Calcium sorbate | Preservative |
| 6. | Dimagnesium phosphate | Nutrient | 29. | Monoammonium L-glutamate | Flavour enhancer, salt substitute |
| 7. | HPC(Hydroxypropyl cellulose) | Tablet binder, emulsifier, thickener | 30. | Sodium aluminium silicate | Anticaking agent |
| 8. | Acetylated distarch adipate | Thickener, binder, stabilizer | 31. | Calcium sorbate | Preservative |
| 9. | Acetylated distarch phosphate | Emulsifier, thickener, binder | 32. | Calcium aluminum | Anticaking agent |
| 10. | Acetylated oxidized starch | Emulsifier, thickener, binder, stabilizer | 33. | Magnesium silicate(synthetic) | Anticaking agent |

*Continued on next page.*

**Table 3. (Continued). Food Additives except Flavoring Agents of International General-Purpose Food Additives**

| | *Name* | *Major purposes* | | *Name* | *Major purposes* |
|---|---|---|---|---|---|
| 11. | Starch sodium octenylsuccinate | Stabilizer, thickener, binder | 34. | β-apo-8'-carotene | Colour |
| 12. | Hydroxypropyl starch | Emulsifier, thickener, binder | 35. | Carmines | |
| 13. | Hydroxypropyl distarch phosphate | | 36. | Canthaxanthin | |
| 14. | Phosphated distarch phosphate | Stabilizer, thickener, binder | 37. | Sodium aluminum phosphate, acidic | Raising agent |
| 15. | Monostarch phosphate | | 38. | Nisin | Preservative |
| 16. | Distarch phosphate | Emulsifier, thickener, binder | 39. | Calcium acetate | Preservative, stabilizer, acidity regulator |
| 17. | Oxidized starch | | 40. | Calcium oxide | Dough conditioner, alkali, yeast food |
| 18. | Starch acetate | | 41. | Potassium sulphate | Salt substitute |
| 19. | Ammonium alginate | Emulsifier, stabilizer, thickener, gelling agent | 42. | Triethyl citrate | Carrier solvent, sequestrant |
| 20. | Potassium alginate | | 43. | Isopropanol | Extract solvent, carrier solvent |
| 21. | Calcium alginate | | 44. | Nitrous oxide | Propellant, gaseous filling agent |
| 22. | Calcium ascorbate | Preservative | 45. | Natamycin | Fungicidal preservative |
| 23. | Calcium saccharin | Sweetener | | | |

This decision was made from the viewpoint of international harmonization of substances that have been internationally proven as safe and have obtained the widespread global use. In recent years, there has been an increase in global food distribution, and imported foods account for approx. 60% of the foods distributed on the Japanese market. Furthermore, there is a growing possibility that imported foods contain additives that are authorized in other countries, but not in Japan; currently, 99 substances (45 food additives except flavoring agents

and 54 flavoring agents) fall into this category. The substances shown in Table 3 are 45 food additives except flavoring agents, and Table 4 are 54 flavoring agents. Discussions are being conducted on substances for which full documentation on safety and usefulness is available.

As of December, 2013, 88 out of the 99 substances have already been designated, such as sodium stearoyl lactylate and calcium saccharin.

**Table 4. Flavoring Agents of International General-Purpose Food Additive**

| *No.* | *Substance* | *No.* | *Substance* |
|---|---|---|---|
| 1. | Isobutanol | 28. | 5,6,7,8-Tetrahydroquinoxaline |
| 2. | 2,3,5,6-Tetramethylpyrazine | 29. | 2-Ethyl-5-methylpyrazine |
| 3. | 2-Ethyl-3,(5 or 6)-dimethylpyrazine | 30. | Isopentylamine |
| 4. | Propanol | 31. | Butylamine |
| 5. | Isopropanol | 32. | Phenethylamine |
| 6. | 2,3,5-Trimethylpyrazine | 33. | Piperidine |
| 7. | Amyl alcohol | 34. | Pyrrolidine |
| 8. | Isoamyl alcohol | 35. | 2,6-Dimethylpyridine |
| 9. | Acetaldehyde | 36. | 5-Ethyl-2-methylpyridine |
| 10. | 2-Ethyl-3-methylpyrazine | 37. | 2,3-Diethyl-5-methylpyrazine |
| 11. | 5-Methylquinoxaline | 38. | 2-(3-Phenylpropyl)pyridine |
| 12. | Butanol | 39. | 5-Methyl-6,7-dihydro-5H-cyclopentapyrazine |
| 13. | 2-Methylbutanol | 40. | 1-Penten-3-ol |
| 14. | Isobutyraldehyde | 41. | 3-Methyl-2-butenol |
| 15. | Butyraldehyde | 42. | Pyrazine |
| 16. | Isovaleraldehyde | 43. | 3-Methyl-2-butenal |
| 17. | Valeraldehyde | 44. | Isoquinoline |
| 18. | 2,3-Dimethylpyrazine | 45. | Pyrrole |
| 19. | 2,5-Dimethylpyrazine | 46. | *trans*-2-Pentenal |
| 20. | 2,6-Dimethylpyrazine | 47. | Trimethylamine |
| 21. | 2-Methylpyrazine | 48. | 2-Ethyl-6-methylpyrazine |
| 22. | 2-Ethylpyrazine | 49. | *trans*-2-Methyl-2-butenal |

*Continued on next page.*

**Table 4. (Continued). Flavoring Agents of International General-Purpose Food Additive**

| *No.* | *Substance* | *No.* | *Substance* |
|---|---|---|---|
| 23. | 2-Methylbutyralde-hyde | 50. | (3-Amino-3-carboxypropyl)dimethylsulfonium chloride |
| 24. | 2-Pentanol | 51. | 3-Ethylpyridine |
| 25. | Propionaldehyde | 52. | Ammonium isovalerate |
| 26. | 6-Methylquinoline | 53. | 2,3-Diethylpyrazine |
| 27. | 3-Methyl-2-butanol | 54. | 1-Methylnaphthalene |

## Establishment of Specifications and Standards for Food Additives

Typically, people consume a multitude of food additives over the course of their lifetime. Thus, food additives must be subject to stringent regulations.

All designated additives and some natural additives (existing food additives) are currently regulated by the specifications and/or standards.

These specifications and standards include those concerning chemical and physical characteristics, manufacturing, storage, and use. These standards, along with specifications for labeling and storage, are published in an official compilation entitled "Japan's Specifications and Standards for use of Food Additives." Its 9th edition will be published in 2016.

In the Specification, the various types of physicochemical information discussed in the section "Guidelines for designation of food additives" are included.

As an example, the standard for use level of benzoic acid as a preservative is as follows: Caviar, 2.5 g/kg; Margarine, 1.0 g/kg; Nonalcoholic beverages, 0.60 g/kg; Soy sauce, 0.60 g/kg; Syrup, 0.60 g/kg. When benzoic acid is used as an additive in margarine with sorbic acid or potassium sorbate, or as a preparation containing either of the two additives, the total amount of these combined additives shall not exceed 1.0 g/kg.

As another example, the standard for use level of copper chlorophyll as a food colorant is as follows: Agar jelly in MITSUMAME, 0.00040 g/kg (as copper); Chewing gum, 0.050 g/kg; Chocolate; 0.0010 g/kg; Fish-paste products (excluding SURIMI), 0.030 g/kg; Fruits and vegetables for preservation (including those dried, salted, pickled in vinegar, and preserved in syrup), 0.10 g/kg; KONBU (kelp), 0.15 g/kg of dry kelp; moist cakes with sweet fillings or toppings, 0.0064 g/kg.

The NIHS has researched and developed the analytical methodology in terms of specifications and standards for designated food additives (*7–14*).

**Table 5. Estimated Daily Intake of Sweeteners, Preservatives, and Colorants for Adults**

| *Functional class* | *Food additive* | *Estimated daily intake (mg/person/day)* | *ADI (mg/kg body weight/day)** | *ADI per person (mg/person/day)* | *Ratio to ADI (%)* |
|---|---|---|---|---|---|
| Sweetener | Aspartame | 0.14 | 0-40 | 2000 | 0.01 |
| | Acesulfame potassium | 0.57 | 0-15 | 750 | 0.08 |
| | Saccharin | 0.16 | 0-5 | 250 | 0.06 |
| | Sucralose | 0.10 | 0-15 | 750 | 0.01 |
| | Xylitol | 37 | not specified | | |
| | D-Sorbitol | 452 | not specified | | |
| | D-Mannitol | 92 | not specified | | |
| Preservative | Benzoic acid | 1.45 | 0-5 | 250 | 0.58 |
| | Sorbic acid | 6.36 | 0-25 | 1250 | 0.51 |
| | Sulfur dioxide | 0.17 | 0-0.7 | 35 | 0.46 |
| | Ethyl p-hydroxybenzoate | 0 | 0-10 | 500 | 0 |
| | Propionic acid | 4.26 | not limited | | |

*Continued on next page.*

**Table 5. (Continued). Estimated Daily Intake of Sweeteners, Preservatives, and Colorants for Adults**

| *Functional class* | *Food additive* | *Estimated daily intake (mg/person/day)* | *ADI (mg/kg body weight/day)** | *ADI per person (mg/person/day)* | *Ratio to ADI (%)* |
|---|---|---|---|---|---|
| | Norbixin | 0.06 | 0-0.6 | 30 | 0.19 |
| | Bixin | 0.002 | 0-12 | 600 | 0.00 |
| | Food Red No.2 | 0.005 | 0-0.5 | 25 | 0.02 |
| | Food Red No.3 | 0.002 | 0-0.1 | 5 | 0.05 |
| | Food Red No.40 | 0 | 0-7 | 350 | 0 |
| Colorants | Food Red No.102 | 0.037 | 0-4 | 200 | 0.02 |
| | Food Yellow No.4 | 0.087 | 0-7.5 | 375 | 0.02 |
| | Food Yellow No.5 | 0.014 | 0-2.5 | 125 | 0.01 |
| | Food Green No.3 | 0 | 0-25 | 1250 | 0 |
| | Food Blue No.1 | 0.002 | 0-12.5 | 625 | 0.00 |
| | Food Blue No.2 | 0.000 | 0-5 | 250 | 0.00 |

* ADI were calculated with 50 kg as Japanese average body weight.

**Estimated Daily Intake of Food Additives**

At the NIHS, to ensure that the daily intake of food additives is below an established ADI, and that the maximum use limits and/or the target foods and/or the purpose of use is appropriate under the use standards, the daily intake of food additives is estimated using the market basket method. The method is briefly described as follows. Processed foods are collected and categorized. Data regarding the daily consumption of processed foods are based on the National Health and Nutrition survey. Group samples are prepared and then analyzed to determine the estimated daily intake.

Table 5 shows the estimated daily intake of sweeteners, preservatives, and colorants for adults in Japan. The estimated daily intake for all food additives examined in this study was far below the ADI. Therefore, we can confirm that the daily intake of food additives from consumption of typical foodstuffs is in a range considered to be safe.

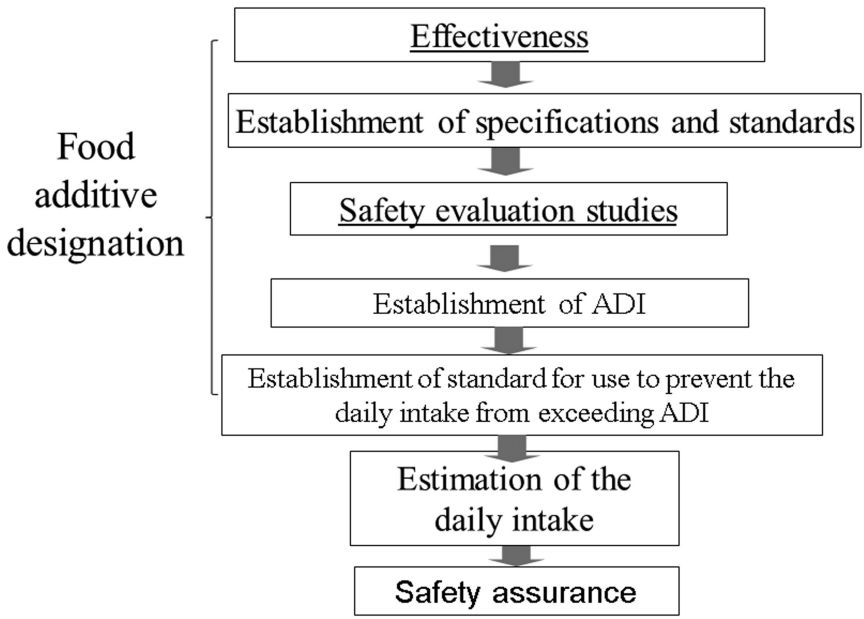



*Figure 4. Safety assurance system for food additives.*

## Conclusion

The safety assurance system for food additives in Japan is summarized and shown in Figure 4. Food additive designation consists of evaluation of effectiveness, establishment of specifications and standards, safety evaluation studies, establishment of an ADI, and standards of use to prevent the daily intake from exceeding the ADI. As demonstrated by the market basket method, an estimated daily intake of food additives is confirmed to be lower than the ADI, showing that the current system results in safety assurance for food additives in Japan.

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Chapter 3

# Framework for FDA's Review of Food Additives, Color Additives, GRAS Substances, and Food Contact Substances

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The 1958 Food Additives Amendment to the Federal Food, Drug, and Cosmetic Act (FD&C Act) defined the term *food additive* and required producers to demonstrate to FDA the safety of a food additive under the intended conditions of use before it enters the market. In enacting this amendment, Congress recognized that many substances intentionally added to food would not require premarket approval to assure their safety because they were generally recognized as safe by experts qualified by scientific training and experience to evaluate their safety. Thus, the intended use of such substances was excluded from the definition of a food additive, and was termed generally recognized as safe (GRAS). Also, in 1960, Congress amended the FD&C Act again to establish premarket review for color additives. Lastly, in 1997, Congress amended the FD&C Act to establish a mandatory pre-market notification process for food contact substances, formerly known as indirect food additives. This chapter will discuss how the agency uses its authorities to ensure that substances and packaging materials in the food supply are safe.

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## Introduction

The food "ingredient" universe is composed of many different entities: food additives, color additives, GRAS substances, food contact substances, food irradiation equipment, and foods/ingredients produced via biotechnology. FDA's Office of Food Additive Safety (OFAS) is responsible for ensuring the safety of these food ingredients.

FDA's modern regulatory functions concerning food safety began with the passage of the 1906 Pure Food and Drug Act. Years later, to improve upon this Act by adding additional regulatory oversight, Congress passed the Federal Food, Drug, and Cosmetic Act (FD&C Act) of 1938. Importantly for OFAS (and its predecessor organizations), over the next 60 years, the FD&C Act was amended a few times to include expanded areas of regulatory oversight, including food additives, GRAS substances, color additives, and food contact substances.

## Legislative History

The first of the important amendments to the FD&C Act was the passage of the 1958 Food Additives Amendment (FAA) to the FD&C Act. Though food additives had been in use for some time, it was not until the passage of the FAA that the term "food additive" was actually defined (see below for definition). The FAA required pre-market approval of new uses of food additives, established the standard of safety and review for food additives, and set in place a formal rulemaking process that would grant legal use of the additive. The FAA also excluded from the definition of a food additive those substances whose uses are GRAS.

A second important amendment to the FD&C Act was the passage of the 1960 Color Additive Amendments (CAA). Federal oversight of color additives began in the 1880s. However, it wasn't until the passage of the CAA in 1960 that the term "color additive" was defined. The CAA required that only color additives listed as "suitable and safe" for a given use, be used in FDA-regulated products. The CAA also made pre-market review for all color additives used in food mandatory; however, there is no GRAS provision in the definition of a color additive.

A third major amendment to the FD&C Act was the passage of the 1997 Food and Drug Administration Modernization Act (FDAMA). Prior to the passage of FDAMA, substances that had an intended use as an indirect additive or a substance that may come in contact with food, but is not directly added to it, were reviewed as food additive petitions (FAPs), unless their intended uses were GRAS or prior sanctioned (that is, had a history of use in food contact materials prior to enactment of the 1958 law). After FDAMA, these indirect substances were termed and defined as "food contact substances (FCSs)". FDAMA established a pre-market notification process for authorizing new uses of FCSs.

The preceding amendments to the FD&C Act have allowed OFAS to provide three pre-market review programs: a petition process for food additives, a petition process for color additives, and a notification process for food contact substances. Though not established through an amendment to the FD&C Act, OFAS also offers

a voluntary notification process for GRAS substances. Each program area has similarities in the safety assessment, but details and processes differ. Ensuring the safety of the food supply is the over-arching goal for all of OFAS' pre-market programs.

## Overarching Programmatic Themes

Regardless of whether a company submits a FAP, color additive petition (CAP), GRAS notice (GRN), or food contact notification (FCN), there are certain similarities across program areas. For example, the standard of safety is the same, regardless of program area. Congressional intent laid out the concept that safety requires evidence of a reasonable certainty that no harm will result from the proposed use of an additive (*1*). Congress recognized that there can never be absolute certainty of safety, but that there should be a reasonable certainty of no harm to ensure safety. Though the FD&C Act and implementing regulations do not define "harm," the legislative history is clear in that an effect is harmful if it adversely affects health, not if it is simply an undesirable or unexpected effect that has no adverse health consequences. Furthermore, a decision on a substance's use in food is based solely on whether the use is safe under the conditions of intended use, and not a risk-benefit analysis.

Additionally, the safety review standard is the same across program areas such that there is a fair, science-based evaluation of all data associated with a submission. All submissions to OFAS's premarket programs are sponsored by companies and include the data needed to support a safety decision. Agency scientists are not limited to the data in the submission and may consider any relevant available data in their evaluation. FDA recognizes that with scientific advancement over time, new information about a substance may become available; therefore, decisions are time-dependent. In addition, decisions that are made on a substance's use with food must be able to withstand scientific, procedural, and legal challenges.

When assessing the safety of a food substance across program areas, it is important to ask two specific questions: 1) what is the food substance and the resulting dietary intake and 2) is it safe for its intended use? The answer to the former lies within the substance itself: its identity and composition, method of manufacture, specifications and its use level and exposure. The answer to the latter lies within data and information on the substance, which may include the absorption, distribution, metabolism, and excretion of the substance, preclinical or clinical studies as appropriate, and any other special studies deemed necessary. All submissions to OFAS must discuss relevant areas in detail.

Environmental informational must also be considered when deciding upon submitting a petition or notification to the agency. The National Environmental Policy Act (NEPA) of 1969 and its implementing regulation at Title 21 of the Code of Federal Regulations (CFR) Part 25, requires Federal agencies to consider the environmental impact of major and final agency actions such as FAPs, CAPs and FCNs. Because GRNs are not agency-initiated actions, environmental information is not required.

## Food and Color Additive Petitions

As mentioned earlier, amendments to the FD&C Act in 1958 and 1960 defined what a food additive and color additive (respectively) was. These amendments also set in place a requirement for pre-market review, via a petition process, for new uses of food additives and both existing, as well as new uses of color additives.

Section 201(s) of the FD&C Act defines a food additive as any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food (including any substance intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food; and including any source of radiation intended for any such use), if such substance is not generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures (or, in the case of a substance used in food prior to January 1, 1958, through either scientific procedures or experience based on common use in food) to be safe under the conditions of its intended use.

Section 201(t) of the FD&C Act defines a color additive as a dye, pigment, or other substance made by a process of synthesis or similar artifice, or extracted, isolated, or otherwise derived, with or without intermediate or final change of identity, from a vegetable, animal, mineral, or other source, and when added or applied to a food, drug, or cosmetic, or to the human body or any part thereof, is capable (alone or through reaction with other substance) of imparting color thereto. Unlike the definition for food additive, there is no GRAS provision in the definition for color additives.

Sections 409 and 721 of the FD&C Act lay out the requirements for substances meeting the definitions of food and color additives, respectively. Those sections of the law and the implementing regulations at 21 CFR Part 171.1 (FAP) and Part 71.1 (CAP) also set forth the petition process that the agency follows when a petitioner wants authorization for a new use of a food or color additive. These regulations also discuss what information should be included in a FAP and CAP. FAPs consists of the following components: 1) Identity and composition of the food substance; 2) Manufacture and specifications; 3) Intended use in food (e.g., food categories, levels, and intended effects); 4) Analytical methodology; 5) Full reports of safety data, including toxicological and other studies – Acceptable Daily Intake; 6) Proposed tolerances, if needed; and 7) Environmental information. CAPs have many of the same components as a FAP, but also require the petitioner to provide both a rationale for whether a color is exempt from batch certification and also submit a prescribed fee for listing.

OFAS encourages a pre-submission consultation for any potential petitioner wanting to discuss a submission prior to submitting it for official review. When FDA receives a food or color additive petition, an initial review is made to determine whether the petition is adequate for filing (see 21 CFR Parts 171.1 and 71.1, respectively for FAP and CAP requirements). If the petition is adequate for filing, a filing notice is published in the Federal Register (FR). The notice includes basic information about the petition, including petitioner name, proposed use, and is the agency's means of alerting the public to receipt of the petition.

During the review process, FDA communicates with the petitioner to address any issues, clarifications, or questions that might arise as a result of the review. The agency then has 180 days to either grant or deny the petition. However, if additional information is needed to complete the review, additional time may be necessary. The amount of time for completion of the safety review of a petition is based on several factors, such as amount of information provided in the petition, complexity of the data, need for supplemental information, as well as the responsiveness of the petitioner.

When all questions/issues have been answered or resolved, the agency makes a safety determination for the petitioned use of the substance and, if found to be safe, publishes a final rule permitting use in the FR. The preamble to the final rule provides the rationale for the agency's safety decision. It is important to note that the approved use is generic—anyone can use the additive as long as the use complies with the regulation—the use is not exclusive to the petitioner. Once the final rule has published, there is a 30-day objection period in which anyone can object to the rule and request a hearing.

The agency also notifies the World Trade Organization of the petitioned substance both after filing and after a final rule is published. In this manner, our trading partners are free to comment on the proposed use or object to the final rule.

## Generally Recognized as Safe Notices

One of the notable exemptions to the definition of a food additive is for substances that are generally recognized as safe or GRAS. GRAS is a legal concept, derived from Sections 201(s) of the FD&C Act. A substance is GRAS if it is generally recognized by qualified experts, as safe through scientific procedures or, in the case of a substance used in food prior to January 1, 1958, through either scientific procedures or experience based on common use, for its intended use. GRAS is meant to be a flexible regulatory tool. In fact, Congressional intent for GRAS was as a practical approach to allocating resources using scientific judgment without giving away the agency's post-market authority (*1*). Though GRAS is an exemption in the definition of a food additive, the standard of safety is the same for GRAS substances as it is for food additives.

Food additives and GRAS substances must both have a technical knowledge element, which consists of the safety data information that is adequate for its intended use. One of the main distinguishing factors for food additives and GRAS substances lies with the common knowledge element. In order for a substance to be considered GRAS for an intended use, its key data must be both generally available (in the public domain) and generally accepted. The intended use is considered as a food additive if both these criteria are not met (*2*).

Under the FD&C Act, the sponsor of a food substance is allowed to make its own independent determination of GRAS status, whether for a new substance in the food supply or for a new use of a substance already in the food supply. A sponsor is not required to inform FDA of this GRAS determination. After the enactment of this provision in 1958, FDA listed a number of substances as GRAS

(21 CFR Part 182). Later, FDA also affirmed as GRAS a number of substances as part of the agency's review of substances considered to be GRAS or in response to industry-sponsored GRAS affirmation petitions (21 CFR Part 184). Currently, in place of the voluntary GRAS affirmation petition process, FDA operates a program under a proposed regulation whereby companies may notify the agency (in a submission called a GRN) about their independent GRAS determinations (*2*).

While the types of information supporting safety in a GRN and a FAP are similar, there are differences. For example, information including raw data from safety studies is provided in petitions, whereas GRNs contain summary information for the relevant studies. As discussed above, since a GRN does not result in agency action, environmental information under NEPA is not required. GRNs contain a GRAS exemption claim signed by the notifier, a discussion of the identity and properties of the substance, method of manufacture, and a discussion of the notifier's reasons for concluding the substance is GRAS for its intended use. These reasons include information supporting the GRAS determination (e.g., estimated daily intake and toxicological studies), information that would appear to be inconsistent with GRAS determination, and why, in light of the totality of the information, the notifier concludes the substance is GRAS for the intended use.

OFAS also offers a pre-submission consultation for potential notifiers to discuss an independent GRAS determination before submitting a formal GRN for review. When OFAS receives a GRN, it is first assessed for completeness before it is accepted for filing. Once filed, it undergoes a thorough evaluation to determine if it meets the GRAS criteria. If needed, communication between OFAS and the notifier (or agent if applicable) occurs to address deficiencies that are associated with the submission.

While there is no statutory deadline for FDA's response to a company's GRN, OFAS sets a goal for a response of 180 days from filing. Complexity, as well as responsiveness by the notifier to FDA's requests for additional information could affect this timeline. FDA responds by letter to a GRAS notice in one of the following categories: 1) the agency does not question the basis for the notifier's GRAS determination; 2) the agency concludes that the notice does not provide a sufficient basis for a GRAS determination; or 3) the agency may also cease to evaluate the notice upon the notifier's request.

## Food Contact Notifications

The passage of FDAMA established a mandatory premarket notification process in 21 CFR 170.100-170.106 (*3*) for FCSs termed "food contact notifications" (FCNs). FDAMA also provided a definition for FCSs as any substance intended for use as a component of materials used in manufacturing, packing, packaging, transporting, or holding food if such use is not intended to have any technical effect in such food. Components of food processing equipment and other substances used in processing food are included.

The FCN process is the primary means by which FDA authorizes new uses of food additives that are food contact substances. In particular, the process is used to review the use of new FCSs (this includes FCSs that are the subject of regulations, but whose previous conditions of use are now being modified). The FCN process is exclusive to the manufacturer and/or supplier. As such, the FCN review process and authorization for use of the new FCS is effective only for the manufacturer and/or supplier who submitted the notice. Any change in the manufacturing process may require a new FCN submission. The FCN process streamlines the previous FAP process for indirect food additives, in that the notification constitutes the sponsor's decision that a food contact material is safe for the proposed use. Prior to submitting an FCN to FDA, a potential notifier may submit a pre-notification consultation (PNC) request to FDA to discuss the eligibility of the FCS, and the adequacy of the information supporting the proposed use of the FCS. The FCN itself has the same basic data requirements (i.e., chemistry, toxicology, and environmental information) as an FAP. If the exposure to the FCS under its proposed intended use is higher than is typically seen for FCSs, FDA can decide that the FAP process is more appropriate. The FDA has 120 days of receipt of a complete FCN notification to object if it disagrees with the assessment. If no agency action is taken, the notification becomes effective and the sponsor may market the material. As mentioned above, the standard of safety for an FCN is the same as for a FAP, CAP, or GRN. If FDA concludes that there is a reasonable certainty of no harm from the intended use, the agency will allow the FCN to become effective. Finally, effective FCNs are listed on FDA's website.

In 1995, FDA established a threshold of regulations exemption process in 21 CFR 170.39 whereby those uses of food contact articles that results in dietary concentrations at or below 0.5 ppb could undergo premarket approval via an abbreviated review process (*4*).

## Summary

The universe of food ingredients is governed by statutory requirements related to safe use. Based on the common knowledge element for data and information to support the safety of a substance (FAP versus GRN) and exposure levels (FAP versus FCN), companies have several regulatory pathways for seeking FDA review of the intended use of their substances in conjunction with food. Though each program has its differences in terms of processes (see Table 1), the standard of safety is the same for all.

Decisions regarding the safety of a substance must first and foremost protect public health. Because new data or information could raise questions about the safety of a substance, safety decisions are subject to post-market review based on advances in toxicology information over time. Decisions must also withstand both scientific and legal challenges. For more information on FDA regulatory programs for food ingredients, please visit www.fda.gov/food.

**Table 1. Comparison of the Regulatory Approaches for Various Food Ingredients**

| *Petitions* | *Gras* | *Fcs* |
|---|---|---|
| For food additives since 1958 and for color additives since 1960 | 1997 to now (previously a GRAS affirmation petition process) | Since 1997 (previously handled as "indirect" food additive petitions |
| Mandatory | Voluntary | Mandatory |
| Industry submits a petition asking FDA to issue a regulation | Notifier informs FDA of their view that a use of a substance is GRAS | Industry submits a notification |
| FDA owns the safety decision | Notifier owns the safety decision; FDA evaluates the notifier's basis | FDA owns the safety decision, but there is a 120-day "hammer" |
| FDA publishes a regulation | FDA responds by letter (no questions, no basis, withdrawal) | FDA responds by letter (deficiency, effective, objection) |
| Environmental information is required | Environment information is not required | Environmental information is required |
| Petition, with the exception of trade secret information, is available publically through FOIA | FDA responses, and more recently entire gras notices, are published on FDA's website | FDA maintains a database of effective notifications on its website |
| Generic | Generic | Exclusive |

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## Chapter 4

# An Industry Representative's Views on the Regulation of GRAS Food Contact Substances and Ingredients

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The concept of general recognition of safety is central to the regulatory scheme applicable to food products, food additives, and food contact substances in the United States. This paper will examine that regulatory scheme and the place of GRAS in it, and will provide an evaluation of recent suggestions that additional regulatory intervention is necessary to assure the safety of food ingredients and food contact substances.

To understand the concept of "general recognition of safety," or "generally recognized as safe," or GRAS, it is necessary to understand the concept of "food additive" as it is defined within the U.S. Federal Food, Drug, and Cosmetic Act (*1*). As important as the concept of GRAS is, ironically, it only appears in FFDCA as a negative conceptual framework within the definition of "food additive." In short, the FFDCA defines "food additive" as essentially any substance that is added to or might migrate into food that is not generally recognized as safe or is not separately regulated because it has some other status as, for example, a pesticide residue or color additive, or has a prior sanction from a government agency. Specifically, the definition reads as follows:

(s) The term "food additive" means any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food (including any substance intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food; and

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including any source of radiation intended for any such use), if such substance is not generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures (or, in the case of a substance used in food prior to January 1, 1958, through either scientific procedures or experience based on common use in food) to be safe under the conditions of its intended use; except that such term does not include—

(1) a pesticide chemical residue in or on a raw agricultural commodity or processed food; or
(2) a pesticide chemical; or
(3) a color additive; or
(4) any substance used in accordance with a sanction or approval granted prior to the enactment of this paragraph 4 pursuant to this Act [enacted Sept. 6, 1958], the Poultry Products Inspection Act (21 U.S.C. 451 and the following) or the Meat Inspection Act of March 4, 1907 (34 Stat. 1260), as amended and extended (21 U.S.C. 71 and the following);
(5) a new animal drug; or
(6) an ingredient described in paragraph (ff) in, or intended for use in, a dietary supplement (*1*).

There can be no doubt from reading this definition that Congress intended the legal scope of food additives to go far beyond what is probably the common understanding of the term among consumers, namely, substances such as preservatives, flavorings and other functional participants in a food formulation. The wording of the definition begins with a very broad statement of substances encompassed within the definition, then, as if to reassure the reader that the broad sweep of the definition is intentional, it provides a listing of specific functions that substances might serve that are encompassed within the definition, specifically listing substances "intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food."

After introducing and reinforcing the broad scope of the definition, it then carves out an exception from coverage of the definition for substances that are GRAS.

Here is how the definition explains the concept of general recognition of safety:

> "if such substance is not generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures (or, in the case of a substance used in food prior to January 1, 1958, through either scientific procedures or experience based on common use in food) to be safe under the conditions of its intended use."

In other words, the law says that we must examine and defer to the opinion of relevant qualified experts, (not otherwise identified), unless we are relying on the fact that a substance was commonly used in food, safely, since before January 1,

1958. And specifically what we seek to find out is whether such experts recognize the safety of the use of a substance under the intended conditions of its use. This is a crucial element of the definition of GRAS as well as the definition food additive: that what is being evaluated is not so much a substance as a specific use of the substance, taking into account the amount of it being used, the food types with which it is being used, the function it serves, and other factors. Therefore it is most useful to think of GRAS uses of substances rather than GRAS substances. Importantly, it follows that a substance that experts consider GRAS for one intended use is not necessarily GRAS for any other intended use.

What one might have expected to see in this definition and is notable for its absence is any reference within the definition to approval, concurrence, license, review or even notice to the relevant regulatory agency, the US FDA. That is an aspect of the regulatory scheme that some observers consider objectionable.

GRAS status is the highest level of recognition that use of a substance can achieve, reflecting both the safety of the substance and widespread knowledge and acceptance of the fact of its safety in that given use. As the name indicates, there are two elements to a GRAS conclusion: first, that a use of a substance is safe, and second, that there is general recognition of that fact among qualified experts. The safety standard employed is the same as that used for evaluating the safety of food additives, often summarized as reasonable certainty of no harm. 21 CFR Sec. 170.3(i) (*2*). The general recognition requirement relies on publicly available information, ideally published facts in peer reviewed scientific or medical publications or reference books. In a sense, the GRAS regulatory scheme, incorporating as it does a kind of freedom and self-determination for individual companies, reflects the American tradition preferring free markets whenever possible.

In recent years, at least two entities have raised important questions about the current regulatory framework applicable uses of substances considered GRAS. The US government's Government Accountability Office (GAO) issued a 2010 report (*3*) recommending steps to strengthen FDA's oversight of GRAS determinations, and the Pew Health Group has issued a series of reports (*4*) about GRAS changes as well.

The GAO report recommended these six measures:

1. Require any company that conducts a GRAS determination to provide FDA with notice, and make it public;
2. Minimize the potential for conflicts of interest in companies' GRAS determinations;
3. Monitor the appropriateness of companies' GRAS determinations through random audits or some other means, including issuing guidance on how to document GRAS determinations;
4. Finalize the [1997] FDA proposed rule (*5*, *6*) that governs the voluntary notification program,
5. Conduct reconsiderations of the safety of GRAS substances in a more systematic manner,
6. Ensure safety of engineered nanomaterials

As evidence of FDA's inadequate process for reconsidering safety conclusions, the GAO report pointed to 11 petitions that have been filed seeking reconsideration by FDA of GRAS conclusions that had been pending for as many as six years without FDA response.

In turn, among the concerns raised in a 2012 report from the PEW Health group (*4*) were:

1. FDA is unaware of a large number of chemical uses in food and, therefore, cannot ensure that safety decisions regarding these uses were properly made.
2. Food manufacturers are not required to notify FDA of relevant health and safety studies, thereby placing FDA in the difficult position of tracking safety information for more than 10,000 chemicals with limited resources and information.
3. The agency's expedited approach to reviewing safety decisions since 1995 occurs with little public engagement.
4. FDA lacks the resources and information needed to identify and prevent potential health problems or to set priorities for systematic reevaluation of safety decisions made during the past half-century.

The concerns raised by both of these groups appear to be more theoretical than real. Re-evaluations of the safety of a substance's use can occur under the current scheme when warranted. Nevertheless, a relatively small number of substances have been the subject of re-evaluations and removals from use. For example, the Flavor and Extract Manufacturers Association ("FEMA"), has for decades operated a careful, thorough and well-respected GRAS evaluation program for flavorings and related substances used in food. FEMA (*7*) has only removed 11 out of 2,600 substances from its lists. Moreover, when, in 1997, the US FDA changed its program from accepting and reviewing GRAS determinations and issuing "affirmations" of the GRAS status of a use, replacing it instead with the GRAS notification program which resulted only in letters confirming that FDA "has no questions" about a submitter's conclusion that a use is GRAS, the agency made remarks seeming to indicate that the evaluation of uses of substances perceived to be GRAS was a realm of general safety. (*5*).

This is especially so when the uses of substances being evaluated are food contact materials rather than food ingredients, because exposure to food contact substances is generally quite significantly lower in quantity than exposure to food ingredients. FDA works from the common principle that the higher the level of exposure to substance, the higher the risk tends to pose. (*2*).

It must be noted that critics of the current system are unable to point to examples of a significant number of substances that have long been widely used but later were discovered to present safety issues. If the system was inadequate or inappropriate, one would expect to see more examples of its failures.

One seemingly middle-ground suggestion is the call for users to give FDA notice of their uses of substances on the basis of a conclusion that the use is GRAS. This was one of the suggestions in the GAO report, and the Pew Health Group raised the concern that FDA was unaware of many uses. However, we must be

careful not to create a solution where there is no problem. Even a notification program would carry with it significant burdens on both industry and FDA that would not appear to be necessary unless there was a perception of significant safety gaps because of the current system.

Remember, other protections against incorrect and faulty decision-making by companies will continue to exist with or without a role for FDA. These include civil tort liability should consumers be injured by product, FDA regulatory remedies including seizure of product, injunction against distribution, criminal prosecution should the agency conclude the food is adulterated, and adverse publicity for the company involved.

Balancing the benefits that regulatory intervention might bring against the costs in light of the perceived problems, it does not appear that the suggested changes in the regulatory scheme put forth by GAO and the Pew Health Group are warranted. Going forward, this is bound to be an issue that will be the subject of healthy public debate.

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## Chapter 5

# Food Additives, Enzymes, and Flavourings Legislation in the European Union

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The European Union adopted in 2008 a package of regulations which further upgrades and further harmonises the rules for food additives and flavourings to be used in and on food and introduces harmonised EU legislation on food enzymes for the first time. In addition, a simplified common approval procedure for food additives, flavourings and enzymes, was created which is based on the scientific opinions from the European Food Safety Authority (EFSA). These regulations often are referred to as the "Food Improvement Agent Package" (FIAP). For additives and flavourings that were already covered by EU legislation, the regulations bring the rules into line with the latest scientific and technological developments and will improve the clarity of the legislation. With regard to food enzymes, the new regulation replaces divergent national legislation with new, harmonised EU rules, including a procedure for the establishment of a Union list of authorised food enzymes. This chapter provides an overview of this new EU legislation.

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# Introduction

The use of food additives in the 28 member states of the European Union (EU) is completely harmonised. In the past, differences between national laws relating to food additives and the conditions for their use could cause barriers to trade between EU member states. A single market for food products cannot exist without harmonised rules for the authorisation of food additives and the conditions for their use. In the beginning, EU legislation on food additives focused on the need to create common European lists for each functional group of additives.

The first directive was on colours (1962) and the E-number classification system was used for the first time. This was followed by directives for preservatives (1964), antioxidants (1970) and emulsifiers, stabilisers, thickeners and gelling agents (1974). Adoption of these directives was slow and they only specified the permitted substances. Member States were still free to lay down which foods could contain the substances and the maximum permitted levels.

In order to create the internal market, further harmonisation was needed. For food additives, this was achieved during the period 1988-1995. In 1989 a Framework Directive (89/107/EEC) was adopted which set out the criteria for the use of food additives and provided for the adoption of three specific technical directives establishing the list of additives. In addition Council Directive 88/388/EEC established also the general principles applicable to flavouring for use in foods.

In 2008 the EU legislation on food improvement agents was adopted. This includes regulations on food additives, food enzymes and flavourings for use in and on foods and a common procedure for their approval. Often they are referred to as the Food Improvement Agent Package. This legislative package replaces the earlier directives on food additives and flavourings and introduces rules for the use of enzymes. The Food Improvement Agent Package comprises the following regulations:

- Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives (*1*).
- Regulation (EC) No 1332/2008 of the European Parliament and of the Council on food enzymes (*2*).
- Regulation (EC) No 1334/2008 of the European Parliament and of the Council on flavourings and certain food ingredients with flavouring properties (*3*).
- Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings (*4*).

This new set of legislation ensures an increased level of human health and consumer protection, including fair practices in food trade, taking into account, where appropriate, the protection of the environment and at the same time ensures the effective functioning of the internal market. The single instrument establishing

a common authorisation procedure for additives, enzymes and flavourings ensures that the risk assessment and approval of these substances is carried out in a uniform and transparent way.

## Food Additives

**Framework Regulation**

The framework Regulation (EC) No 1333/2008 on food additives provides for:

- Definitions.
- General conditions for inclusion and use of food additives in the Union lists.
  A food additive may be included in the Union lists if it meets the following conditions:

    - It does not, on the basis of the scientific evidence available, pose a safety concern to the health of the consumer at the level of use proposed;
    - There is a reasonable technological need that cannot be achieved by other economically and technologically practicable means;
    - Its use does not mislead the consumer.
      Misleading the consumer includes, but is not limited to, issues related to the nature, freshness, quality of ingredients used, the naturalness of a product or of the production process, or the nutritional quality of the product, including its fruit and vegetable content.

- Specific conditions for sweeteners

    (a) Replace sugars for the production of energy-reduced food (at least 30%), non-cariogenic food or food with no added sugars;
    (b) Replace sugars where this permits an increase in the shelf-life of the food;
    (c) Produce food intended for particular nutritional use.

- Specific conditions for colours

    (a) Restoring the original appearance of food of which the colour has been affected by processing, storage, packaging and distribution, whereby visual acceptability may have been impaired;
    (b) Making food more visually appealing;
    (c) Giving colour to food otherwise colourless.

- The principle of carry-over.
- Rules for the labelling of food additives sold as such.
- Need for the adoption of a programme for the re-valuation of food additives.
- Functional classes of food additives in foods and of food additives in food additives and food enzymes.
- Union list of food additives approved for use in foods and conditions of use.
- Union list of food additives including carriers approved for use in food additives, food enzymes, food flavourings, nutrients and their conditions of use.
- Traditional foods for which certain Member States may continue to prohibit the use of certain categories of food additives.
- List of the food colours referred to in Article 24 for which the labelling of foods shall include additional information.

Food additives are defined as any substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food, whether or not it has nutritive value, the intentional addition of which to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food results, or may be reasonably expected to result, in it or its by-products becoming directly or indirectly a component of such foods.

The following are not considered to be food additives:

(i) Monosaccharides, disaccharides or oligosaccharides and foods containing these substances used for their sweetening properties;
(ii) Foods, whether dried or in concentrated form, including flavourings incorporated during the manufacturing of compound foods, because of their aromatic, sapid or nutritive properties together with a secondary colouring effect;
(iii) Substances used in covering or coating materials, which do not form part of foods and are not intended to be consumed together with those foods;
(iv) Products containing pectin and derived from dried apple pomace or peel of citrus fruits or quinces, or from a mixture of them, by the action of dilute acid followed by partial neutralisation with sodium or potassium salts (liquid pectin);
(v) Chewing gum bases;
(vi) White or yellow dextrin, roasted or dextrinated starch, starch modified by acid or alkali treatment, bleached starch, physically modified starch and starch treated by amylolitic enzymes;
(vii) Ammonium chloride;
(viii) Blood plasma, edible gelatin, protein hydrolysates and their salts, milk protein and gluten;
(ix) Amino acids and their salts other than glutamic acid, glycine, cysteine and cystine and their salts having no technological function;
(x) Caseinates and casein;
(xi) Inulin.

The regulation prohibits the placing on the market of a food additive or any food in which such a food additive is present if the use of the food additive does not comply with the requirements set out in the Regulation. Only food additives included in the Union list may be placed on the market as such and used in foods under the conditions of use specified in the Regulation.

All additives in the EU must be authorised and listed in the EU's 'positive' list based on the conditions specified in the Regulation. The approval of food additives should also take into account other factors relevant to the matter under considerations including societal, economic, traditional, ethical and environmental factors, the precautionary principle and the feasibility of controls.

The use and maximum levels of a food additive should take into account the intake of the food additive from other sources and the exposure to the food additive by special groups of consumers.

The Regulation (EC) No 1333/2008 replaces the following legislation:

- Framework Directive 89/107/EEC;
- Directive 94/35/EC on sweeteners for use in foodstuffs;
- Directive 94/36/EC on food colours for use in foodstuffs;
- Directive 95/2/EC on food additives, other than colours and sweeteners.

**List of Authorised Additives**

The Union list of food additives authorised in food is included in Annex II to Regulation (EC) No 1333/2008.

Food additives that were permitted for use in foods under European Parliament and Council Directives 94/35/EC on sweeteners, 94/36/EC on colours 95/2/EC on food additives other than colours and sweeteners are included in the Union list in Annex II to Regulation (EC) No 1333/2008 after a review of their compliance with the conditions of use in the Regulation. Food additives and uses which are no longer needed were not entered in the list.

The list was adopted in November 2011 and applies since 1 June 2013.

Only food additives included in the Union list may be placed on the market and used in foods under the conditions of use specified therein. The food additives are listed on the basis of the categories of food to which they may be added. In order to facilitate the transfer and to enhance transparency, a new food categorisation system which forms the basis of Annex II was established.

The food category system established by the Codex Alimentarius General Standard for Food Additives has been used as a starting point for developing the Union system. However, that system has been adapted to take into account the specificity of the existing food additive authorisations in the European Union. Specific EU legislation that exists for certain foodstuffs has been taken into account, e.g. EU legislation on jam, chocolate sprit drinks etc. The categories were created with the sole purpose of listing the authorised additives and their conditions of use. The European Commission provides guidance by describing the different categories in order to ensure uniform interpretation.

Transparency is the major benefit of the new legislation. The additives are listed in a clear way, according to the category of food to which they may be added. e.g. fish and fish products, fruit and vegetables, dairy products, confectionery, etc.

The new list is more accessible for all persons involved in any component of the food chain, including consumer, control authorities or food industry. The improved transparency allows correct and therefore safer use of food additives.

This Union list includes:

- The name of the food additive and its E number;
- The foods to which the food additive may be added;
- The conditions under which the food additive may be used;
- Restrictions on the sale of the food additive directly to the final consumer.

Annex II includes furthermore:

- List of foods in which the presence of an additive may not be permitted by virtue of the carry over principle set out in Article 18(1)(a) of Regulation (EC) No 1333/2008;
- List of foods in which the presence of a food colour may not be permitted by virtue of the carry over principle set out in Article 18(1)(a) of Regulation (EC) No 1333/2008 are listed in table 2;
- List of colours that may be used in the form of lakes.

The Union list of food additives authorised for use in food and their conditions of use can be consulted via the food additives database which is available online (*5*).

Annex III to Regulation (EC) No 1333/2008: Union list of food additives including carriers approved for use in food additives, food enzymes, food flavourings, nutrients and their conditions of use.

Annex III consists of the following parts:

- Part 1: Carriers in food additives.
- Part 2: Food additives other than carriers in food additives.
- Part 3: Food additives including carriers in food enzymes.
- Part 4: Food additives including carriers in food flavourings.
- Part 5: Carriers in nutrients and other substances added for nutritional and/or for other physiological purposes.

**Specifications for Food Additives**

Specifications for food additives that are listed in the Union lists in Annex II and III to Regulation (EC) No 1333/2008 relating to origin, purity criteria, and any other necessary information were adopted by Regulation (EU) No 231/2012 (*6*).

The regulation contains and updates the specifications previously developed for food additives in Commission Directive 2008/60/EC laying down specific purity criteria concerning sweeteners for use in foodstuffs, Commission Directive 2008/128/EC laying down specific purity criteria concerning colours for use in

foodstuffs and Commission Directive 2008/84/EC laying down specific purity criteria on food additives other than colours and sweeteners. The specifications take into account the specifications and analytical techniques as set out in the Codex Alimentarius drafted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

**Re-Evaluation of Food Additives**

Regulation (EU) No 257/2010 (*7*) sets up a programme for the re-evaluation by the European Food Safety Authority (EFSA) of the safety of food additives that were already permitted in the Union before 20 January 2009.

The deadlines for these re-evaluations are:

- All food colours by the end of 2015;
- Preservatives, antioxidants, glutamates, silicon dioxide by the end of 2016;
- All other additives except sweeteners by the end of 2018;
- Sweeteners by the end of 2020.

When re-evaluating an approved food additive, EFSA shall:

(a) Examine the original opinion and the working documents of the Scientific Committee on Food ('SCF') or EFSA;
(b) Examine, where available, the original dossier;
(c) Examine the data submitted by the interested business operator(s) and/or any other interested party;
(d) Examine any data made available by the Commission and Member States;
(e) Identify any relevant literature published since the last evaluation of each food additive.

In order to acquire the data from the interested business operators and/or other interested parties, EFSA makes open calls for data for the food additives under re-evaluation, specifying the timetable for data submission.

This data comprises among others:

- Study reports from the original dossier as evaluated by the SCF or EFSA or the Joint FAO/WHO Expert Committee on Food Additives (JECFA);
- Information on the data on the safety of the food additive concerned not previously reviewed by the SCF or the JECFA;
- Information on the specifications of the food additives presently in use, including information on particle size and relevant physicochemical characteristics and properties;
- Information on the manufacturing process;
- Information on analytical methods available for determination in food;
- Information on the human exposure to the food additives from food (e.g. consumption pattern and uses, actual use levels and maximum use levels, frequency of consumption and other factors influencing exposure).

Where the requested information has not been submitted to EFSA within the set deadlines, the food additive may be removed from the Union list.

## Enzymes

### Introduction

Enzymes are naturally-occurring proteins that enhance chemical reactions. They can be obtained by extraction from plants or animals or by fermentation from micro-organisms. They are normally added to perform a technological function in the manufacture, processing, preparation and treatment of foods.

Enzymes are classified by the type of reaction they catalyse and the substrate they act upon.

### Background

Over the last thirty years, the use of enzymes and enzyme preparations has steadily increased in all sectors of the food industry. Enzymes are generally used and considered as processing aids, since most are used during food processing. Food enzymes other than those used as food additives were not regulated or were regulated as processing aids under the legislation of the member states. Only two food enzymes (invertase and lysozyme) fell within the definition of food additives in accordance with Regulation (EC) No 1333/2008 on food additives.

The Scientific Committee on Food (SCF) drew up guidelines for the presentation of data on food enzymes (*8*) (published in 27th report series 1992) and specified that, whilst the distinction between processing aids and food additives may be of administrative importance (some being labelled, others not), from a toxicological point of view it was not pertinent to distinguish these two categories since, in both cases, the enzyme preparation may remain in the food. Very few enzymes were evaluated by the SCF.

The White Paper on food safety (*9*) drawn up by the European Commission in January 2000 proposed, among other things, that the status of enzymes should be clarified. Moreover, the Commission, as part of the actions plans on food safety, proposed to lay down specific provisions in respect of food enzymes.

The Commission therefore was reflecting on the need to consider the legal status and the safety of enzymes throughout the European Union. For that purpose, a Task (study of the enzymes used in foodstuffs and collection of data on their safety) was undertaken in the framework of Council Directive 93/5/EEC (*10*) on the assistance to the Commission and co-operation by the Member States in the scientific examination of questions relating to food. The main objectives of the Task were to draw up inventories of the uses of enzymes, the approval system and the safety evaluation procedures concerning the enzyme preparations used in the EU food industry. In order to fulfil the objectives, nine volunteered Member States, the Commission and the Association of Manufacturers of Fermentation Enzyme Product (AMFEP) participated in the Task. The report of the Task was published in December 2000 (*11*).

The report indicated that a list of food enzymes and their uses in food manufacturing in the European Union (EU) were not available. Only two member states, Denmark and France, were able to provide an official list of food enzymes that are permitted for use in accordance with their national regulations. It was concluded that procedures for the safety evaluation and approval as well as for the control of the uses of enzymes would be useful in all the EU Member States. It was also agreed to support an approach for harmonisation of the Regulations through the usual procedures.

**Legal Framework**

Following the outcome of the Task report and due to the fact that differences between national laws, regulations and administrative provisions concerning the assessment and authorisation of food enzymes may hinder their free movement, creating conditions for unequal and unfair competitions, it was necessary to adopt EU rules harmonising national provisions relating to the use of enzymes in foods. In this regard, Regulation (EC) No 1332/2008 on food enzymes (enzyme Regulation) harmonises for the first time the rules for food enzymes in the EU. Regulation (EC) No 1331/2008 introduced a common approval procedure for additives, enzymes and flavourings used in food.

The enzyme Regulation provides for a Union list of approved food enzymes, conditions of use of food enzymes in foods and rules on the labelling of food enzymes sold as such. The enzyme Regulation does not cover enzymes intended for human consumption, for example, those used for nutritional or digestive purposes, or food enzymes used in the production of food additives (as defined by Regulation EC No 1333/2008 on food additives). The Regulation does not cover microbial cultures that are traditionally used in the production of food and which may incidentally produce enzymes, but which are not specifically used to produce them.

With regard to the Union list of food enzymes, the enzyme Regulation requires that all food enzymes currently on the EU market as well as new food enzymes shall be subject to safety evaluation by the European Food Safety Authority (EFSA). In order to allow industry to make available the information necessary for the risk assessment of food enzymes a two-year period was fixed in the enzyme Regulation for submission of applications on existing enzymes and new enzymes. This period started from 11 September 2011 as prescribed by Regulation (EU) No 234/2011 implementing Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. However, the experience gained in the meantime showed that the initial deadline for submitting applications was insufficient in order to allow stakeholders and in particular small and medium sized enterprises to produce all necessary data within that period. Therefore, the enzyme Regulation was amended by Commission Regulation (EU) No 1056/2012 (*12*) in order to extend the initial 24-months period to 42 months (deadline by 11 March 2015).

Regulation (EU) No 234/2011 lays down the content, drafting and presentation of an application. A further amendment by Regulation (EU) No 562/2012 (*13*) amending Commission Regulation (EU) No 234/2011 with regard

to specific data required for risk assessment of food enzymes (*1*) introduced the possibility of a derogation from submitting toxicological data under certain conditions and the grouping of food enzymes under one application to improve the efficiency of the evaluation process. The latter only applies to enzymes with the same catalytic class, manufactured substantially by the same process and originating from the same source, and for enzymes obtained from:

- Edible parts of non-genetically modified plants and animals;
- Microorganisms having the EFSA's status of Qualified Presumption of Safety;
- Micro-organisms which have been used in the production of food enzymes that have been evaluated by France or Denmark (with the exception of those produced by genetically-modified plants, animals or micro-organisms) in accordance with the SCF guidelines of 1992.

The requirements of the content of the Union list are set out in the enzyme regulations. The Union list shall be drawn up in a single step once EFSA has issued an opinion on each food enzyme. As a significant number of applications is expected to be submitted during the submission period, a lengthy period will therefore be needed before the risk assessment of these applications can be completed and the Union list is drawn up.

Concerning the conditions of use, a food enzyme will be included in the Union list if it does not pose a health concern to the consumer; there is a technological need for its use; and its use does not mislead consumers.

The enzyme regulation provides rules on the labelling of food enzymes and food enzyme preparations intended and not intended for sale to the final consumer.

## Flavourings

### Framework Regulation

Most of the Flavourings are naturally present in foodstuffs or are formed during the normal preparation of food. Flavourings can also be added to foodstuffs to impart odour and/or taste.

Regulation (EC) No 1334/2008 of the European Parliament and of the Council on flavourings and certain food ingredients with flavouring properties for use in and on foods lays down rules on flavourings and food ingredients with flavouring properties with a view to ensuring the effective functioning of the internal market whilst ensuring a high level of protection of human health and a high level of consumer protection. The Regulation was adopted on 16 December 2008 and applies since 20 January 2011. It repeals Council Directive 88/388/EEC.

Regulation (EC) No 1334 applies to:

(a) Flavourings which are used or intended to be used in or on foods, with the exception of smoke flavourings falling within the scope of Regulation (EC) No 2065/2003 (*14*);
(b) Food ingredients with flavouring properties;

(c) Food containing flavourings and food ingredients with flavouring properties;
(d) Source materials for flavourings and food ingredients with flavouring properties.

The regulation does not apply to:

(a) Substances which have exclusively a sweet, sour or salty taste;
(b) Raw or non-compound foods.

Definitions are provided for flavouring substances, flavouring preparations, thermal process flavourings, smoke flavourings, flavour precursors and other flavourings. Food ingredients with flavouring properties are defined as a food ingredient other than flavourings which may be added to food for the main purpose of adding flavour to it or modifying its flavour and which contribute significantly to the presence in food of certain naturally occurring undesirable substances.

Flavouring preparations obtained from food and thermal process flavourings that comply with the conditions for their production, described in Annex V of the Regulation, and the maximum limits set for certain undesired substances, described in Annex III of the regulation, do not require an authorisation and approval.

For other flavourings an evaluation and approval is required:

(a) Flavouring substances;
(b) Flavouring preparations obtained from non-food sources;
(c) Thermal process flavourings obtained from non-food sources or for which the conditions for their production are not met, or that do not comply with the maximum levels for certain undesirable substances;
(d) Flavour precursors other than food;
(e) Other flavourings;
(f) Source materials for the production of flavourings other than food referred.

The regulation lays down specific requirements for the labelling of flavourings, including the use of the term 'natural':

- The term '*natural*' for the description of a flavouring may only be used if the flavouring component comprises only flavouring preparations and/or natural flavouring substances.
- The term '*natural flavouring substance(s)*' may only be used for flavourings in which the flavouring component contains exclusively natural flavouring substances.
- The term 'natural' may only be used in combination with a reference to a food, food category or a vegetable or animal flavouring source if the flavouring component has been obtained exclusively or by at least 95 % w/w from the source material referred to. The description shall read '*natural "food(s) or food category or source(s)" flavouring'*.

- The term '*natural "food(s) or food category or source(s)" flavouring with other natural flavourings*' may only be used if the flavouring component is partially derived from the source material referred to, the flavour of which can easily be recognised.
- The term '*natural flavouring*' may only be used if the flavouring component is derived from different source materials and where a reference to the source materials would not reflect their flavour or taste.

**List of Authorised Flavourings**

Commission implementing Regulation (EU) No 872/2012 (*15*) adopted a new Union list of flavouring substances that can be used in food. It entered into force in October 2012 and applies as of 22 April 2013. The EU food industry will only be able to use flavouring substances that are on the EU list. Flavouring substances not on the list will be banned after an 18-months phasing-out period.

The flavouring substances may be used in or on foods in accordance with the good manufacturing practices and, if necessary, under specific conditions. The list contains information on the unique identification number of the substance (FL-No), the name of the substance (Chemical name), the Chemical Abstracts Service registry number (CAS), the JECFA number, the Council of Europe number, the purity, the specific conditions of use and reference to the scientific body that has carried or is carrying out the evaluation.

For the establishment of that list all flavourings substances have been evaluated by the European Food Safety Authority following a stepwise approach that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. Also substances that have been evaluated by the Scientific Committee on Food (SCF), the Council of Europe and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and of which the use is considered safe are included in the list.

For certain substances in the list, the evaluation by EFSA has to be completed. A footnote is allocated to these substances, including where necessary time limits for applicants to comply with EFSA's requests for information as expressed in the published opinions. Where the necessary information is not provided by the time requested, the flavouring substance in question will be withdrawn from the Union list. EFSA will evaluate the submitted data within nine months from the receipt of such data.

The Union list has been already updated to take into account these new EFSA assessments and also the removal of certain substances.

**Smoke Flavourings**

Specific rules about the authorisation and the use of smoke flavourings are laid down in Regulation (EC) No 2065/2003 (*14*) on smoke flavourings used or intended for use in or on foods.

It includes definitions, general use and safety requirements, conditions for the production of smoke flavouring primary products, a procedure for the safety assessment and the authorisation and provisions for labelling.

Smoke flavourings are defined as:

(a) The purified water based part of condensed smoke;
(b) The purified fraction of the water-insoluble high-density tar phase of condensed smoke;
(c) Flavourings produced as a result of the further processing of these primary products.

(a) and (b) are also called "primary products". A list of authorised primary products that can be used as such or from which smoke flavourings can be derived is planned for adoption by the end of 2013. The list provides for each authorised primary product, a unique product code, the name of the product, the name and address of the authorisation holder, a description and characterisation of the product, the conditions of its use in or on specific foods or food categories, the date from which the product is authorised, and the date until which the product is authorised. The authorisation of the primary products is granted for five years.

When authorised smoke flavourings are used in or on food, their use must be in accordance with the conditions of use, including maximum levels, set in the Regulation. When authorised smoke flavourings are used in combination, the individual levels should be reduced proportionally.

Smoking by regenerated smoke is a process of treating food by exposing it to smoke which is regenerated by atomizing smoke flavourings in a smoking chamber under the time and temperature conditions similar to those for hot or cold smoking. In that case it is difficult to estimate how much of the smoke flavourings will be present in the final food as marketed due to loss of smoke flavouring during smoking. Therefore, the use should be in accordance with good manufacturing practices.

Smoke flavourings have to be labelled on the list of ingredients if the flavouring component imparts a smoky flavour to the food.

## Common Authorisation Procedure of Food Additives, Food Enzymes, and Flavourings

A common procedure for the evaluation and authorisation of food additives, food enzymes and flavourings is provided by Regulation (EC) No 1331/2008. The Commission, a member State or an interested party can start the procedure through an application for updating the Union lists.

"Updating the Union list" means, adding a substance; removing a substance; adding, removing or changing conditions, specifications or restrictions related to the presence of a substance.

The main stages of the authorisation procedure are the following:

1) An application is sent to the European Commission.
2) The Commission will ask the European Food Safety Authority (EFSA) for an opinion.
   An opinion of EFSA is not required if the updates in question are not liable to have an effect on human health.
3) EFSA gives an opinion within nine months of receipt of a valid application. A derogation is given in the food enzyme regulation, meaning that this period will be not applicable to food enzymes until the Union list of food enzymes is established.
4) Within nine months after receiving the opinion of EFSA, the Commission submits a draft regulation to the Standing Committee. A derogation is given in the food enzyme regulation, meaning that this period will be not applicable to food enzymes until the Union list of food enzymes is established.
5) The proposed regulation can be adopted after a two months scrutiny procedure by the European Parliament and the Council.

The time limits established in the Regulation (EC) No 1331/2008 for various stages of the authorisation procedure apply at present time for food additives and food flavourings. Regarding food enzymes these time limits will be valid once the Union list of enzymes is established and applied.

Regulation (EU) No 234/2011 (*16*) implementing Regulation (EC) No 1331/2008 lays down requirements concerning the content, drafting and presentation of applications to establish or update the Union lists of food additives, food enzymes and food flavourings; arrangements for checking the validity of applications and the type of information that must be included in the opinion of the European Food Safety Authority (EFSA).

Application dossiers shall include:

- Administrative data;
- General data required for risk assessment;
- Specific data required for risk assessment;
- Data required for risk management of food additives, food enzymes and flavourings.

Applicants are requested to follow the scientific opinions of EFSA on data requirements for the evaluation of food additives (*17*), food enzymes (*18*) and flavourings (*19*) to be used in or on foods. Where needed, applicants should consult the Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed (*20*) and the EFSA scientific opinion on Guidance on the risk assessment of the application of nano-science and nanotechnologies in the food and feed chain (*21*).

An application may be considered as valid even if it does not contain all the elements required provided that the applicant has submitted verifiable justification for each missing element.

The Commission may end the authorisation procedure and decide not to proceed with a planned update, at any stage of the procedure, if it judges that such an update is not justified. In such cases the Commission will inform the applicant indicating in its letter the reasons for not considering the update justified.

Further practical information for applicants is available on the European Commission Health and Consumers website (*22*).

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Chapter 6

# History of U.S. Regulation of Color Additives and Colorants

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Color additives are dyes, pigments, or other substances that impart color to foods, drugs, cosmetics, and certain medical devices. Colorants are similar substances that impart color to food contact materials such as packaging. The U.S. Food and Drug Administration has the responsibility for regulating color additives and colorants, and both have premarket approval requirements. Federal oversight began in the late 19th Century and continued with the 1906 Food and Drugs Act, 1938 Federal Food, Drug, and Cosmetic Act, and multiple amendments. This chapter combines the history of U.S. regulation of color additives and colorants.

## Introduction

Color additives are dyes, pigments, and other substances that impart color to foods, drugs, cosmetics, and certain medical devices. Colorants are similar substances that impart color to food contact materials such as packaging. The U.S. Food and Drug Administration (FDA) has the responsibility for regulating color additives and colorants and lists permitted substances in Title 21 of the Code of Federal Regulations (CFR) (*1*). Color additives are required to be approved by the FDA prior to their use in FDA-regulated products. Most colorants have the same requirement, and exceptions are described below.

Permitted color additives include water-soluble dyes, extracts, spices, inorganic substances, lakes, and mixtures. Lakes are insoluble pigments formed from water-soluble dyes combined with precipitants and substrata, and mixtures are one or more color additives mixed with each other or with non-colored

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diluents. Most color additives have purity specifications, including requirements for total dye content and limits for impurities such as lead, arsenic, and mercury. In addition, some color additives are subject to batch certification by the FDA to ensure compliance with their requirements.

Permitted colorants include organic dyes and pigments, inorganic pigments, optical brighteners, and fluorescent whiteners. The substances are used in paper packaging, plastic containers, marking inks, freshness labels, and tracers in boiler water. Colorants are not permitted to migrate to foods in an amount that will contribute any color apparent to the naked eye. They originally were called indirect food additives because they are not food ingredients but today are categorized as food contact substances. Because colorants are not considered food ingredients, they are not required to be declared on food labels.

This chapter combines the history of U.S. regulation of color additives and colorants. The term color additive was defined by U.S. law in 1960, and the definition of colorant as a component of food contact materials was codified in 1983. Color additives originally were called coloring matters or colors and today are often are called colors. The European Union uses the term "colourant" for dyes and pigments in both products and packaging. Therefore, color additives frequently are referred to as colorants or colourants throughout the world.

## Early Use of Food Coloring

Food coloring dates back to ancient times and frequently was used for concealing inferiorities in products offered in the marketplace (*2–7*). Bread, cayenne pepper, coffee, milk, mustard, tea, vinegar, and wine are some of the products that were mixed with other substances to improve their appearance and marketability. Sometimes toxic pigments were added to foods with complete disregard for human health (*5*). Confectionery, in particular, was commonly colored with lead chromate, lead oxide, mercury sulfide, and copper arsenite (*5*, *8*).

Attempts to regulate food coloring also go back to early history (*2–7*). For example, wine inspectors were appointed in ancient Rome and Greece and a 1396 edict in Paris prohibited the coloring of butter (*5*, *6*). As commerce expanded in the sixteenth and seventeenth centuries, the demand for tea, coffee, chocolate, and sugar increased, as well as skill in adulteration that could not be detected by the inspectors and health officers of the time (*2*, *5*, *6*, *8*). Development of the analytical balance and microscope provided the tools needed for the identification of foreign substances in foods (*5*, *6*).

Analysts of food adulteration in the first half of the 19th century included Frederick Accum and Arthur H. Hassall in London, who not only reported many dangerous additions to foods but also published the names and addresses of the merchants selling the foods (*2*, *4–8*). These efforts were not enough, however, because in 1860, a green-colored pudding was served at a public dinner that turned out to contain copper arsenite (*5*, *7*). The resulting deaths led to passage of the Adulteration of Food and Drink Act in 1860, the first general food law in England (*5*, *6*).

In 1856, Sir William Henry Perkin of the Royal College of Chemistry, London, discovered that a purple dye could be obtained from the distillation of coal tar (*9*, *10*). Perkin obtained "mauveine" by nitrating a fraction containing benzene and toluene, reducing it to a mixture of aniline, o-toluidine, and p-toluidine, and oxidizing the mixture with potassium dichromate (*10*, *11*). After obtaining a patent for his product, Perkin began commercial production of the first truly synthetic dye (*9*). Although mauveine proved to be less suitable for use on silk and cotton than dyes discovered soon afterward, it became known as the first "aniline" or "coal-tar" dye, terms still in use today (*9*, *10*). As more aniline dyes were discovered, they began being used not only for dyeing cloth but also for coloring food (*4*, *7*, *8*, *10*, *12*).

## USDA Regulation of Food Colors

The U.S. Department of Agriculture (USDA) and FDA both trace their roots to the U.S. Patent Office (*6*, *13*). Established in 1836, the Patent Office immediately turned its attention to the science and progress of agriculture, the biggest industry of the nation. In 1842, the Patent Office's Agricultural Division began publishing reports on topics of interest at the time, such as insecticides for crops and medicines for domestic animals. In 1861, Pennsylvania dairy farmer Isaac Newton was appointed Superintendent of the Division and called on Congress to establish a separate Department of Agriculture (*13*).

In 1862, President Abraham Lincoln appointed Newton the first Commissioner of the new U.S. Department of Agriculture (USDA) and appointed Charles M. Wetherill the USDA's first Chief Chemist (*2*, *13–15*). Under Wetherill and succeeding Chief Chemists, the USDA's Division of Chemistry mostly analyzed soils, fertilizers, and other agricultural products. Expertise in analytical chemistry gradually grew, and on September 8, 1884, the USDA founded the Association of Official Agricultural Chemists to establish uniform methods for analyzing products such as foods (*16*).

The first federal authorization of food colors was in an 1886 act passed by Congress that defined butter as the product made from milk or cream "with or without additional coloring matter" and oleomargarine as a manufactured substance that could include "annotto" [sic] and "other coloring matter" (*3*, *7*, *17*). Ten years later, another act similarly defined cheese "with or without additional coloring matter" (*18*). Added color also became a source of controversy when the dairy industry strongly objected to the efforts by the oleomargarine industry to make their product look like butter (*4*).

The increased use of coloring materials was recognized as a threat to the public health (*7*). In 1879, USDA's Division of Chemistry began an investigation of food and drug adulteration, and in 1880, Chief Chemist Peter Collier recommended the enactment of a national food and drug law (*2*, *19*). Between 1879 and 1905, both houses of Congress considered more than 100 bills addressing the adulteration and misbranding of food and drugs but none was enacted into law (*6*, *7*, *19*). Individual states also took action. For example, in 1900, Virginia passed a law prohibiting

food adulteration and misbranding that included the coloring, coating, or staining of foods to conceal damage or inferiority and the use of "poisonous colors" in candy and chocolate (*6*).

Starting in 1900, the USDA was given funding to "enable the Secretary of Agriculture to investigate the character of proposed food preservatives and coloring matters; to determine their relation to digestion and to health, and to establish the principles which should guide their use" (*7*, *20*). The assignment was given to USDA's Division of Chemistry, which became the Bureau of Chemistry in 1901 (*6*, *19*).

In 1902, Dr. Harvey Wiley, chief of USDA's Bureau of Chemistry, began his "hygienic table trials" of preservatives (*7*, *21*). Groups of young men, called the "Poison Squad" by the press, volunteered to eat all of their meals under Dr. Wiley's supervision so he could study the effects of preservatives and food colors on their digestion and health (*6*, *7*, *21*). Among the substances studied were borax, salicylic acid, sulfuric acid, sodium benzoate, formaldehyde, and copper sulfate. The study was conducted over five years with new groups of young men every year (*21*). Although controversial, the study paved the way for a new law establishing federal regulation of food and drugs.

In 1904 and 1906, the USDA published two Food Inspection Decisions (FIDs) that declared a food adulterated if color was added with intent to deceive and stopped importation of macaroni colored with Martius Yellow (*7*). Publicity from the "poison squad" and a book called "The Jungle" by Upton Sinclair, an exposé of the Chicago meatpacking industry, helped convince Congress to pass the Food and Drugs Act, signed by President Theodore Roosevelt on June 25, 1906 (*22*).

The 1906 Food and Drugs Act (popularly known as the "Pure Food and Drugs Act" and the "Wiley Act") prohibited the manufacture, sale, or transportation of adulterated or misbranded or poisonous or deleterious foods, drugs, medicines, and liquors (*22*). The substances "terra alba [gypsum], barytes, talc, chrome yellow, or other mineral substance or poisonous color or flavor" were prohibited in confectionery. Food was deemed to be adulterated "if it be mixed, colored, powdered, coated, or stained in a manner whereby damage or inferiority is concealed."

The USDA was given the authority to enforce the 1906 Act, with the burden placed on USDA's Bureau of Chemistry. Dr. Bernhard C. Hesse, an expert on the German dye industry, was hired as a consultant and proceeded to study 695 food colors used throughout the world (*7*, *23*). The result of Dr. Hesse's research was Food Inspection Decision (FID) 76, issued on July 13, 1907, which listed seven permitted food colors (Table 1) (*24*). These were all "coal-tar" colors and were the only ones found to be safe out of the 80 being sold in the U.S. at that time. Only three are still in use today because listings for the other four were terminated in later years (*25–29*).

Four more FIDs published from 1907 to 1910 established a voluntary certification program for individual batches of food colors, extended the definitions of batches and mixtures, officially recommended the use of certified colors in foods, and authorized repackaging of color additives so they could be sold in smaller quantities (*3*, *4*, *23*). Additional food colors were authorized by subsequent FIDs and USDA bulletins (*23*).

**Table 1. Food Colors in FID 76**

| *Common name* | *Listed name* | *Current status* |
|---|---|---|
| Amaranth | FD&C Red No. 2 | Listing terminated in 1976 (*25*) |
| Erythrosine | FD&C Red No. 3 | Permitted in foods and drugs |
| Indigotine | FD&C Blue No. 2 | Permitted in foods, drugs, and medical devices |
| Light Green SF Yellowish | FD&C Green No. 2 | Listing terminated in 1966 (*26*) |
| Naphthol Yellow S | FD&C Yellow No. 1 | Listing terminated in 1959 (*27*) |
| Orange 1 | FD&C Orange No. 1 | Listing terminated in 1955 (*28*) |
| Ponceau 3R | FD&C Red No. 1 | Listing terminated in 1977 (*29*) |

The 1906 Food and Drugs Act prohibited food ingredients which were "poisonous or deleterious" but did not define those terms. However, in 1914, an important decision by the U.S. Supreme Court laid the foundation for federal regulation of "food additives" (*4*, *6*, *13*). In the case of *United States v. Lexington Mill & Elevator Co.,* the court stated that Congress had intended the law to prohibit adding substances which may render a food injurious to health (*6*, *30*). However, the court also determined that if a food bears a small addition of poisonous or deleterious ingredients but the food cannot injure the health of any consumer, the food cannot be condemned under the Act (*30*). The federal government must show a relationship between a food additive and the harm it allegedly causes in humans before banning it from food (*6*).

## FDA Regulation of Food, Drug, and Cosmetic Colors

In 1927, USDA's Bureau of Chemistry was reorganized into the Bureau of Chemistry and Soil for non-regulatory research and the Food, Drug, and Insecticide Administration for regulatory functions (*31*). The FDA was given its current name in 1930 and remained under the control of the USDA until it was transferred to the Federal Security Agency in 1940; the Department of Health, Education, and Welfare in 1953; and finally the Department of Health and Human Services in 1980 (*7*, *31*).

In the early 1930's, serious problems with two cosmetics occurred. "Lash Lure," which was marketed as an eyelash dye, caused serious injuries including blindness attributed to the presence of *p*-phenylenediamine, an aniline derivative used as an ingredient in hair dyes (*23*, *32*, *33*). "Koremlu," which was marketed as a depilatory, caused paralysis, optic nerve damage, and baldness because it contained thallium acetate, a rat poison (*32*).

The newly established FDA had been assigned the responsibility for enforcing the 1906 Food and Drugs Act, which included oversight of food colors. However, the agency did not have the authority to regulate the colors used in drugs and cosmetics, and coal-tar colors in particular were of concern. These shortcomings

prompted the FDA to recommend that the 1906 Act be completely revised. This launched a 5-year legislative battle (*6*, *7*) but Congress finally passed the 1938 Food, Drug, and Cosmetic Act, signed by President Franklin D. Roosevelt on June 25, 1938 (*34*).

The 1938 Food, Drug, and Cosmetic Act contained a safety standard of "harmless and suitable" for coal-tar colors (*34*). Those that met the safety standard, as determined by the FDA, could be listed for use in coloring foods, drugs, or cosmetics. Announcements of the listings would be published in the Federal Register, which had started in 1936. The actual listings would be published in the Code of Federal Regulations, which started in 1938.

Under the 1938 Act, the previously voluntary certification program was made mandatory for new batches of listed coal-tar colors. The Secretary of Agriculture (who still had oversight of the FDA) was authorized to issue regulations providing for the listing of coal-tar colors "which are harmless and suitable" for use in food, drugs, and cosmetics and for the certification of batches of such colors (*34*). The listing and certification of coal-tar colors were authorized to be performed "only upon payment of such fees, which shall be specified in such regulations, as may be necessary to provide, maintain, and equip an adequate service for such purposes." Thus, the batch certification program became the FDA's first user fee program. The new law also contained adulteration and misbranding provisions for coal-tar colors that were based on certification and labeling requirements.

In the Federal Register of January 7, 1939, the FDA announced that the agency intended to list 82 coal-tar colors for use in foods, drugs, and cosmetics (*35*). The colors were identified by their common names. Following public hearings, final regulations for the colors were published on May 9, 1939 (*36*). The colors were listed with new nomenclature in order to distinguish the certified materials from their technical-grade equivalents (*7*). Those permitted in foods, drugs, and cosmetics were assigned "FD&C" numbers, those permitted in drugs and cosmetics were assigned "D&C" numbers, and those permitted in externally applied drugs and cosmetics were assigned "Ext. D&C" numbers (*36*). Identity and purity specifications were established for all of the listed food and color additives. The listings included a requirement for batch certification.

The 1938 Act established an important exception to the listing requirements for coal-tar hair dyes (*34*). Those synthetic organic dyes do not have to be listed for use in hair dyes. Instead, hair dye products must be labeled with a caution statement for possible skin irritation and directions for preliminary testing for irritation. In response to the events of the 1930s that led up to the law's passage, the law also states that no listed color additive or other coal-tar dye may be used for dyeing the eyebrows or eyelashes.

## 1958 and 1960 Amendments to the Federal Food, Drug, and Cosmetic Act

In the early 1950s, children became ill from eating an orange Halloween candy that contained FD&C Orange No. 1 (*4*, *7*). Also at that time, U.S. House Representative James Delaney began holding hearings to determine whether

any food additives caused cancer (*7*). In the next few years, FDA delisted FD&C Orange No. 1 and several other colors based on results from animal testing. FDA's delistings were upheld by the Supreme Court, to the dismay of the color manufacturers and the food and cosmetic industries (*7*, *37*). Further disagreements with FDA about the safety of colors and weaknesses in the existing law made it obvious that new regulations were needed. As a result, Congress amended the 1938 Act with the 1958 Food Additives Amendment and 1960 Color Additive Amendments (*38*, *39*). A safety standard for food additives of "reasonable certainty of no harm" was established based on the determination by Congress that proof beyond any possible doubt was not required. The new safety standard was defined in the legislative history of the amendments and was added to the CFR (*1*, *38*, *39*).

The 1958 Amendment defined "food additive" as any substance that through its intended use may reasonably be expected to result, directly or indirectly, in its becoming a component of food (*38*). The dyes and pigments used in food packaging materials were not yet classified as colorants and were considered indirect food additives. Important exceptions to the definition of food additive were for substances generally recognized as safe ("GRAS"), among experts qualified by scientific training and experience to evaluate their safety, and ingredients that had been already permitted as of September 6, 1958. The latter became identified as "prior-sanctioned" substances. Sanctioning was in the form of a letter issued by the FDA or USDA before 1958 offering no objection to a specific use of a specific substance in contact with food. The 1958 Amendment required premarket approval by FDA of food additives and established the petition process by which an individual obtain approval, and listing in the CFR.

The 1960 Amendments defined "color additive" as any dye, pigment, or other substance that is capable of imparting color when added to a food, drug, or cosmetic or to the human body (*39*). The 1958 definition of food additive was amended to add another exception for color additives and a "provisional" list of the approximately 233 color additives in use at that time was established. These color additives could continue to be used while testing against the new safety standard was conducted. Just as for food additives, permanent listing of color additives in the CFR could be accomplished by petitioning FDA. The petition process could be used for listing new color additives and new uses for listed color additives. Exemption from certification could be given to substances such as plant extracts and inorganic pigments if the agency did not have safety concerns about variations in composition from batch to batch.

The 1958 and 1960 amendments included a "general safety clause," which is the requirement that only safe food or color additives can be listed, and established four requirements for determining the safety of a food or color additive (*38*, *39*). These are probable consumption or exposure from use, cumulative effect in the diet, evaluation of safety by experts qualified by scientific training and experience, and, for color additives, the availability of analytical methods for determining their purity and acceptable levels of impurities. Using these criteria, the FDA began permanently listing color additives that were either exempt from or subject to batch certification.

## The Delaney Clause and Constituents Policy

The 1958 and 1960 amendments both contained what became known as the Delaney Clause, after Representative James Delaney, which states that no food or color additive shall be deemed safe if it is found to induce cancer when ingested by man or animal (*4*, *38*, *39*). Based on this clause, several color additives were delisted due to carcinogenic impurities (*4*). In addition, analytical chemists began postulating and finding trace levels of carcinogenic impurities (*2*). Because applying the Delaney Clause threatened to remove many color additives from the market, a compromise was needed.

In 1979, in the case of *Monsanto v. Kennedy,* the U.S. Court of Appeals endorsed the concept that there is "administrative discretion, inherent in the statutory scheme, to deal appropriately with de minimis situations" (*40*). The court held that FDA has discretion to find that low-level migration into food of substances in indirect additives is so insignificant as to present no public health or safety concern (*41*). This finding supported FDA's conclusion that, after appropriate tests, the agency could assess the upper limit of risk of a carcinogenic impurity in a non-carcinogenic food or color additive and set a limiting specification if FDA determines that there is a reasonable certainty of no harm from the impurity. This conclusion became known as the constituents policy (*12*, *42*).

Following the 1979 court decision, FDA established a regulatory approach to clarify exactly what an additive is and estimate human exposure to it, to interpret the Delaney Clause to apply only when the additive itself has been shown to cause cancer, and to use risk assessment of the impurities as one of the tools for determining whether the additive is safe under the general safety clause. The constituents policy began being applied in 1982 to the permanent listings of D&C Green No. 5 and D&C Green No. 6 (*43*, *44*). FDA's interpretation of the constituents policy was challenged in the case of Scott v. FDA and was upheld by the U.S. Court of Appeals for the Sixth District in 1984 (*45*).

## Other New Amendments and Laws Affecting Color Additives

The 1976 Medical Device Amendments to the Federal Food, Drug, and Cosmetic Act, signed by President Gerald R. Ford on May 28, 1976, added oversight of color additives in medical devices in direct contact with the body for a significant period of time (*46*). Prior to 1976, sutures were classified as drugs. The first color additive permanently listed for suture use was D&C Green No. 6 in 1962, and the color additive was listed in the drugs section of 21 CFR part 74 (*47*). After 1976, color additives for suture use were listed in the medical devices sections of the color additive regulations. In 1983, color additives began being listed for use in coloring contact lenses. The first color additive listed for that use was D&C Green No. 6 (*48*).

In 1966 and 1990, two new laws directly affected color additive regulation. The 1966 Fair Packaging and Labeling Act signed by President Lyndon B. Johnson on November 3, 1966, added requirements for ingredient declarations on food labels (*49*). This law was passed in response to numerous requests from consumers

for information on the many processed foods being added to the marketplace. In response, FDA issued new regulations for the declaration of color additives on the labels of foods, drugs, and cosmetics. The 1990 Nutrition Labeling and Education Act, signed by President George H. W. Bush, on November 8, 1990, established "Nutrition Facts" labeling and the award winning designs seen today on food packages (*50*). New labeling requirements were established for certified color additives and certification exempt color additives.

The Public Health Security and Bioterrorism Preparedness and Response Act, known as the "Bioterrorism Act," was signed by President George W. Bush on June 12, 2002 (*51*). Requirements of the law include registration with the FDA as food facilities, notification prior to importing food shipments, establishment and maintenance of records of the immediate previous sources and the immediate subsequent recipients of food products, and administrative detention of food that presents a serious health threat. Manufacturers of color additives for food use are subject to this law (*52*).

## FDA Regulation of Color Additives

Between 1960 and 1990, color additives were gradually removed from the provisional list and either were permanently listed or their listings were revoked. Today the provisional list, which is in 21 CFR part 81, includes most of the color additive lakes. Specific requirements for the provisionally listed lakes are in 21 CFR part 82. Descriptions of many revoked listings also are in part 81.

The color additives currently permitted for use in foods, drugs, cosmetics, and medical devices are listed in 21 CFR parts 73, 74, and 82. Color additives permitted in foods usually are listed either for general use or for use in specific products. Those permitted in drugs and cosmetics are listed either for general use or external use only. Those permitted in medical devices are listed for use in specific products. Extracts, spices, and inorganic substances are listed in 21 CFR part 73. Examples include β-carotene, paprika, and zinc oxide. Synthetic organic dyes and pigments subject to batch certification are listed in 21 CFR parts 74 and 82. Examples include FD&C Blue No. 1, D&C Red No. 7, and FD&C Yellow No. 5—Aluminum Lake. Titanium dioxide is the only color additive permitted in all four types of products. The color additive listings include the listed names, identities, specifications, uses and restrictions, labeling requirements for the marketed material, and requirement for or exemption from certification.

Since 1960, uses for some certified color additives have been amended but their nomenclature has not been changed. Therefore, product manufacturers need to check the individual listing regulations to make sure they are using color additives only for their approved uses. For example, FD&C Red No. 3 is permitted in foods and ingested drugs, FD&C Red No. 4 is permitted in externally applied drugs and cosmetics, and FD&C Blue No. 2 is permitted in foods, ingested drugs, and medical devices.

Labeling requirements for products containing color additives are described in 21 CFR parts 101 (foods), 201 (drugs), and 701 (cosmetics). Color additives must be declared by their listed names, with some exceptions. Certified color

additives may be declared by appropriate abbreviations on food and cosmetic labels. Certification exempt color additives may be declared on food labels by their listed names or as "artificial color" or similar terms. FDA does not permit color additives to be declared as "natural" food ingredients because they are artificially added to foods.

## Colorants as Food Contact Substances

Following passage of the 1958 Amendment, FDA began receiving many requests for exemptions from the listing requirements for substances that migrated from food packaging into foods at very low levels (*53*, *54*). The 1979 court decision had made it clear that FDA had the authority to exempt these substances from the formal petition process based on the determination that the substances do not present health or safety concerns. Moreover, in the 1980s, large collections of toxicity data on which to base a threshold level for low level migration became available (*53*). Therefore, in 1986, FDA proposed a probabilistic approach to a "threshold of regulation" (TOR) that met the safety standard of reasonable certainty of no harm and did not authorize the use of known carcinogens (*54–56*). Following publication of the final rule in 1995, FDA began issuing TOR exemptions for food contact articles resulting in a dietary concentration of 0.5 ppb or dietary exposure at or below 1 percent of the acceptable dietary intake (*57*). C.I. Solvent Blue 129 and C.I. Solvent Yellow 143 were among the first colorants given TOR exemptions (*31*).

In 1959, FDA began listing GRAS substances in 21 CFR part 121, subsequently recodified as 21 CFR part 182 (*58*). FDA established an affirmation process for the evaluation of GRAS substances in the 1970s and listed affirmed substances in 21 CFR parts 184 and 186 (*31*, *41*). In 1997, FDA replaced the GRAS affirmation process with a voluntary notification program, which is in place today (*31*, *56*). Ferric oxide is a colorant listed under 21 CFR 186.1300 as an indirect food substance affirmed as GRAS for use in food packaging.

In 1967, FDA published a proposed rule for listing synthetic organic colorants for use in food-packaging paper and paperboard (*59*). The colorants either had to be listed as color additives for food use or specified precautions had to be taken to limit their migration into the food. The proposed rule responded to a petition requesting the use of a large number of colorants in paper and paperboard intended for food wrapping or packaging. A list of synthetic organic colorants in use prior to 1958 had been compiled by the American Paper Institute (comprising most of the pre-1958 "prior-sanctioned" substances), and the intent of the petition was to request that those substances be permanently listed for the proposed uses (*60*). FDA withdrew the proposal in 1979 but sent a letter to the American Paper Institute stating that, in the absence of any safety concerns, the agency did not intend to take regulatory action against the use of prior-sanctioned colorants (*60*, *61*).

In 1972, FDA published a proposed rule for the use of colorants in plastics (*62*). Five petitions had requested that FDA provide for the safe use of colorants in any flexible, semi rigid, or rigid plastic intended for food contact use. In a 1983 final rule, FDA established a new category called "colorants for polymers"

for coloring agents used in polymeric food contact materials (*63*). The term "plastics" was replaced with the scientifically more precise term "polymers." The substances were listed under new 21 CFR 178.3297, which defined colorant as "a dye, pigment, or other substance that is used to impart color to or to alter the color of a food-contact material, but that does not migrate to food in amounts that will contribute to that food any color apparent to the naked eye" (*63*).

The 1997 Food and Drug Modernization Act, signed by President Bill Clinton on November 21, 1997, amended the 1938 Act to streamline the way the FDA conducted business (*64*). One of the new procedures for accomplishing this goal was the establishment of a food contact notification (FCN) process for regulation of food contact substances (*65–67*). 2, 9-Dimethylquinacridone and C.I. Pigment Red 122 are colorants that have been subjects of FCNs (*31*).

## FDA Regulation of Colorants

Colorants permitted for use in food-packaging materials include organic dyes and pigments, inorganic pigments, optical brighteners, and fluorescent whiteners. Listed color additives may be used as colorants, but optical brighteners and fluorescent whiteners are categories of pigments that may not be used as color additives (*11*). Colorants may be used in paper packaging, plastic containers, marking inks, freshness labels, and as tracers in boiler water. They must be used in accordance with good manufacturing practices and are required to have no or limited migration into products. Extraction testing guidelines are available from FDA.

Colorants may be authorized by a regulation listed in Title 21 of the CFR, prior sanction, a GRAS notification, a TOR exemption, or an effective FCN. Colorants permitted in paper and paperboard are listed in 21 CFR 176.170. As discussed above, colorants permitted in polymers are listed in 21 CFR 178.3297, which references the listed color additives and provides instructions for obtaining FDA's extraction testing guidelines. Colorants given TOR exemptions or that are subjects of FCNs and GRAS notifications are available on FDA's web site (*31*).

Colorant listings may include their chemical names, C.I. numbers, Chemical Abstracts Service (CAS) Registry Numbers, and specific use limitations. An example of a colorant listed under 21 CFR 178.3297 is 1, 4-bis[(2,4,6-trimethylphenyl)amino]-9,10-anthracenedione (CAS Reg. No. 116-75-6), which is permitted for use at levels not to exceed 0.0004 percent by weight of polyethylene phthalate polymers complying with 21 CFR 177.1630.

## Summary

The history of FDA regulation of color additives and colorants has been summarized in numerous articles, and some of that information has been presented here. A bibliography of useful sources and additional information about color additives and colorants can be found on FDA's web site at www.fda.gov (*31*).

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## Chapter 7

# Food Additives and Packaging in Thailand

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Thailand's food additives and food packaging are governed by the Food Act of B.E. 2522 (1979). The Act gives the Ministry of Public Health the authority to implement the Act through the Food and Drug Administration (FDA), which is a department within the Ministry. In general, imports of food for sale in the Kingdom require an import license and standard labeling according to domestic regulations. According to the Ministry of Public Health Announcement, there are four notifications under the Food Act to regulate use of food additives which are Notification No. 281, B.E. 2547 (2004), Notification No. 359, B.E. 2556 (2013), Notification No. 360, B.E. 2556 (2013), and Notification No. 363, B.E. 2556 (2013) and three notifications under this Act to control all food containers and packaging materials which are the Ministerial Notification No. 92, B.E. 2528 (1985), No. 117 B.E. 2532 (1989), No. 295, BE. 2548 (2005). Aside from the Thai FDA where is responsible for legislating the Food Act of food additives and packaging, there are two organizations, namely, the Thai Industrial Standards Institute (TISI) and the Office of the Consumer Protection Board (OCPB) also responsible for setting up regulations. TISI develops both mandatory and voluntary standards for food packaging for consumer protection and for the need of industry and trade while OCPB regulates the label of plastic packaging for consumer protection mainly. In addition, the Department of Science Service (DSS), which was appointed to be ASEAN Food Reference Laboratory (AFRL) in the area of food contact

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materials by the ASEAN Consultative Committee on Standard and Quality - Prepared Foodstuff Product Working Group (ACCSQ-PFPWG), is actively working on standard of food contact material for ASEAN.

## Regulation of Food Additives in Thailand

Thailand has employed the principles of risk assessment to issue safety protection for consumers and to facilitate international trade. For regulations on food additives, the General Standard for Food Additives (GSFA) and Codex Advisory Specification for the Identity and Purity of Food Additives have been applied as the reference standards to issue Thailand regulations on food additives because of their international recognition, conscientious preparation and reliable scientific information. Codex member countries are involved in establishing and adopting them as international reference standards. The Thai regulations provide the list of authorized food additives approved for use in foods as well as use condition according to GSFA. The labeling of food additives also complies with the Codex General Standard for the Labeling of Food Additives when sold as such. This information helps food manufacturers to select the food additives that are allowed to be used according to the regulations. If they would like to use the food additives that are not included in such regulations, they need to submit the safety information and additional evidence documents for application to the Thai FDA in order to approve the use of those additives.

## Regulatory Enforcement

According to the Ministry of Public Health Announcement, there are four notifications under the Food Act related to food additives.

1. **Notification of Food and Drug Administration (No.281) B.E. 2547 (2004)** in which it contains provisions in relation to the restriction on the usage of food additives i.e. the list of authorized substances and their quality/ standard and use condition that are prescribed in Codex standards. Nevertheless, the Thai FDA also issues additional authorized food additives beyond those listed in the Codex standards. Currently, there are 317 food additives authorized for use and passed the safety assessment (*1*).

Clause 2 of Notification defines that "Food additives mean substances that are not usually used as foods or as essential ingredients of foods whether the substances will or will not provide food value, but are added to foods for the benefits of processing technology, food coloring, food enhancing, packaging, storage or transportation that will affect the qualities or standards or characteristics of foods. Food additives shall also include substances not added to foods but are in the packaging containers together with the foods for the mentioned purposes such as moisture absorbers, oxygen absorbers, etc.

The statement in the first phrase shall not include nutrients added to supplement or adjust the nutritive values of foods such as protein, fat, carbohydrate, vitamins and minerals."

Therefore, the food additives that are covered by these announcements include food additives used for production technology, processing aid and substances for quality maintenance or standard, e.g. moisture absorbers, oxygen absorbers etc., except for nutrients and flavoring materials that are regulated by other announcements.

**Table 1. Cases of Authorized Food Additives and Information for Consideration to Permit the Use of Food Additives**

| ***Authorized food additives*** | ***Information for consideration*** |
|---|---|
| (1) New food additive | Submit information in accordance with the Environmental Health Criteria 70: Principle for the Safety Assessment of Food Additives and Contaminant in Food (Being revised to conform with the new criteria of Environmental Health Criteria 240: Principles and Methods for the Risk Assessment of Chemicals in Food) as follows:<br>(1) Requirements of quality and standards (Specifications) consist of components, production methods, raw materials, impurities in the manufacturing process and stability. |
| | (2) Toxicity and studies in humans, e.g. absorption, distribution, metabolism, and excretion including residues of toxicological concern, general systemic toxicity, acute toxicity, genotoxicity, carcinogenicity, chronic toxicity, reproductive and developmental toxicity, neurotoxicity, immunotoxicity, food allergy and other food hypersensitivities, general principles of studies in humans and gut flora.<br>These data are utilized to determine the safety for human exposure from an acceptable daily intake (ADI) and dietary exposure assessment of chemicals in food. |
| (2) New conditional use | Submit information as follows:<br>1. Information on substances, e.g. safety, quality or standards, components, etc.<br>2. Conditional use in food required.<br>3. Legal permission of use in other countries.<br>4. Research results representing the requirement for a reliable technology. |

Clause 4 of Notification informs that the use of food additives must pass safety evaluation and their quality or standard of food additives must meet the requirement of the Codex Advisory Specification for the Identity and Purity of Food Additives or requirement of Food and Drug Administration for food additives

additional permitted beyond the listed in the Codex standards. Moreover, clause 6 of Notification provides information about the conditions for the use of food additives which may be defined differently from the Codex standard depending on types of food additives, the actual technological requirement and necessity, and the exposure assessments based on the GSFA and the Thai national food consumption data, B.E. 2549 and current scientific evidence, for example, the use of colors, preservatives, and sweeteners.

However, the use of food additives according to the categories defined by Codex does not cover all kinds of foods in Thailand. In certain cases, the specific conditions of use of food additives are defined in the commodity standard such as that of cow's milk, margarine, blends, fat spreads and fat spread blends similar to the Codex standard for specific food products.

The use of food additives that are not included in these notifications must be approved based on their safety evaluation and technological justification prior to issuance of the Notification of the Food and Drug Administration to assign their quality or standard and condition of use. According to the procedure of law issuing, the information is required for consideration (under Clause 4(3) of this Notification) as described in Table 1.

2. **Notification of Food and Drug Administration No. 359 B.E. 2556 (2013)** in which it contains provisions in relation to the use of cyclamate and reissues the requirement of non-permitted use of cyclamates corresponding to Codex standards referred to Codex Advisory Specification for Identity and Purity of Food additives as well as specifies the conditional use according to the requirement of GSFA. However, the non-permitted use of cyclamates or sweeteners is restricted to some food products such as milk, flavored milk, jam, jelly, marmalade in a sealed container, chocolate and electrolyte drink. The qualities or standards and the maximum use of cyclamate and sodium salts or calcium salts of acid- are prescribed (*2*).

**3. Notification of Food and Drug Administration No. 360 B.E. 2556 (2013)** in which it contains provisions in relation to the use of steviol glycosides and reissues the requirement of non-permitted use of star gooseberry corresponding to Codex standards referred to Codex Advisory Specification for Identity and Purity of Food additives as well as specifies the conditional use according to the requirement of General Standard for GSFA. There are requirements for some foods which are different from GSFA such as drink containing milk as main ingredient in both liquid and powder milk (Food category code: 06.8.1), flavored beverage (Food category code: 14.1.4), and gum (Food category code: 05.3). However, all food products which are not permitted to use a sweetener such as milk, flavored milk, jam, jelly, marmalade in a sealed containers, chocolate and electrolyte drink are still banned (*3*).

4. **Notification of Food and Drug Administration No. 363 B.E. 2556 (2013)** in which it contains provisions in relation to food additives (issue 2): Labeling of food additives referred to Codex General Standard for the Labeling of Food Additives when sold as such in order to more effectively protect the consumers and provide information for food manufacturers to use food additives required by regulation.

**Summary**

Thailand has permitted the use of food additives in accordance with the Codex standards which relate to food additives in order to protect the safety of consumers and enhance the international trade. However, the Codex standards are continuously being updated; therefore the Thai FDA Notifications have to be updated accordingly to comply with the Codex standards including oversight of the use of food additives in accordance with the laws and regulations.

## Regulation and Standard of Food Contact Materials in Thailand

Food packaging in Thailand is regulated under the Food Act of B.E. 2522 (1979). Primarily, the food packaging needs to be clean and must not be contaminated of any hazardous substances to human health, pathogenic microorganisms, and artificial food colorings. This Food Act also specifies that the used containers are not permitted to be reused, unless it is glass, ceramic, or plastic. In addition, containers must not be previously used with fertilizer, poisonous substance, or substance likely to be harmful to human health. Moreover, there are specifications of packaging depending on the type of materials used.

According to the Ministry of Public Health Announcement, there are three notifications under the Food Act related to food packaging.

1. **Notification No. 92 B.E. 2528 (1985)** is for food containers, use of food containers, and prohibition of food containers material. This notification specifies the migration limits of lead and cadmium that leach from ceramic and enameled metal containers. The limits are specific to container/vessel shapes, for example, small deep vessels, large deep vessels, and so on (*4*).

2. **Notification No. 295 B.E. 2548 (2005)**. This notification regulates 12 types of plastic food packaging which are polyvinylchloride, polyethylene, polypropylene, polystyrene, polyvinylidene chloride, polyethylene terephthalate, polycarbonate, nylon, polyvinyl alcohol, polymethyl methacrylate, polymethyl pentene, and melamine.

Specifications under Notification No. 295 are divided into two categories. The first part sets limits for heavy metals such as lead, cadmium arsenic and barium in the plastic itself and other toxic substances which can migrate into food depending on the type of plastic used such as vinyl chloride monomer from polyvinylchloride, bisphenol A from polycarbonate and so on.

The second part sets limits for substances migration into four food simulants. The limits are specific to particular types of plastic, for example, polyvinylchloride, polyethylene, polypropylene, and polystyrene. Clause 5 of this Notification states that "the analysis of qualities or standards of dispersion of plastic containers shall be carried out by the methods prescribed by the Food and Drug Administration." Based on the present best knowledge, the migration tests are done using four food simulants: water for food with pH > 5, 4% acetic acid for food with pH < 5, n-heptane for fatty food, and 20% ethanol for alcoholic food.

Clause 6 of Notification No. 295 specifies that plastic containers for milk or milk products and other products shall be made of polyethylene, ethylene, 1-alkene copolymerized resin, polypropylene, polystyrene, or polyethylene terephthalate. Two additional limits which are extracted substance by *n*-hexane and substance dissolved in xylene are also listed.

Clause 7 of Notification No. 295 prohibits the use of colored plastic containers to pack food, except in the following cases:

(a) Laminate plastics specifically the layer that does not directly contact with food;
(b) Plastics used to pack fruits with peel;
(c) Other cases which approval has been obtained from the Food and Drug Administration.

Clause 8 of Notification No. 295 prohibits the use of recycled plastic except using for packing food with peel.

Clauses 9 and 10 of Notification No. 295 prohibit using plastic containers that have previously been used for fertilizer, toxic substances or other hazardous substances to health. They also prohibit using plastic containers that have been used to pack other products which are not food and that bear a design of statement that may mislead the consumer (*5*).

3. **Notification No 117 B.E. 2532 (1989)** is for feeding bottles to be used by infants and children, which consist of bottle, lid, rubber teat, and rubber teat cover. The bottle, rubber teat, rubber teat cover shall be clean and shall have no color that can contaminate the food. In case the bottle is made of plastic, the plastic shall be of polycarbonate. The specifications of the container are divided into two parts. The first part sets the migration limits of lead and cadmium of the plastic material. The second part sets the migration limits of other heavy metals, potassium permanganate, and evaporation residues.

Although polycarbonate has been used safely as a baby milk bottle for over 40 years, recent research studies have implicated that bisphenol A (BPA), which is a raw material used for the production of polycarbonate, is a toxic substance.

The toxicology of BPA has been extensively studied by industry, government, and academic research groups using short and long term animal tests, including several reproduction studies. Therefore, the Thai FDA is highly concerned about the potential effect of BPA on infants and children. The Notification No. 117 is revised to use polypropylene, polyethersulfone and borosilicate glass instead of polycarbonate. In addition, the specifications for substances that migrate into food simulants are also revised.

The rubber teat can be made of natural rubber or synthetic rubber. They must not contain nitrosamine at a level greater than 0.01 mg/kg. This Notification regulates the migration limits of formaldehyde, zinc, volatile compound content,2-mercaptobenzothiazole (MBT), 2,6-bis(1,1-dimethylethyl) -4-methyl-phenol (BHT) and 2,2´-methylenebis(6-(1,1-dimethylethyl)-4-methyl-phenol) (*6*).

The Notification also requires that milk container must be clean, and must not be contaminated with any hazardous substances to human health, pathogenic microorganisms, and artificial food colorings.

The second organization is the Thai Industrial Standards Institute (TISI) which is responsible for developing a set of industrial product standards in the food contact materials industry. TISI standards provide guidelines on quality and other properties of product and related processes. TISI develops both mandatory and voluntary Thai Industrial Standards (TIS) to suit the need and the growth of industry and trade, consumer protection, industrial promotion to be competitive in world market, environmental protection, and natural resources preservation.

At present, TISI has already developed 37 packaging standards. Among them are two mandatory standards for food packaging. These two standards are TIS 1136-2536 (Cling film) and TIS 2440-2552 (Stainless steel: seamed stockpots) (*7*).

The third organization is the Office of the Consumer Protection Board (OCPB) that is responsible for developing the mechanism for consumer protection as there are increasing numbers of products and services offered to the people. While the business operators have applied the advertising and marketing for sales promotion, this might be disadvantage to the consumers since they do not know the market situation, the fact about the product quality, and the reasonable price. Therefore, OCPB regulates the label of plastic packaging through the Notification No.9 B.E. 2544 (2001) and the label of melamine food packaging through the Notification No.18 B.E. 2547 (2004) to control the safety of melamine packaging (*8*).

It is worth noting that the Department of Science Service (DSS) is the governmental organization which is responsible for providing testing services of food additives and food packaging products and is involved in every committee concerning the regulation and standard of food packaging in Thailand.

Since 2012, the DSS has issued the Certificate of Analysis of food contact materials for Thai exporters. Importantly, DSS was appointed to be ASEAN Food Reference Laboratory (AFRL) in the area of food contact materials by the ASEAN Consultative Committee on Standard and Quality- Prepared Foodstuff Product Working Group (ACCSQ-PFPWG) in the 11th Meeting of PFPWG in 2010. The meeting requested that the DSS survey standards and regulations of food contact materials among ASEAN member countries. Then the DSS conducted such survey in 2011-2012. It was found that Brunei Darussalam, Cambodia, Myanmar and The Lao People's Democratic Republic (Lao PDR) have not developed the regulations for food contact material for prepared food products. Singapore has developed its own regulation while Indonesia, Malaysia, Philippines, Thailand and Vietnam developed their regulations based on Japanese Standard, European Commission, and the U.S. Code of Federal Regulations. The information obtained will be beneficial for the DSS to harmonize the standard of food contact material for ASEAN which will be implemented in 2015 when ASEAN community becomes a single market.

## Summary

Although it seems that several notifications and mandatory standard of food packaging in Thailand have not been updated for a long time, the revising process will be initiated whenever there is scientific information concerning a new positive

list. Developing the ASEAN standard of food contact materials, which will be accepted among ASEAN member countries, will be a key factor leading to a revising process for notifications and standards of Thailand.

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## Chapter 8

# EU Legislation on Food Contact Materials

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The chapter is giving a comprehensive overview over the EU regulatory framework on food contact materials and explains the link between EU and national measures. It introduces the basic requirements applicable to all food contact materials including requirements on good manufacturing practice. It focusses on requirements set out for plastic food contact materials, active and intelligent materials and plastic recycling processes including the EU approval system established for those materials.

## Introduction

This chapter discusses the scope of EU legislation on food contact materials (FCM) and its links to Member States' national legislation. It gives an introduction on the basic principles according to which EU legislation is being established, followed by an overview of the general rules applicable to all food contact materials, as well as specific EU legislation for certain types of materials.

## The Objective of EU Legislation on Food Contact Materials

The EU was created to establish a common market in Europe. Before its creation, Member States had more or less extensive national legislation on FCMs in place. A harmonised legislation was deemed necessary to remove barriers to trade and to ensure a high level of consumer protection. A full harmonisation at EU level is not yet in place. When putting EU legislation into place, the principles of subsidiarity and proportionality are applied.

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Under the subsidiarity principle, a determination is made as to whether action at the EU level is necessary and justified or if action at national level would be more effective to achieve the goals. Under the principle of proportionality, the action is limited to what is necessary to achieve the objective and thus to avoid overregulation.

### What Are Food Contact Materials (FCM)?

In EU legislation, FCMs cover three categories. The first category covers all materials and articles that are already in contact with food. This means the packaging of packaged food, for example, a water bottle. The second category covers materials and articles that are intended to come into contact with food but not yet in contact with food. This means for example, an empty food container before it is filled either at the food business operator or at home. The third category covers materials and articles that can reasonably be expected to come into contact with food. This means for example, the work surfaces in food preparation areas, or some secondary packaging if the primary packaging is not a sufficient barrier to the transfer of substances from the secondary packaging into the food. In summary, FCMs cover all types of articles such as food packaging, food processing and distribution machinery, kitchenware and table ware. Excluded from the scope of EU legislation are fixed, public and private water supplies, which are covered under legislation on construction products.

## Horizontal EU Measures

### The Framework Regulation (EC) No 1935/2004

In the EU, the Framework Regulation (EC) No 1935/2004 (*1*) is the horizontal basic act that applies to all FCMs setting out the general principles that apply to manufacturing, marketing and control of FCMs. It sets out the principles of safety and inertness of the FCMs. The safety principle requires that materials and articles shall not release their constituents into food in concentrations that could endanger human health. As regards to inertness, the materials should not release their constituents into food in concentrations that could change the composition of the food, its taste or odour in an unacceptable way. Materials and articles have to be manufactured in accordance with good manufacturing practices (GMP) and should not be presented in a way that could mislead the consumer.

Other principles set out in the framework regulation are traceability and labelling. Traceability is similar to the traceability set out in the General Food Law. Traceability has to be ensured, one step up and one step down in the FCM supply chain. One step up means that the suppliers of raw materials are known and one step down means that the customers to which the products have been sold are known.

Labelling requirements cover for example, a symbol for the suitability of a material to come into contact with food, the glass & fork symbol, but also the responsible person in the EU for placing the product on the market and if necessary any information on the safe use of the material and article.

The Framework Regulation also specifies that enforcement and control is performed by Member States. In support of a European Reference Laboratory, a network of national reference laboratories is established. The European Commission is empowered to adopt specific measures on specific materials and articles.

### Good Manufacturing Practice – Regulation (EC) No 2023/2006

Good manufacturing practice (GMP) has to be applied in the production of FCMs. Business operators need to have in place well documented quality assurance and quality control systems. The quality assurance system has to cover the suitability of the starting materials. This means materials should be selected with a view to the safety and inertness of the final articles, including the purities of the starting substances. The process has to be designed and operated so that the reaction and degradation products created are not posing a risk to food composition or health of the consumers. Premises and equipment have to be adequate for the purpose and the staffs have to be qualified and aware of the critical stages in the production.

GMP applies at all manufacturing stages, except the starting materials, which means the chemical industry or the production of the wood, primary wood and the production of glass and so on. These are covered by other legislations, for example the legislation on chemicals.

In summary, the EU horizontal legislation on FCMs covers the Framework Regulation, which lays out the general requirements for all FCMs and articles and Regulation (EC) No 2023/2006 (GMP Regulation) (*2*). Under these 2 horizontal measures, material specific legislation is in place which covers the following five materials: ceramics, regenerated cellulose film, plastics, recycled plastics, and active and intelligent materials. Two EU measures (Directive 93/11/EFC and Regulation 1895/2005/EC) cover specific substances, nitrosamines in rubber teats and soothers, and bisphenol A diglycidyl ether (BADGE), bisphenol F diglycidyl ether (BFDGE) and novolac glycidyl ether (NOGE) in plastics, coatings and adhesives.

## Material Specific Measures

### The Relation between EU and National Measures

At this moment only five materials are covered by specific EU measures, as described above. In the absence of EU specific measures, Member States may keep their national legislation or even adopt a new national legislation. This covers materials such as adhesives, coatings, cork, glass, metals, paper, printing inks, rubber, silicones, and wood. National measures in place have to be respected when a FCM is placed on the market in the respective Member State.

### Approaches To Implement General Principles of the Framework Regulation in Material Specific Measures

In EU specific measures, different instruments are applied to implement the general principles set out in the Framework Regulation. To ensure the safety of materials, the following three instruments can be distinguished. The first instrument is a positive list, meaning only substances, processes, materials which are explicitly authorised can be used in the manufacture of a certain material. This approach applies to the Union List for monomers and additives used in plastics, the substances used in regenerated cellulose film, the plastic recycling processes, and the substances in active and intelligent components. The second instrument is the restriction of use, which means setting out limits of migration of the substance into the food or residual content in the material itself, the use in contact with certain type of foods or certain use conditions of the material. Examples are the migration limits established in plastics, cadmium and lead release limits from ceramics, nitrosamines limits in rubber teats and soothers, residual content of substances in plastics or regenerated cellulose film. The third instrument is the prohibition of use, certain substances are actively prohibited which are, BFDGE and NOGE in plastics, coatings and adhesives, Bisphenol A in infant feeding bottles, and certain phthalates in FCMs intended for use by infants and young children.

## Plastic Materials and Articles - Regulation (EU) No 10/2011

### Scope

Plastic materials and articles are covered by Regulation (EU) No 10/2011 (Plastics Regulation) (*3*), which is combining all the rules previously distributed over several directives. The Plastics Regulation applies to materials and articles exclusively made of plastics, plastic multilayers bound together by adhesives, plastic layers and coatings forming gaskets in lids, plastic layers in multi-material articles; this means for example the plastic layer in a beverage carton. All plastics can be coated and/or printed. The fact is that if they are coated or printed on another substrate, this does not rule them out of the scope of the Plastics Regulation. However, adhesives, printing inks and the coatings used in these combined articles are only concerned with respect to the migration limits established for substances in plastics. A specific EU measure for those materials is not yet established in place and thus they may be covered by national legislation. In articles made of multi-materials only the plastic layer is covered, but layers made of other materials such as paper or aluminium are not covered by the Plastics Regulation. Clearly excluded from the scope of the Plastic Regulation are Ion-exchange resins, rubber and silicones.

**Compositional Requirements**

The Plastics Regulation lays out the compositional requirements for plastics. All substances that are being used in plastic materials have to be of good technical quality and suitable to produce safe food contact plastics. A Union List of authorized substances is established for monomers and additives used in plastic FCMs. Only the substances on the list can be used as monomer or additive to the exclusion of all others. The Union List also contains some polymer production aids; in addition national lists can exist and other polymer production aids listed in the national legislation can be used. Substances can be added to the Union List following a procedure for authorization established in the Framework Regulation. The Union List sets out restrictions and specifications for the substances on the list. Certain substance classes are not including on the Union List, namely colorants, solvents, aids to polymerization. These substances may be covered by a national law or can be used subject to risk assessment by business operator. Reaction- and degradation- products, as well as impurities of starting substances, can be used subject to risk assessment by the business operator. The Union List contains general information on the substances, its name and CAS number. It may limit the use of the substance to certain applications and sets out requirements like restrictions, specific migration limit, group restrictions, residual content of the substance in the material, specification on impurities and if necessary, notes on how compliance testing can be performed. The Union List is accessible via a searchable internet based database under the following link https://webgate.ec.europa.eu/sanco_foods/main/?event=display.

Two types of restrictions for substances are set out in the Plastics Regulation: the specific migration limit (SML) and an overall migration limit (OML). SMLs are safety limits established for individual substances or a group of substances based on the risk assessment performed by the European Food Safety Authority (EFSA). The SML is listed in the Union List. If no SML is given for a substance in this Union List, then the generic SML of 60 mg per kg of food applies. The SML is related to food, but can also be tested in food simulants. In cases where the Union List for a substance specifies "non-detectable" in the SML column in the Union List, the substance should not be detected at a level of 10 ppb.

The OML is the limit for the inertness of the material. Therefore, it is linked to the food contact surface area and is established as 10 mg/dm$^2$ of food contact surface. It does not cover volatile substances due to the method that is being applied. For infant food it is expressed in mg per kg in food simulants.

In addition to the Union List of authorized substances, the following general restrictions apply: general migration limits for heavy metals, general restrictions for primary aromatic amines and the limitation on the use of substances in nanoform to those that have been subject to risk assessment and authorisation in this form. Substances that have been subject to risk assessment in nanoform, even though the nano aspect was not specifically assessed in the past, were silicon dioxide and carbon black. A substance for which the nano aspects were specifically assessed is titanium nitrite. The definition of nano is not included in the Regulation but a Commission Recommendation (*4*) on nanomaterials exists.

## The Declaration of Compliance (DoC) and Supporting Documentation

In order to produce safe FCMs, GMP has to be applied at each manufacturing step in the manufacturing process. Moreover, a system has to be in place to inform all the manufacturers in the production chain about the compliance work that has been performed during previous manufacturing stages. To this end, the legislation sets out that a DoC has to be issued in order to ensure that information is exchanged on compliance throughout the manufacturing chain. Supporting documentation contains all the aspects that are being performed on compliance. The supporting documentation is kept in house and is the basis for issuing the DoC. The DoC is aimed at informing the customer to which the product is sold. The supporting documentation is only aimed for your own dossier and has to be available to control authorities. The system of DoC and supporting documentation is applied throughout the entire FCM production chain from the starting materials over intermediate stages up to the final material. As soon as a substance is sold to be used in FCMs, a DoC needs to be available to the customer. For non-plastics parts used in a plastics material and article such as adhesives, printing inks and coatings, adequate information has to be provided as regards substances that are listed on the Union List of authorised substances with an SML.

## Verification of Compliance

After having chosen the components of the FCM, in accordance with the Union List and having performed the manufacturing in accordance with GMP, it still may be necessary to verify if the produced material is compliant with the migration limits set out in the legislation. Two different ways are described in the Plastics Regulation for verification of compliance. First the screening methods and second the verification methods.

The screening methods are aimed at business operators to verify compliance. These comprise simple methodologies that are more severe to ensure having a large margin of safety. They comprise measuring the residual content of a substance in the material, extracting the substance with solvents like ethanol or isooctane, or employing, based on the residual content or the composition, migration modelling which is mathematically modelling the migration of the substance into food or simulants.

The verification methods have to be used by official control laboratories when confirming non-compliance of a material and articles and before taking the material off the market. These methods can be applied to materials in contact with food and not yet in contact with food. The two approaches for materials not yet in contact with food, are to either test migration into food or into food simulants under defined test conditions. These food simulants are described in legislation together with the testing conditions covering testing times and testing temperatures.

Guidance on the provisions of the Plastics Regulation (*5*, *6*) is made available on the website of the European Commission (see useful websites at the end of the Chapter).

## Recycled Plastics – Regulation (EC) No 282/2008

In view of making the best use of our resources and support sustainability, recycling of plastics is becoming more and prominent as a manufacturing technique. The current legislation on plastics is not sufficient to ensure the safety of the plastic which is being used in the recycling as it can be contaminated by substances from its previous use. The recycled plastic may be contaminated with substances from previous use or contain substances from plastics that were not food contact grade. Both would not be suitable to come into contact with food. Therefore, the recycling process has to be efficient to reduce any contaminants from previous uses. The quality of input material that can be accepted for the recycling also needs to be established. If necessary, restrictions need to be established on the use of the recycled materials.

The aim of the Regulation (EC) No 282/2008 (*7*) is to allow a risk assessment of individual combinations of input characteristics, efficiency of the process to reduce contaminants taking into account the intended use of the final materials. If a recycling process is authorized it can be applied at several production sites and can be licensed out to other companies. The authorizations will be individual authorizations. Authorization of the recycling process will apply to all recycled plastics which are to be placed on the EU market. At the moment we are in a transitional phase of establishing an authorization scheme. This means that applications for authorization have been received, the risk assessment by EFSA is on-going - some opinions have been issued-, but authorizations have not yet been granted. The authorizations will be granted at EU level by the European Commission once all the risk assessments are finalized for all applications received during the transitional phase. The progress on the risk assessments can be followed on the EFSA website. A register of valid applications for authorization is available on the website of the European Commission.

## Active Materials and Articles – Regulation (EC) No 450/2009

Active materials are those intended to extend the shelf life or to maintain or improve the conditions of packaged food. They are designed to deliberately incorporate components that would release or absorb substances into or from the food or the environment surrounding the food. Examples are oxygen absorbers, meat pads or releasers of flavourings or preservatives. The release of a natural constituent of a material, for example, a wood barrel in the maturing of wine, is not covered by the legislation.

Intelligent materials monitor the condition of packaged food or the environment surrounding the food. Examples are time and temperature indicators that change colour when the packaged food has been stored above a certain temperature.

For active and intelligent materials in Regulation (EC) No 450/2009 (*8*), a general authorization is established for substances that constitute the active or intelligent component of the material or article but it does not cover the passive part. This means, using an absorber as an example, only those substances that

are necessary for the absorption function need authorization under this legislation but not the plastic material in which this absorber is embedded, as the plastic is covered by the Plastics Regulation.

Exempted from the authorisation are released substances because they have to comply with food legislation. For example, if an additive is released into the food then it has to comply with the food additives legislation, and as such a released preservative has to be authorized in the food in which it is released. Substances which are grafted on to the material but which exert a function on the food itself have to comply with food legislation. Examples for grafted substances could be preservatives or enzymes that exert a function on the food which is in contact with its packaging. A labelling of parts than can be mistaken for food, have to be labelled either with words "do not eat" or if possible with a specified symbol. For active and intelligent materials and articles, requirements exist to issue a declaration of compliance and to keep supporting documentation.

Also for active and intelligent materials, we are in a transitional phase where applications for authorizations have been received and the risk assessment is on-going. Once all the risk assessments are finalized for those applications that were received during the transitional phase, generic authorizations will be granted at EU level by the European Commission and a Union List of authorised substances will be established. The progress on the risk assessments can be followed on the EFSA website. On the website of the European Commission a register of valid applications for authorisation is available.

## Useful Websites

The European Commission is making available on its website a searchable database of the Union List of authorised substances, links to legislation in place, guidance documents, registers and other useful documents. http://ec.europa.eu/food/food/chemicalsafety/foodcontact/index_en.htm.

A roadmap for future initiatives on FCMs can be consulted at the following link. http://ec.europa.eu/governance/impact/planned_ia/docs/2014_sanco_005_fcm_specific_provisions_for_materials_other_than_plastics_en.pdf.

The risk assessment performed by EFSA is available on their website http://www.efsa.europa.eu/en/panels/fip.htm.

Information on migration testing is available on the website of the European Reference Laboratory hosted at the EU Joint Research Centre http://ihcp.jrc.ec.europa.eu/our_labs/eurl_food_c_m.

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2. Commission Regulation (EC) No. 2023/2006 of 22 December 2006 on good manufacturing practice for materials and articles intended to come into contact with food (OJ L 384, 29.12.2006, p 75).
3. Commission Regulation (EU) No. 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food (OJ L 12, 15.1.2011, p 1).
4. Commission Recommendation of 18 October 2011 on the definition of nanomaterial (2011/696/EU), OJL 275, 20.10.2011, p 38.
5. Union Guidelines on Regulation (EU) No. 10/2011 on plastic materials and articles intended to come into contact with food.
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8. Commission Regulation (EC) No. 450/2009 of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food (OJ L 135, 30.5.2009, p 3).

Chapter 9

# U. S. Food and Drug Administration's (FDA) Safety Assessment of Food Ingredients

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The FDA's Center for Food Safety and Applied Nutrition (CFSAN) and Center for Veterinary Medicine have regulatory responsibility for approximately 80% of foods and food-related products consumed in the United States. The safety assessment of ingredients added directly to foods meant for human consumption is an important component of this responsibility. Before food ingredients such as certified colors, colors derived from natural sources, artificial sweeteners, leavening agents, etc., can be introduced into the food supply they require a safety decision that they present "… a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use." This section will present a brief summary of the safety assessment process conducted for chemicals added to our food supply as food additives or color additives. It will also briefly discuss the current FDA draft regulatory guidance for the use of food-related products developed using nanotechnology, as well as some of the issues associated with toxicology testing of food ingredients developed using this technology. Lastly, it will discuss the potential for the incorporation of alternative *in vitro* and *in vivo* toxicity testing methods into the overall safety assessment paradigm.

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## Chemicals Added to Food

The FDA regulates chemicals added to foods per the requirements in section 409 of the Federal Food, Drug, and Cosmetic Act (FD&C Act). Section 409 of the FD&C Act establishes the processes to be used for authorizing new uses of food additives. Similarly, Section 721 of the FD&C Act establishes the requirements for listing new uses of color additives in food as well as in other FDA regulated products. In both cases, the additives must be determined to be safe by FDA before they are authorized for their intended use. The term safe means a "reasonable certainty of no harm" and is defined in Title 21 of the Code of Federal Regulations Part 170.3(i) (21 CFR 170.3(i)). When the FDA is petitioned to allow the addition of a substance (food additive or color additive used in food) to the US food supply, the petitioner is required to present evidence that the petitioned compound is safe for its intended use(s). The guidance to industry that FDA provides for assessing toxicity is its *Redbook 2000: Toxicological Principles for the Safety Assessment of Food Ingredients* (*1*). This guidance is intended to assist petitioners in 1) determining the minimum toxicity studies needed to establish safety, 2) designing and conducting toxicology studies, 3) reporting the results of toxicity studies, 4) conducting statistical analyses of toxicology data, and 5) submitting this information to the FDA as part of the overall petition package. The petitioner is also encouraged to solicit feedback from FDA prior to conducting any safety assessment studies.

The need for specific toxicity testing can vary based on the level of potential exposure of the petitioned compound to the population, its structure and its history of published research (i.e., available literature on safety research conducted for the product). Petitioned products that have the potential for high population exposure levels, or have chemical structures associated with cancer initiation, may require a greater number and variety of toxicity studies. For instance, a petition for an ingredient for which high exposure might be anticipated may contain multiple different *in vitro* and *in vivo* toxicity studies, including two-year rodent carcinogenicity bioassays. Although not required by FDA, a submission of this type may also include human clinical tolerance studies. On the other hand, a petition for an additive in which exposure is estimated to be low for the intended conditions of use or for which there is already a large body of safety data may only require a small number of additional safety assessment studies. A petitioner would also need to identify the impurities in the additive, determine their concentrations, and assess their potential to produce toxicity. If the petitioner were requesting additional uses of an already approved additive such that there will be an increase in exposure, a determination will need to be made whether the increased exposure would represent a safety concern and thus would require additional toxicity testing.

The toxicology data submitted in support of a petitioned additive is reviewed and FDA determines whether the information demonstrates that the proposed use of the additive is safe. In some cases, it may be necessary to request additional studies to answer questions that arise during the review process. Such additional studies may involve testing conducted to address specific issues, such as an immunotoxicology study to address questions regarding potential effects on the

immune system (e.g., on immune function or alterations of immune cells). It also may be necessary to request assistance from specialists in evaluating the submitted data, such as the review of pathology information. Once all data requirements have been satisfied and the review is completed, an acceptable daily intake (ADI) may be calculated based on the study results in the most sensitive animal species. The ADI is calculated using the No Observed Effect Level (NOEL) or No Observed Adverse Effect Level (NOAEL) dose of the test compound, which is then divided by a safety factor (e.g., for a well conducted chronic, 1 year study the safety factor would typically be 100 (a factor of 10 for interspecies difference multiplied by a factor of 10 for differences between humans)). The safety factor may be higher, or lower, depending on the quality and/or quantity of the supporting toxicology information. The use of an additive is determined to be safe if the Estimated Daily Intake (EDI) for the additive does not exceed the ADI.

## Nanomaterials as Food Additives or Color Additives

The emerging field of nanotechnology presents some new challenges for regulatory agencies when products developed using this technology are added to the food supply. Although at present there have been no petitioned uses for nanomaterials as food additives or color additives, they are present in other FDA-regulated products (*2*). FDA has prepared draft guidance for industry that addresses the use of nanomaterials in regulated products. The first of these, *Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology* (*3*) sets forth the points to consider when determining whether an FDA-regulated product involves the application of nanotechnology. A second guidance for industry, *Assessing the Effects of Significant Manufacturing Process Changes, Including Emerging Technologies, on the Safety and Regulatory Status of Food Ingredients and Food Contact Substances, Including Food Ingredients that are Color Additives* (*4*), describes the factors manufacturers should consider when determining whether changes in the manufacturing processes, including those that involve nanotechnology, affect the identity, safety, or regulatory status of a food substance. At present, FDA is taking a case-by-case approach to the safety evaluation of nanomaterials in products under its regulatory purview.

Safety assessment methodologies for nanomaterials for use in food ingredients are under development. Assays that are currently used to assess the safety of chemical substances as food additives and color additives may or may not be appropriate for toxicity testing of some nanomaterials. As an example, the Ames test (bacterial reverse mutation test), which is recommended by FDA for *in vitro* analysis of compounds for their potential as mutagens, may not be useful for additives such as titanium dioxide or silver nanoparticles due to the inability of these particulate nanomaterials to penetrate the walls of the bacteria used in the assay (*5*). Other issues also may need to be addressed before a test method can be validated for assessing the safety of a nanomaterial. Further research will need to be conducted on the optimization of *in vitro* and *in vivo* toxicity testing paradigms for different types of nanomaterials (e.g., metallic nanoparticles,

liposome nanocapsules, etc.) and on improved tests for determining the fate of nanomaterials in the alimentary tract and their bioaccessibility in the small intestine.

## Alternative in Vitro and in Vivo Safety Assessment Paradigms

In recent years, there has been increased concern over the expense of some of the more commonly recommended safety assessment tests, as well as the large number of animals that may be used in such tests. For instance, a standard chronic/carcinogenicity bioassay lasts one to two years, can cost several millions of dollars, and include nearly 900 animals in the *in utero*, chronic and carcinogenicity phases. In light of this, FDA CFSAN has started evaluating the potential utility of alternative approaches to traditional animal safety studies. To this end we are currently evaluating a number of alternate safety assessment models such as, the use of non-mammalian species (e.g., zebra fish, *C. elegans*), computational toxicology (Quantitative Structure Activity Relationships (QSAR)), high throughput *in vitro* test systems (e.g., Tox21), organ-on-a-chip systems, short-term mammalian alternatives, and other novel *in vitro* assays that would result in reduced numbers of study animals, lower overall cost, and/or shorter study durations. We have previously adopted alternative methods such as the QSAR computational toxicology method that is used to evaluate the mutagenic and/or carcinogenic potential for contaminants that may be found in food contact materials for which there are little toxicity data (*6*). The FDA also currently receives data from *in vitro* genetic toxicity and mutagenicity studies in support of the approval process for food contact materials, for comparison with the QSAR results. The main concerns regarding these alternative testing paradigms are their ability to give dose/response and time-related information on food-related products that may be consumed at various concentrations over a lifetime. Overall, our focus in our evaluation of these alternative methods is to determine whether they can produce results that will satisfy the determination of a "reasonable certainty of no harm" for a food additive or color additive.

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Chapter 10

# Environmental Review of Food Additive Submissions

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Under the National Environmental Policy Act (NEPA) of 1969, federal agencies are required to consider environmental factors in their decision-making, and examine the environmental impacts of major, final actions. At FDA, these actions include–but are not limited to--petitions, (food- and color-additive, and citizen petitions), Threshold of Regulation (TOR) determinations, and allowing a Food Contact Notification (FCN) to become effective. Under FDA's NEPA implementing regulations, each of these submission types is required, to include an environmental component in the form of either a claim of categorical exclusion or an environmental assessment (EA). NEPA does not supplement or replace FDA's enabling statute, the Federal Food, Drug and Cosmetic Act (FFDCA). Categorical exclusions apply to actions that the agency has found do not individually or cumulatively affect the environment. Thus, categorical exclusions are very narrowly defined and precisely worded to fit very specific actions. EAs are required for any action for which there is no categorical exclusion, or where a categorical exclusion exists, but there are extraordinary circumstances that require further examination in an EA. The agency has developed guidance for what information to include in an EA to ensure that it provides the agency with sufficient information to determine whether an Environmental Impact Statement (EIS) or a Findings of No Significant Impact (FONSI) is appropriate.

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## National Environmental Policy Act (NEPA)

The National Environmental Policy Act was passed by the Senate in 1969 and signed into law in 1970 by President Nixon. Its stated purpose is "To declare a national policy which will encourage productive and enjoyable harmony between man and his environment; to promote efforts which will prevent or eliminate damage to the environment and biosphere and stimulate the health and welfare of man; to enrich the understanding of the ecological systems and natural resources important to the Nation; and to establish a Council on Environmental Quality (*1*)."

In practical terms, NEPA requires consideration of environmental factors in agency decision-making, and examination of environmental impacts of major (as defined in 40 CFR 1508.18) and final (i.e. meeting the finality test in the Administrative Procedure Act) actions.

Unlike other environmental statutes such as the Clean Water Act or Clean Air Act, NEPA is not a regulatory statute. Rather, it lays out national environmental policy and goals, and serves as a planning tool for agency decision-making.

As indicated by the Declaration of Purpose excerpted above, NEPA required the creation of a Council on Environmental Quality (CEQ). The CEQ was established in 1970, and is housed within the Executive Office of the President. One of CEQ's first duties was to promulgate NEPA Regulations that delineate action-forcing provisions ensuring that federal agencies meet the letter and spirit of NEPA. These regulations are codified in 40 CFR 1500-1508. Section 1500.2(a) requires federal agencies to "Interpret and administer the policies, regulations, and public laws of the United States in accordance with the policies set forth in the Act and these regulations (*2*)." As a result, FDA promulgated—and has from time to time updated—NEPA implementing regulations in 21 CFR Part 25. Although part of FDA's regulations, the NEPA requirements do not supplement or supplant FDA's duties under FFDCA.

## Environmental Information Required for FDA Submissions

The process for assessing environmental impacts is the same for FCN, TOR, and petition submissions. However, it is important to note that the public disclosure requirements in NEPA differ greatly from those in the FFDCA. Whereas the FFDCA requires that the Agency safeguard information identified by the submitter as confidential, NEPA is a full disclosure statute that requires federal agencies to "Make diligent efforts to involve the public in preparing and implementing their NEPA procedures (*3*)." While, on the face of it, this would seem to create tension between the two statutes, as mentioned above, NEPA does not trump or replace the FFDCA. This means that, in the case of a petition, the Federal Register notes the availability of the environmental information in the appropriate FDA Docket at the time the petition is accepted. By contrast, the FFDCA requires that a FCN remain confidential during the 120-day review period. Since NEPA does not supersede the FFDCA, the environmental record associated with a FCN is not made publicly available until permitted under the FFDCA, namely after it becomes effective.

**Claim of Categorical Exclusion**

CEQ required agencies to define categories of actions that do not individually or cumulatively affect the environment (*4*). FDA, therefore, has identified such categories based on agency experience in reviewing EAs for actions which resulted in Findings of No Significant Impact (FONSI). Thus, these categories of actions are excluded from the need for an EA or Environmental Impact Statement (EIS). Categorical exclusion regulations are narrowly defined and precisely worded to fit very specific actions, such that it becomes clear upon reading the regulation, whether or not the proposed action meets the criteria of a particular categorical exclusion.

All submissions—i.e. applications or petitions requesting agency action—must include either a claim of categorical exclusion or an EA. In accordance with 21 CFR 25.25(a), an adequate claim of categorical exclusion must contain three parts:

1. A citation of the categorical exclusion claimed (e.g. 21 CFR 25.32(i)),
2. A statement of compliance with the criteria of that categorical exclusion, and
3. A statement that, to the applicant's knowledge, no extraordinary circumstances exist that would require the preparation of an EA.

A claim of categorical exclusion lacking any of the above items is not adequate, and the submission will be deemed not to have a complete environmental component.

Extraordinary circumstances, as defined in 21 CFR 25.21, require an EA for any action that, ordinarily, would qualify for categorical exclusion. Examples of such extraordinary circumstances, as provided in 21 CFR 25.21(a) and (b,) include actions for which data show that, at the expected exposure, serious environmental harm could occur; or, actions that adversely affect species or critical habitat of species "determined under the Endangered Species Act or the Convention on International Trade in Endangered Species of Wild Flora and Fauna to be endangered or threatened (*5*)…"

It is important to note that the determination as to whether extraordinary circumstances exist is applied to production, use and disposal of a substance, and that data used in that determination include information received by the agency historically, as well as that submitted by the industry sponsor. Hence, though the submitter may conclude that no extraordinary circumstances exist, FDA may, based on experience from past submissions, require the submission of an EA for an otherwise categorically excluded action.

Furthermore, "significantly" is defined in the CEQ regulations at 40 CFR 1508.27(a) and (b), in terms of context and intensity:

"*Context*: This means that the significance of an action must be analyzed in several contexts such as society as a whole (human, national), the affected region, the affected interests, and the locality. Significance varies with the setting of the proposed action. […] Both short and long-term effects are relevant.

*Intensity*: This refers to the severity of impact. Responsible officials must bear in mind that more than one agency may make decisions about partial aspects of a major action (*6*)…"

Crucially, section 1508.27(b) provides that impacts may be both beneficial and adverse. Thus, applicants are encouraged to use care when making claims of "significant" beneficial impacts as such conclusions could jeopardize a FONSI.

Below are the most commonly used categorical exclusions for food additives (21 CFR 25.32).

1. Approval of a food additive petition, GRAS affirmation petition, the granting of a request for exemption, or allowing a notification to become effective, when the substance is present in finished food-packaging material at not greater than 5 percent-by-weight and is expected to remain with finished food-packaging material through use by consumers or when the substance is a component of a coating of a finished food-packaging material (§25.32(i)) (*7*);
2. Approval of a food additive petition, GRAS affirmation petition, the granting of a request for exemption, or allowing a notification to become effective, when the substance is to be used as a component of a food-contact surface of permanent or semi-permanent equipment or of another food-contact article intended for repeated use (§ 25.32(j)) (*8*);
3. Approval of a food additive petition, the granting of a request for exemption, or allowing a notification to become effective, for a substance registered by the U.S. Environmental Protection Agency (EPA) under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) for the same use requested in the petition (§ 25.32(q)) (*9*)

**Environmental Assessment**

For actions for which there is no categorical exclusion or in the case of extraordinary circumstances, the preparation of an EA is required. The required elements of an adequate EA are codified in 21 CFR 25.40. In summary, an EA is a concise public document providing the agency with sufficient information to determine whether an EIS or a FONSI is appropriate. Detailed information and recommendations for EA content are provided in the agency's Environmental Guidance at http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/IngredientsAdditivesGRASPackaging/ucm081049.htm, but generally, an EA must discuss the need for the proposed action, introductions, fate, and effects of substances in the environment, and environmental impact of the proposed use and disposal of the substance.

Since EAs are public documents, any confidential business information must be clearly designated, and placed in a separate confidential attachment to the EA. Physical measurements or chemical properties do not qualify as confidential information.

In general, there are three main use profiles requiring the preparation of an EA:

1. Substances used in the production or processing of food that are not intended to remain with food (e.g. antimicrobial washes)
2. Processing aids used in the manufacture of food-packaging material that are not intended to remain with finished food packaging (e.g. plasticizers)
3. Components of food packaging present at greater than 5 percent by-weight (% b.w.).

In this last instance, it is worth noting that in certain circumstances, substances present at ≤ 5% b.w. may still require the preparation of an EA. This is the case when the food-contact substance covalently bonds with the polymer, thus resulting in a new food additive in the form of a modified polymer. When determining the environmental impact, it is the resulting food additive (i.e. 100% b.w.), not the food-contact substance (≤ 5% b.w.), which has the potential to (a) migrate from food packaging into landfill leachate and (b) impact the recycling stream. Therefore, when applying the categorical exclusion at 21 CFR 25.32(i) the agency looks at the potential impact of the substance entering the environment (i.e., the new, modified polymer). Thus, such submissions generally require the preparation of an EA examining the environmental impacts resulting from use and disposal of the modified polymer.

*Polymeric Substances*

However, in FDA's experience, most polymeric substances, regardless of percent-by-weight, whether blended or covalently bound, will not be introduced to any significant amount into the environment. This is because they are generally incorporated into the finished food-packaging material, and are expected to remain throughout the lifecycle of the product. The EA will examine that lifecycle through use and disposal by the consumer, which, in the case of polymers, typically involves disposal as municipal solid waste (MSW) to a landfill or combustion facility, and recycling. Due to EPA regulations governing landfills (40 CFR Part 258) and waste combustion facilities (40 CFR Part 60), it can generally be assumed that no significant environmental releases are expected from the use and disposal of polymeric materials.

Under NEPA, FDA is responsible for considering the impacts of its actions on the use of natural resources and energy ("…it is the continuing responsibility of the Federal Government to … enhance the quality of renewable resources and approach the maximum attainable recycling of depletable resources." (*10*). Issues may arise in the area of recycling if the substance could be expected to interfere with recyclability of similar food-packaging products (e.g. water or soda bottles). In such instances, the agency may require a use limitation requiring that the substance not be used in items that are frequently recycled.

*Other Substances*

For substances not intended to remain with food or food packaging, the EA must provide the agency with information about the amounts, fates, and effects of those substances that will be released to the any compartment of the environment (water, soil, air). Such information can include data obtainable from public sources such as EPA (ECOTOX - Ecotoxicology database; EPIWIN – environmental partitioning and fate; ECOSAR – Ecological Structure Activity Relationships), or data obtained from testing conducted by the submitter. Those concentrations expected to be released to a certain environmental compartment (known as EEC – expected environmental concentration) are compared to ecotoxicity endpoints for the most sensitive organisms for that compartment. Detailed information on how to obtain and report EECs is available at the aforementioned Environmental Guidance document.

**Inadequacies in EAs**

The submission of an inadequate EA—i.e. one that does not provide the agency with sufficient information to determine whether a FONSI or EIS is appropriate—can cause substantial delays in the review process, even requiring withdrawal and subsequent re-submission. The above-mentioned Environmental Guidance documents are important and useful resources, and careful consideration of their recommendations and suggestions can prevent common mistakes, such as:

- Incorrect use statement: If the use described in the EA does not match that in the rest of the submission, the EA will be deemed inadequate and a revised EA will be required.
- EA does not meet "sufficient evidence" criterion: As defined in 21 CFR 25.40, an EA is a stand-alone document that must contain sufficient evidence for the agency to determine whether a FONSI or EIA is appropriate for the action. Therefore, the EA may not refer the reader to another part of the submission for that evidence—it must appear in the EA.
- Incorporation by reference: Similar to above, the submitter may not refer the reader to another source to retrieve crucial environmental information. Such material must be quoted, summarized, or otherwise included in the EA, and the source document listed as a reference. The source document must be readily available for inspection by interested parties and may not include confidential information (see below).
- Confidential information in the EA: Since an EA is a public document it may not contain confidential business information (CBI). Instead, the submitter must include CBI in a confidential attachment to the EA, with a summary of the information in the EA itself.

## References


1. Congressional Declaration of Purpose; NEPA § 2; 42 U.S.C. 4321; available at http://www.epw.senate.gov/nepa69.pdf.
2. 40 CFR 1500.2(a) Policy; Regulations for Implementing the Procedural Provisions of the National Environmental Policy Act; available at http://www.gpo.gov/fdsys/pkg/CFR-2004-title40-vol30/pdf/CFR-2004-title40-vol30-part1500.pdf.
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4. 40 CFR 1500.5(k) Reducing delay; Regulations for Implementing the Procedural Provisions of the National Environmental Policy Act; available at http://www.gpo.gov/fdsys/pkg/CFR-2004-title40-vol30/pdf/CFR-2004-title40-vol30-part1500.pdf.
5. 21 CFR 25.21 Extraordinary circumstances; available at http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=25.21.
6. 40 CFR 1508.27 Significantly; Regulations for Implementing the Procedural Provisions of the National Environmental Policy Act; available at http://www.gpo.gov/fdsys/pkg/CFR-2004-title40-vol30/pdf/CFR-2004-title40-vol30-part1508.pdf.
7. 21 CFR 25.32(i); available at http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=25.32.
8. 21 CFR 25.32(j); available at http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=25.32.
9. 21 CFR 25.32(q); available at http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=25.32.
10. Congressional Declaration of National Environmental Policy; NEPA § 101; 42 U.S.C 4331; available at http://www.epw.senate.gov/nepa69.pdf.

## Chapter 11

# Managing FDA Food Contact Compliance Through the Packaging Supply Chain

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The packaging industry is one of the most rapidly growing global industries. It is also an industry that is dominated by continuous innovation, combined with new, and emerging technologies. For example, new polymer and additives technologies, including active packaging, nanotechnology, sterilization processes, advanced colorants technology, bio-based polymers, recycling processes, and new multi-layer laminate technology.

This chapter will address the complex process of managing and ensuring the compliance of the components of food-contact articles (FCA), known as food-contact substances (FCS), throughout the supply chain with the laws and regulations administered by the U.S. Food and Drug Administration (FDA). The brief discussion covers all possible options potentially available for establishing compliance, including a listing in FDA's food additive regulations in 21 CFR Parts 170 to 199, threshold of regulation (TOR) exemptions as per 21 CFR 170.39, and food-contact substance notifications (FCN) (all requiring FDA review); and "no migration," generally recognized as safe (GRAS) status, and prior sanctions (all company self-determinations). These approaches apply to all types of FCAs and their substances, including plastics, metal, paper, glass, additives/adjuvants, adhesives, inks, colorants, and coatings.

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## The Packaging Supply Chain

The packaging supply chain is complex and the regulatory compliance of the components through that supply chain is equally complex and often poorly understood and communicated along the supply chain. The principal aim is for the end use article (aka FCA) to be safe and in compliance with the FDA regulations for its intended use.

Figure 1 illustrates the various steps along the packaging supply chain, from manufacture of the additives to the packaged food available to the consumer.

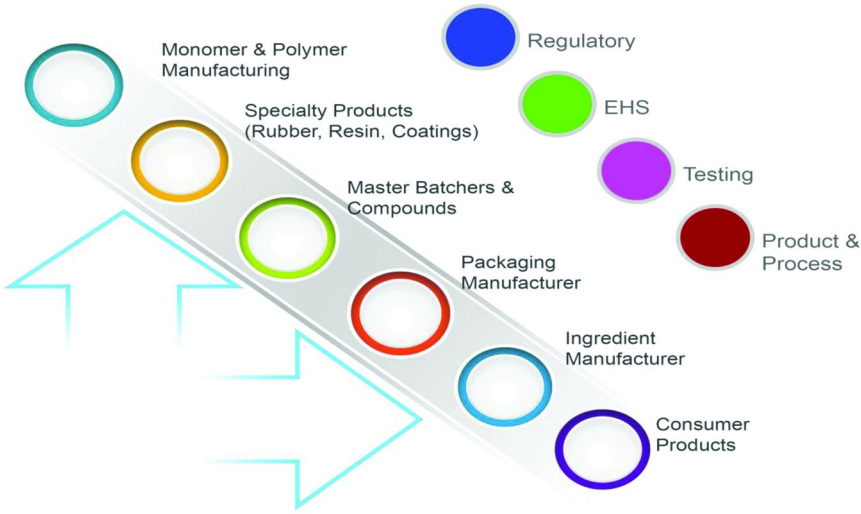


*Figure 1. Supply Chain Regulatory Requirements.*

## FDA Compliance of Food-Contact Articles (FCA) and Components

How do we ensure that the food packaging and other FCAs comply with the FDA regulations for their intended use?

FCA are constructed from many different types of base materials, including paper, plastics, metals, and glass, and generally contain other additives or adjuvants, such as stabilizers, colorants, inks, coatings and adhesives either within or on each of the base materials.

As elaborated on below, these FCAs and their components may be food additives for purposes of determining FDA compliance. The Federal Food, Drug and Cosmetic Act (the "Act") states the following definition of "food additive":

Under Section 201(s) of the Act, a food additive is defined as "*any substance the intended use of which results in or may reasonably be expected to result ….in it's becoming a component of food.*

## Direct and Indirect Food Additives

Direct food additives are not naturally a part of the food but are cleared by the FDA for direct addition to food in order to perform a specific function or technical effect. There must be intent to affect some characteristic of the food by the addition of the food additive. Examples of typical direct food additives include preservatives and flavorings agents.

Substances passing into food from their use in packaging or other FCAs traditionally have been regarded as "indirect food additives". Indirect food additives are not approved for direct addition to food. There is no intent that they have a functional effect in or on the food. However, they may be reasonably expected to migrate into food, that is, become a component of the food from their intended use. As discussed below, at present, such substances are known as food-contact substances (FCS).

FCSs which meet the "food additive" definition require FDA premarket review and clearance through a food additive petition (which results in an "indirect food additive regulation" or, more recently, through a food contact notification (FCN), which results in a listing in the FDA online inventory (available at www.fda.gov).

Any FCS is deemed unsafe unless it is used in conformity with a regulation or notification issued by the FDA. Suppliers and manufacturers are ultimately held responsible for ensuring that the FCA and all of its components comply with the FDA's requirements for safe use as indirect food additives.

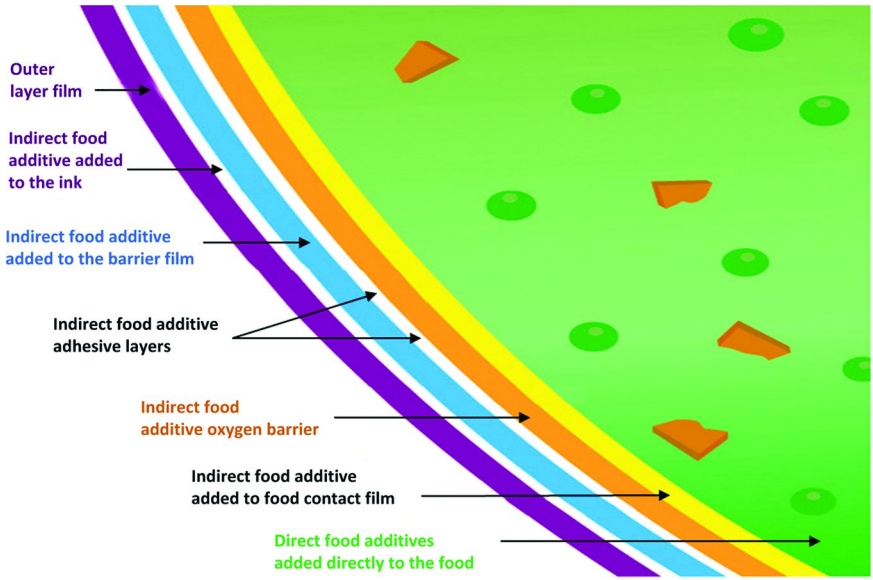


*Figure 2. Direct and Indirect Food Additives.*

## Direct, Indirect, and Incidental Food Contact

Direct food contact refers to a surface that is directly in contact with a food product.

Indirect food contact is an industry-created term used for a surface not in contact with food. An example of this would be a printing ink on the outer layer of multilayer lamination. FDA does not recognize the term indirect food contact.

Incidental food contact refers to substances used on equipment or machinery used for producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food. By virtue of these processes, the substances may contact food although this is not their purpose and any food contact is unintended. There are a few parts at 21 CFR 178 which list substances approved by the FDA for incidental contact. An example would be a substance for use as a lubricant as listed under 21 CFR 178.3570.

Figure 2 illustrates various contact (direct contact, indirect contact, no contact) and use (film, ink, adhesive and oxygen barrier) scenarios.

## FDA FCA and FCS Compliance - Listings

The Federal Register is where FDA orders are published, including orders creating indirect and direct food additive regulations. The Code of Federal Regulations (CFR) is the official collection of all FDA regulations and is updated regularly.

More recently, the "Threshold of Regulation (TOR) Exemptions" listings and the "Inventory of Effective Premarket Notifications for FCS" are the online listing of FCSs that are cleared by FDA under the TOR and FCN program, respectively.

Typical sections of 21 CFR Parts 170-199 that interpret some of the food contact regulations include:

- 21 CFR Part 175 – Adhesives and Components of Coatings
  21 CFR Part 176 – Paper and Paperboard
- 21 CFR Part 177 – Polymers
- 21 CFR Part 178 – Adjuvants, Production Aids and Sanitizers

## Paths to FDA Clearance Vary with Dietary Exposure to Migrants

Depending on the dietary exposure from migration of an FCS to food under the intended conditions of use, there are three procedures (with increasing data requirements) for getting a substance "cleared" for use as "food additive":

- TOR (Threshold of Regulation)
  (concentration in the daily diet < 0.5 ppb)
- FCN (Food Contact Notification)
  (concentration in the daily diet < 1 ppm)
- FAP (Food Additive Petition)
  concentration in the daily diet > 1 ppm )

## Substances Excluded from the Food Additive Definition Do Not Require FDA Review and Clearance

The food additive definition (above) excludes substances which are not expected to become components of food from their intended use, or are prior sanctioned, or are "generally recognized as safe" (GRAS). The strategy to evaluate the final FCA for regulatory compliance for the intended us is as follows. First, one should determine the intended use of the FCA, such as food type and temperature-time conditions. Second, one should evaluate the regulatory status of each component of the FCA. And, third, this can then be followed by end-use extraction studies (aka end-test) on each component, as needed, to ensure compliance with 21 CFR requirements. The component with the most stringent limitations on food types and conditions of use dictates the proposed uses of the FCA.

If all components are in compliance for the intended use, the manufacturer or supplier could claim that the product complies with FDA requirements. If any of the components are not in compliance, then one of the following steps could be followed in lieu of a full FCN submission:

## No Migration as a Tool for Compliance

If there is no detectable migration of an FCS to food at the appropriate analytical sensitivity, the FCS is not reasonably expected to migrate to food. Therefore, the FCS is not a food additive and does not require premarket review or clearance by FDA. This is an industry interpretation.

The traditional industry standard for "no migration" is no detectable migration at 50 ppb (analytical limit of detection), except for high exposure applications such as milk and carbonated soft drink bottles and for biologically active molecules where a limit of 10 ppb or lower is applied (i.e., BPA, sanitizers and PET). A limit of 50 ppb is based on the 1969 Ramsey Proposal, which was articulated by Dr. Lessel Ramsey of FDA as the level at which the Agency does not need toxicology data to reach a safety determination.

The FCS must not pose special toxicological concerns (e.g., heavy metals or carcinogens), and must not be known to pose toxic reactions at levels of 40 ppb or lower in the diet of man or animals).

To determine migration levels to food, the use of accelerated migration testing under the intended conditions of use, the assumption of 100% migration to food, or the use of migration modelling are considered acceptable methods.

This is a self-determination (although you may prefer someone with expertise and public liability insurance to carry out the calculation). There is no need to notify FDA.

FDA does not formally acknowledge the acceptability of the "no migration" approach and, on when needed; the Agency has taken action against such a determination.

## Functional Barrier as a Tool for Compliance

The "functional barrier" doctrine is a corollary of "no migration." If there is a functional barrier between the FCS and food which prevents migration from the FCS to food, the FCS is not a food additive. Again, this is a self-determination. The idea of a functional barrier to migration is discussed in the adhesives regulation (21 CFR 175.105).

The existence of a functional barrier is determined by the following process:

- Analyzing package structure
- Analyzing exposure conditions anticipated for the package
- Migration testing or calculation
- FDA has the Threshold of Regulation (0.50 ppb dietary exposure)
- Industry has the Ramsey Proposal

Figure 3 illustrates the concepts behind a functional barrier.

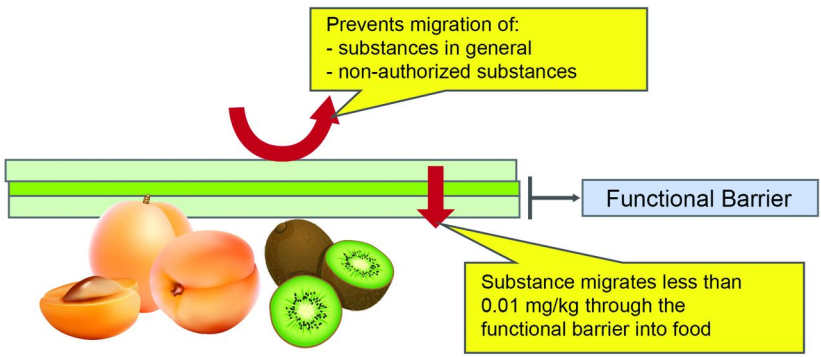


*Figure 3. Functional Barrier Example.*

## Prior Sanction as a Tool for Compliance

- Pre-1958 approval by FDA or USDA
- Approvals are in private letters from FDA or USDA to companies
- 21 CFR Part 181 lists some, but not all, prior sanctions
- Prior sanctions are specific to the terms of the prior sanction letter

## Generally Recognized as Safe (GRAS) as a Tool for Compliance

- Pre- 1958 common use in food is one basis, but this is unlikely to apply to an FCS
- Common knowledge of safety among the scientific community
- Some GRAS substances are listed in FDA's regulations. 21 CFR Parts 182 and 184 list substances that are GRAS for direct addition to food. Part 186 has a short list of substances that FDA has affirmed as GRAS

for food-contact use. Substances that are GRAS for use in food are GRAS for use in packaging or other food-contact articles. 21 CFR §§ 174.5 and 186.1 (a).

- Self-determination. FDA has a voluntary GRAS notification process, but it is almost always used for direct food ingredients.

## Risk Assessment Process

Figure 4 lists steps in the risk assessment process that should be carried out in establishing the safety of any substance that is not in compliance with respect to the conditions of use. This is the type of analysis which would be used in a food additive petition or an FCN.

A generalized safety qualification process that involves exposure determination and toxicological testing of the FCS and its impurities is shown in Figure 5. The testing incorporates two components: the level of migration and dietary exposure for each the substances; and the available toxicological data and other information on each of the substances. The data from the two components are then used as basis for establishing a safe level of consumer exposure to the FCS and its impurities, with a margin of safety. The greater the expected exposure, the more toxicity information required to support safety, which is an exposure-driven tiered approach recommended by FDA for safety evaluation. If the structure of the migrant is similar to a known toxicant found in the literature (such as cancer risk), to the extent feasible, knowledge in predicting potential toxicity based on structure and activity relationships (SAR) may be incorporated into the safety assessment of the migrant. SAR may also be used as part of an overall strategy for assessing the safety of the migrant or to help interpret safety test results.

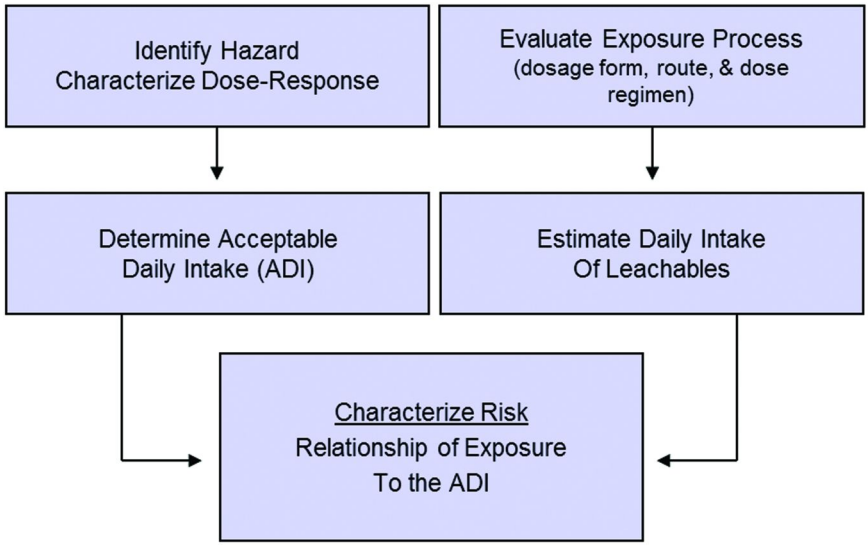


*Figure 4. Steps in the Risk Assessment Process.*

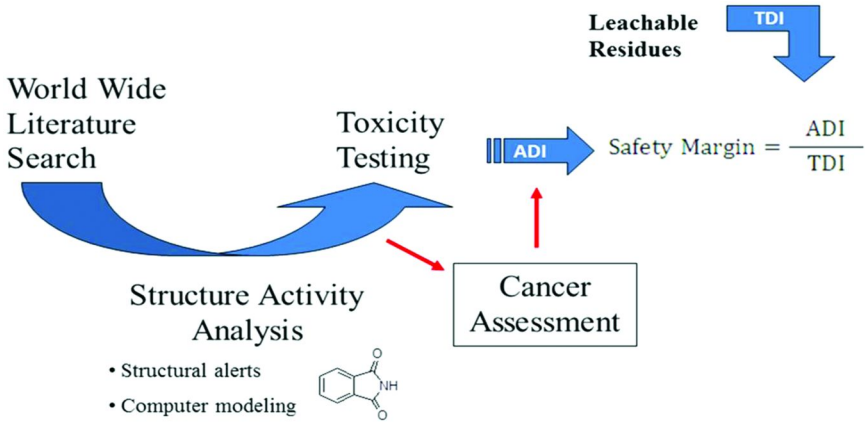


*Figure 5. Generalized Safety Qualification Process.*

## Coatings Compliance

For coatings, which may include metal (cans), plastic, or paper, the following are mandated FDA Regulations useful for compliance:

- 21 CFR §175.300 – "Can coatings," coatings on metal substrates
- 21 CFR §175.320 – Coatings on plastics
- 21 CFR §176.170 – Coatings on paper

Coatings are used as barrier between food or beverage and cans (e.g., Coke cans would be etched away within hours if there wasn't a coating).

There is much recent research and development work currently taking place on can coatings, driven by consumer pressure to move away from Bisphenol-A based epoxies.

## Inks Compliance

For inks, which may include metal (cans), plastic, or paper, the following are mandated FDA Regulations useful for compliance:

21 CFR Part 175 Indirect Food Additives: Adhesives and Components of Coatings
21CFR 175.105 Adhesives
21CFR 175.300 Resinous and Polymeric Coatings
21CFR 175.320 Resinous and Polymeric Coatings for Polyolefin Film

21 CFR Part 176 Indirect Food Additives: Paper and Paperboard
21CFR 176.170 Components of Paper and Paperboard in Contact With Aqueous and Fatty Foods
21CFR 176.180 Components of Paper and Paperboard in Contact With Dry Foods
21CFR 176.200 Defoaming Agents Used in Coatings

21 CFR Part 178 Indirect Food Additives: Adjuvants, Production Aids, and Sanitizers
178.3297 Colorants for Polymers
178.3740 Plasticizers in Polymeric Substances
178.3860 Release Agents
178.3870 Rosins and Rosin Derivatives

See Table 1 for an additional example.

**Table 1. Example of Printing Ink over Polyethylene (PE)**

| *Printing Ink Ingredients* | *21 CFR Citation* |
|---|---|
| Polyamide Resin | 175.320 (limits) |
| Pigment | 178.3297 |
| Polyethylene wax | 175.320 |
| Resin | 178.3870 |

## FDA Requirements for Recycled Plastics

So far in this chapter, we have focused on establishing FDA compliance for a "virgin" FCS. Establishing compliance of a recycled FCS requires a related but somewhat different approach.

## The Three Distinct Approaches to the Recycling of Plastic Packaging Materials

1. **Primary recycling (1°)** refers to the use of pre-consumer industrial scrap and salvage to form new packaging, a common practice in the industry.
2. **Secondary recycling (2°)** refers to the physical reprocessing (e.g., grinding and melting) and reformation of post-consumer plastic packaging materials.
3. **Tertiary recycling (3°)** involves subjecting post-consumer plastic packaging to chemical treatment whereby its components are isolated and reprocessed for use in manufacture.

## Post Industrial/Post Consumer

Post Industrial Scrap Material is defined as material generated in the production process before it has gone out of the factory for its intended end use. Examples would be "out of specification goods" or simply unavoidable scrap such as edge trims. In recycling post- industrial materials, the primary need is to get the industrial scrap back in to a form that can be reused in the production process.

Post-Consumer Scrap Material is defined as material that has been used for its original intended use. Examples would include used bottles from curbside pickup and used carpeting. In recycling post-consumer materials, the primary need is cleaning and separation of the desired materials from undesirable materials.

## FDA No Objection Letter for Recycling Process

- If the recycled plastics are originally in compliance with FDA requirements for the safe use in the intended food contact applications, no FCN is required.
- The FDA recommends a submission describing the recycling process in order to obtain a No Objection Letter (NOL). The FDA website has a list of the NOLs which have been issued.
- The FDA regulates post-consumer recycled (PCR) plastic to be used in the manufacturing of food-contact articles. The FDA web site has a list of **No Objection Letters.**
- Filing a submission for a No Objection Letter for recycled plastics is a recommendation and is not required by law.

## FDA Safety Concerns

FDA's main safety concerns with the use of recycled plastic materials in food-contact articles are:

1. Those contaminants from the post-consumer material may appear in the final food-contact product made from the recycled material.
2. That recycled post-consumer material not regulated for food-contact use may be incorporated into food-contact packaging.
3. That adjuvant in the recycled plastic may not comply with the regulations for food-contact use.

## FDA Recommendations for Secondary Processes

Examples in which Recyclers address these concerns are:

- Implementing controls on the source of the post-consumer polymer
- Adequate sorting procedures for the incoming post-consumer material
- Use limitations on the finished recycled packaging (such as use at room temperature or below), or
- Food-type restrictions (such as dry or aqueous foods only).
- In any submissions to FDA regarding 2° recycling processes, a discussion of these types of actions would be very helpful in FDA's evaluation of the processes.

## FDA Requirements for a NOL for the Use of Recycle Plastics in Food Contact

- A complete description of the recycling process, including a description of the source of the recyclable plastic and a description of any source controls in place intended to ensure that only plastic that initially complied with the applicable regulations is recycled. Also, a description of any steps that are taken to ensure that the recyclable plastic is not contaminated at some point, either before collection for recycling, or during the recycling process.
- The results of any tests performed to show that the recycling process removes possible contaminants.
- It is necessary to either show that there has been no possibility of contamination with substances other than food or to demonstrate that the process will remove any of these contaminations.
- A description of the proposed conditions of use of the plastic (e.g., information on intended temperature of use, type of food with which the plastic will come into contact, the duration of the contact, and whether the food-contact plastic will be for repeated or single-use applications.)

Chapter 12

# Evaluating Packaging Materials for Use during the Irradiation of Prepackaged Food

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Irradiation is an effective means for controlling foodborne pathogens and has gained much interest from the food industry in the past two decades. Irradiation of prepackaged food is a reasonable and practical method to avoid the post-irradiation contamination of food, and additionally, the irradiated foods are ready for shipping to the market immediately after irradiation. Irradiation can induce chemical changes to the packaging materials resulting in the formation of breakdown products that may readily migrate into foods. Therefore, the packaging materials holding food being irradiated are required to undergo premarket authorization prior to use. This chapter addresses the interpretation of the food irradiation regulations, the effects of various types of radiation on food packaging materials, challenges for analyzing breakdown products, and some approaches for use in evaluating packaging materials for use during the irradiation of prepackaged food.

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## Introduction

The U.S. Food and Drug Administration is responsible for regulating the use of irradiation in the treatment of food and food packaging. This authority results from the 1958 Food Additives Amendment to the Federal Food, Drug, and Cosmetic Act (FD&C Act) where Congress explicitly defined a source of radiation as a food additive (Section 201(s) of the FD&C Act). Section 201(s) of the FD&C Act, defines a food additive as: "any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food……including any source of radiation intended for such use…"

The 1958 Food Additives Amendment also provides that a food is adulterated (that is, it cannot be marketed legally) if it has been irradiated, unless the irradiation is carried out in conformity with a regulation prescribing safe conditions of use (Section 403(a)(7) of the FD&C Act). Prior to 1999, FDA regulated the lawful use of irradiation of food packaging materials through the food additive petition (FAP) process, the completion of which resulted in the promulgation of a regulation published in the Federal Register prescribing the approved use. Since 1999, food additives, including radiation, are authorized by the food contact notification (FCN) process as described in 21 CFR §170.100–§170.106 or threshold of regulation (TOR) exemption processes as described in 21 CFR §170.39.

When pre-packed food is irradiated, the packaging materials holding the food are also irradiated together with food, and as such these packaging materials are required to undergo a safety evaluation before they can be used to hold irradiated food. The safety evaluation for the packaging materials relies on an assessment of the radiolysis products (RPs) that are formed and which may migrate to the packaged food. Under Section 409(a) of the Act, a food is deemed adulterated, and thus prohibited from interstate commerce, if it has been intentionally irradiated, unless the irradiation is carried out in compliance with an applicable food additive regulation or effective FCN or is exempted from the requirement of a listing regulation under the TOR exemption process for the specified conditions of use.

This chapter highlights answers to questions regarding food irradiation, irradiation regulations, effects of radiation on food packaging materials, challenges in analyzing radiolysis products, and approaches that may be considered in evaluating packaging materials for use during the irradiation of prepackaged food.

## Why Is There Interest in Food Irradiation?

Although food irradiation technology has been utilized for several decades in many other countries, its use in the U.S. became more prevalent by the food industry in 1990's after the incessant outbreaks of food-borne pathogens that led to many food-borne illnesses (*1*). Irradiation is an effective means of controlling several food-borne pathogens on/in various foods thereby improving the safety of food and extending shelf life (*2*). Several types of foods are currently permitted to be irradiated with ionizing radiation and are listed under Title 21 of Code of

Federal Regulations (denoted 21 CFR) section 179.26 (Ionizing radiation for the treatment of food) (*3*). Since 1997 the list has expanded to include refrigerated and frozen uncooked meat products, fresh shell eggs, seeds for sprouting, fresh and frozen molluscan shellfish, and most recently, fresh iceberg lettuce and fresh spinach.

Foods are preferably prepackaged before irradiation to avoid recontamination, and after irradiation, the irradiated foods are immediately ready to be shipped to the market. However, to ensure food safety, the packaging materials must not be altered upon irradiation in a manner that could result in any substance in the packaging becoming a component of food at unsafe levels.

## What Are the Food Irradiation Regulations?

The food irradiation regulations are codified in accordance with the interpretation of the definition of a food additive, as previously mentioned, by which both foods and food packaging materials that are exposed to ionizing radiation are required to undergo premarket approval to ensure the safety of the irradiated foods.

21 CFR Part 179 (Irradiation in the production, processing and handling of food) consists of Subparts B and C. Subpart B (Radiation and Radiation Sources) includes section 179.26 (denoted as §179.26) that describes radiation or energy sources, conditions of irradiation and a list of foods permitted for irradiation, and labeling. Section 179.25 (General provisions for food irradiation) links food packaging materials as specified in §179.45 to the conditions of irradiation as described in §179.26. Subpart C (Packaging Materials for Irradiated Foods) includes §179.45 (Packaging materials for use during the irradiation of prepackaged foods) and describes approved polymers and adjuvants.

Foods that are not yet permitted to be irradiated as described in 21 CFR §179.26 need to undergo premarket approval via the food additive petition (FAP) process, the completion of which results in the promulgation of a regulation published in the Federal Register prescribing the approved use.

Food packaging materials not yet listed in 21 CFR §179.45 need to undergo premarket approval via the FCN or TOR exemption process as noted above.

## What Food Packaging Materials Are Already Permitted for Holding Food during Irradiation?

There are several food packaging materials permitted for use in contact with foods during the irradiation process and they are listed in 21 CFR §179.45 (Packaging materials for use during the irradiation of pre-packaged foods). The list is not comprehensive as it only contains a limited number of packaging materials, such as films, homogeneous structures, and some adjuvants that they may contain. Packaging that is constructed from these materials may be irradiated by any permitted radiation source (gamma rays, electron e-beams, or X-rays), in either the presence or absence of oxygen, and in contact with food under the defined radiation conditions (e.g., a maximum dose limit). For example, approved

films include polyolefin films complying with 21 CFR §177.1520 (Olefin polymers); polyethylene terephthalate (PET) films complying with 21 CFR §177.1630 (Polyethylene phthalate polymers), subparagraphs (e)(4)(i) and (ii); Nylon 6 films complying with 21 CFR §177.1500 (Nylon resins), subparagraph (a)(6); and ethylene vinyl acetate copolymers complying with 21 CFR §177.1350 (Ethylene-vinyl acetate copolymers).

Keep in mind that the §179.45 list has not been updated since the 1980's, nor does it include modern food packaging materials that are more desirable for use in contact with food during irradiation. Food packaging materials not listed in §179.45 are required to undergo premarket approval via the FCN process as described in 21 CFR §170.100-§170.106, or the TOR exemption process as described in 21 CFR §170.39. Regardless of the regulatory approval process, the safety assessment for packaging materials used to hold food during irradiation is conducted in accordance with FDA recommendations as described in the chemistry, toxicology and environmental guidance documents published by the agency (*4*).

Although there are no effective FCNs for packaging materials that have been authorized for use in irradiation of prepackaged foods, a number of significant authorizations have been granted via the TOR exemption process. TOR exemptions have been issued to permit the irradiation of certain packaging structures under specific conditions of use, i.e., under an oxygen free environment, a nitrogen atmosphere, or while frozen and under vacuum. TOR exemptions granted in 2005 permitted use of polystyrene (PS) foam trays with multilayer food contact coatings for contact with ground beef being irradiated in a nitrogen atmosphere, or under vacuum while frozen, at doses not to exceed 3.0 kGy. The most recent TOR exemptions in 2010 (*5*) permitted all food additives (i.e., food contact substances) listed in 21 CFR §174–186, effective FCNs, and TOR exemptions, for contact with foods being irradiated in a verifiably oxygen-free environment or while frozen and contained under vacuum, at doses not to exceed 4.5 kGy.

## If Food Packaging Materials Are Irradiated before Food Contact, Are They Required to Undergo Premarket Approval?

Irradiation of food packaging materials before food contact is allowed only if the irradiation is considered as part of the manufacturing process (i.e., for cross-linking or sanitizing purposes), provided that the packaging materials are either listed in 21 CFR §179.45 or have been otherwise approved for non-irradiated uses. The irradiation process must be performed under conditions of good manufacturing practice (GMP), and the irradiated packaging materials must comply with the specifications and limitations as described in all applicable authorizations. This implies that the irradiation does not significantly affect the packaging materials, i.e., does not form significant amounts of new chemical substances that could migrate to food.

The irradiated packaging materials must also comply with the general provisions in 21 CFR §174.5. For example, any substance used as a component of food contact articles must be of a purity suitable for its intended use, used in accordance with section 402(a)(3) of the FD&C Act, and use of the irradiated packaging material should not impart odor or taste to food rendering it unfit for human consumption.

## What Are Radiation-Induced Changes in Polymeric Food Packaging Materials?

It is generally known that radiation induces chemical changes in polymeric packaging materials which could result in formation of unique radiolysis products in polymers. The chemical changes are induced by two competing reactions – cross-linking and chain scission/degradation. Both reactions are random and are proportional to the dose, dose rate and oxygen content of the atmosphere in which the polymer is irradiated. Generally, cross-linking dominates when polymers are irradiated under vacuum or in an inert atmosphere such as nitrogen, which is the basis for approving the recent TOR exemptions (*5*). Chain scission dominates when polymers are irradiated in the presence of oxygen, resulting in the formation of oxidative degradation products, or oxidative RPs (ORPs), which are primarily oxygenated volatile and semi-volatile organic compounds. The concentrations of these compounds generally increased with increased radiation dose.

The radiation-induced changes in functionality and properties of packaging materials have been investigated using mechanical and physicochemical testing, colorimetry, Fourier transform infrared spectroscopy (FTIR), rheological testing, and electron spin resonance (ESR) spectroscopy. A review of these reactive changes is available (*6*).

## How To Determine if Radiolysis Products (RPs) Are Formed from Polymers and Adjuvants?

The best method to determine if there are RPs present in irradiated packaging materials, originating from either the polymer and/or adjuvants, is by conducting an irradiation experiment with the materials and then comparing the irradiated materials with their non-irradiated counterparts. Any RPs generated would then be the subject of safety assessment for a new use.

In performing the safety assessment for a new use, the dietary exposure to RPs must be considered in context with the available toxicological information on the RPs. The residual levels of RPs are important for estimating exposures using the simple assumption of 100% migration to food and known thickness, density, food mass-to-surface area ratio, and the consumption factor (CF) for the packaging application. If the dietary concentration (DC) of any one RP exceeds 0.5 µg/kg, other approaches, such as migration modeling or a migration study, may be used as the next step to refine the exposure.

## What Information Is Recommended To Submit to FDA in Support of the Irradiation of Packaging Materials in the Presence of Oxygen?

When searching for regulatory precedent, keep in mind that there are not many approvals for the use of the irradiation of packaging materials in the presence of oxygen because not many requests have been submitted to FDA. The approval process might be more difficult due to the complexity of analyzing for the identities and amounts of all ORPs.

Since chain scission reactions dominate when polymers/adjuvants are irradiated in the presence of oxygen, ORPs are of safety concern and should be evaluated for determining whether the packaging materials are suitable for use during the irradiation of prepackaged food that requires oxygen to maintain quality (e.g., fresh produce). The ORPs include new substances and existing substances that increase in concentration, such as aldehydes, ketones, alcohols, carboxylic acids, all of which are known to affect the organoleptic properties and shelf-life of irradiated foods.

## What Analytical Approaches May Be Considered for Testing Irradiated Packaging Materials?

A real-life or practical approach may be considered for use in testing the irradiated packaging materials. A "real-life" approach involves irradiating the polymer while in contact with a food simulant. The RP migration levels in the simulant are used in estimating exposure. However, this method may be difficult in practice due to analytical problems with small quantities of RPs generated from the food simulant that interfere with the RPs that migrate from the polymer to the simulant.

On the other hand, a practical (or step-wise) approach involves irradiating the polymer alone, followed by analysis of residual RPs by an appropriate method and properly validated procedure. The step-wise approach is intended to pre-identify and quantify low molecular weight (volatile) RPs present in irradiated polymer samples, using headspace/gas chromatography (HS/GC) or thermal desorption, with mass spectrometry (MS) detection. Non-volatile RPs are usually analyzed by total polymer dissolution or solvent extraction, followed by liquid chromatography (LC) with MS detection.

After the RPs are identified and quantified in the polymer, the concentration of each RP may be used to calculate exposure based on an assumption of 100% migration to food. If the assumed 100% migration level produces RP exposures that are too high to be supported by toxicology data, a more realistic level of migration may be estimated by migration modeling. As an alternative, a migration study with food-simulating solvents or actual foods may be conducted under realistic use conditions, to refine the exposure estimate. If a migration study is needed, consult the recommendations on migration testing as described in Chemistry Guidance, which can be accessed from the Internet in the Ingredients, Packaging & Labeling section under the Food topic of www.fda.gov.

Another approach is to conduct a direct migration study, skipping the pre-identification step as describe above. However, this alternate approach is not informative because it does not pre-identify the RPs, an essential step in developing appropriate methods for quantification of the RPs. The drawbacks of the approach are concerns on stability of RPs in the food simulants, the increased possibility that RPs could be missed completely, and it is very difficult to validate the analytical results.

## What Are Special Considerations on Polymer Adjuvants?

Most polymers commonly contain adjuvants, such as antioxidants and stabilizers that enhance polymer processing. Although the §179.45 contains various approved polymers, only few adjuvants are approved (*6*). The lack of approved adjuvants presents a challenge for the use of packaging materials during the irradiation of prepackaged food.

Upon irradiation of a polymer-adjuvant system in the presence of oxygen, adjuvants preferentially degrade over polymers (*7*), resulting in high levels of adjuvant RPs in comparison to polymer RPs. Fortunately, adjuvant RPs may be predicted from their chemical structure or previous studies discussed in the literature. If an adjuvant is not yet approved for non-irradiated uses, additional testing may be needed for both the non-irradiated and irradiated uses.

## How Would Radiolysis Products from Adjuvants Be Predicted or Identified?

RPs may be predicted using a model polymer system instead of a real polymer system to simplify analytical work. Also, a thermal degradation experiment may assist in determining the RPs because it has been reported that irradiation is comparable to accelerated aging by photochemical and/or thermal oxidation (*6*). Thus, a thermal degradation experiment can be supplemental to an irradiation experiment. Moreover, adjuvant RPs may be identified using mass spectrometry (MS). If the cone voltages (collision energy) of MS is set to match the energy (irradiation dose) delivered to the adjuvant, then it may be possible to use the mass spectra for the adjuvant to determine the likelihood of fragments that may be formed from its oxidation (*6*).

## Conclusion

The U.S. Food and Drug Administration is responsible for regulating the use of irradiation in the treatment of food and food packaging materials. Several food packaging materials that are subjected to irradiation incidental to the radiation treatment and processing of prepackaged foods are listed in 21 CFR §179.45. Additional food packaging materials that are recently approved via a TOR exemption expands the 21 CFR §179.45 list to include all authorized FCSs for use in articles to be irradiated, incidental to the radiation of prepackaged foods, when the process meets 21 CFR §179, at doses not to exceed 4.5 kGy,

under an oxygen-free environment or while frozen and contained under vacuum. Packaging materials that are not yet authorized for holding food during irradiation require premarket approval via the FCN or TOR exemption process.

Evaluating the suitability of packaging materials, in particular those that require oxygen to maintain food quality, relies on the identities, quantities and dietary exposures to the RPs. A step-wise approach as described in this chapter is recommended for ORPs. However, the agency is always open to receiving new approaches for the analysis of RPs.

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Chapter 13

# Development of Controlled Release Packaging Technology

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Controlled release packaging (CRP) is an emerging technology by which active compounds such as antimicrobials or antioxidants are first incorporated into the package and then released to the food in a controlled manner to inhibit microbial growth, lipid oxidation, or other food deteriorations, thereby extending the shelf life of the food product. This chapter introduces the CRP technology, provides scientific evidence to support its technical soundness, and suggests a conceptual framework for its further development.

## Introduction

Controlled release packaging (CRP) is a new generation of packaging materials or systems that can release active compounds such as antimicrobials and antioxidants at desirable rates to extend the shelf life of food products. CRP has the ability to provide a sustained supply of active compounds at suitable rates for food protection. Traditionally, active compounds such as antioxidants, antimicrobials, and anti-browning agents are incorporated directly into the food formulation; however, once these active compounds have been consumed in reactions, protection ceases and food quality degrades rapidly. CRP can overcome this limitation by continuously replenishing the active compounds via controlled release from the package to provide sustained food protection.

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Another advantage of CRP over the traditional method of adding active compounds directly to the food formulation is that a smaller quantity of active compounds may sometimes be used to provide the same or better level of food protection. For example, microbial problems are known to occur mostly on the food surface, and CRP has the advantage of releasing antimicrobials targeted to the food surface where microbial inhibition is most needed. Adding antimicrobials directly to food is less effective and may result in overloading, since most of antimicrobials added using this method end up inside the food where microbial growth is of less concern.

Still another advantage of CRP is its ability to protect unstable active compounds from degradation until they are released. For example, when tocopherol (a common antioxidant) and nisin (a common antimicrobial) are added to the food formulation, unused amounts of these active compounds may undergo rapid degradation resulting in significant loss and thus a much reduced level food protection at later time. Our experimental data indicate that CRP can prevent this problem by storing tocopherol and nisin inside the package and thus protecting them from degradation until their release to the food.

CRP is a type of active packaging system that uses the package to deliver active compounds in a controlled manner. Active packaging may be defined as a group of technologies that actively modify the internal package environment through physical, chemical, or biological interactions between the package, the food, and the headspace for the purpose of enhancing food quality and safety (*1*). CRP modifies the internal package environment by releasing active compounds in a controlled and desirable manner. The CRP technology is particularly useful for controlling food degradation reactions that are continuous and increase exponentially, such as microbial growth and lipid oxidation, as constant replenishment of inhibitory active compound can prevent these runaway deterioration processes.

## Useful Terms for CRP

The following terms are useful to understand the concept of CRP. 'Instant addition' means adding the entire amount of active compound to the initial food formulation. 'Slow release' means releasing the active compound over a period of time, either in a controlled or uncontrolled manner. 'Controlled release' is a special form of slow release, in which the active compound is released over time in a controlled manner. Controlled release may be achieved in experiment using a precision device such as a computer controlled syringe pump to release the active compound at predetermined rates, or it may be achieved in practice by using the technology of controlled release packaging. 'Controlled release packaging' is a sophisticated form of controlled release that uses the package as a delivery system to release the active compound in a controlled manner. The ability to control the release is achieved by quantifying the functional relationships in the conceptual framework described later in this paper and using these functional relationships to aid the design of CRP systems.

## Scientific Evidence Supporting the Concept of CRP

This section provides two examples of scientific evidence, one relating to lipid oxidation and the other relating to microbial inhibition, to support the soundness of the CRP concept.

As the first example, Zhu et al. compared the effects of instant addition versus four different controlled release rates of tocopherol on the lipid oxidation of linoleic acid (*2*). For instant addition, an appropriate amount of tocopherol was added to linoleic acid to achieve an instant concentration of 300 mg tocopherol per kg linoleic acid (hereafter shorten as 300 mg/kg). For controlled release, four release rates of 30, 50, 75, and 100 mg tocopherol per kg linoleic acid per day (hereafter shorten as 30, 50, 70, and 100 mg/kg/day, respectively) were achieved by using a computer controlled syringe pump system to precisely add or "release" the appropriate amounts of tocopherol to linoleic acid over time. Tocopherol was continuously added at these rates until the final concentration reached 300 mg/kg, which was the same concentration as that for instant addition. Lipid oxidation was monitored by the generation of conjugated dienes in linoleic acid at 40°C.

Figure 1 shows the release rates of 75 and 100 mg/kg/day resulted in longer induction periods or better antioxidation effects, while the release rates of 30 and 50 resulted in shorter induction periods or poorer antioxidation effects, than that of instant addition. Thus the results clearly show that the effectiveness of tocopherol depends on the release rate. There is also an optimum release rate or "target release rate" at 75 mg/kg/day.

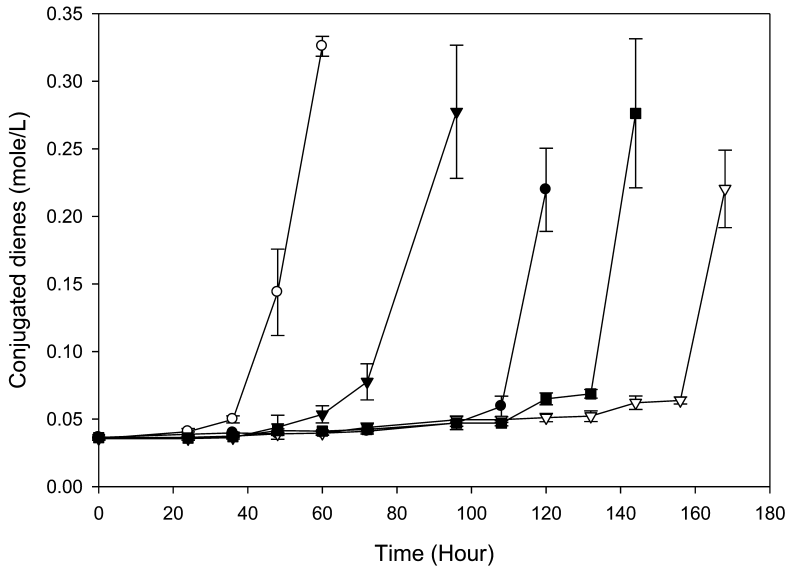


*Figure 1. Generation of conjugated dienes in linoleic acid under the conditions of 40°C, open-air, dark, and rotary shaking. Instant addition: (●) 300 mg/kg. Controlled release rates: (○) 30 mg/kg/day; (▾) 50 mg/kg/day; (▿) 75 mg/kg/day; ■ 100 mg/kg/day. Vertical bars are standard deviations.*

Table 1 reveals more insight of the data, in which the cumulative amounts of tocopherol added at the ends of the induction period are estimated. The release rate of 75 mg/kg/day performed better than the release rate of 100 mg/kg/day and the instant addition, although in all these cases the concentrations of tocopherol at the end of the induction period were the same at 300 mg/kg. The release rates of 30 and 50 mg/kg/day performed worse than instant addition, probably because the concentrations at the end of the induction period (at 44 and 121 mg/kg, respectively) were lower than the 300 mg/kg concentration of instant addition.

**Table 1. Delivery Rates and Tocopherol Added at End of Induction Period**

| *No.* | *Rate of delivery* | *Total tocopherol added* |
|---|---|---|
| 1 | Instant addition | 300 mg/kg |
| 2 | 30 mg/kg/day | 44 mg/kg |
| 3 | 50 mg/kg/day | 121 mg/kg |
| 4 | 75 mg/kg/day | 300 mg/kg |
| 5 | 100 mg/kg/day | 300 mg/kg |

As the second example to investigate the soundness of CRP, Balasubramanian et al. compared the effects of instant addition versus four different controlled release profiles of nisin on the microbial inhibition of *M. luteus* (*3*). The term "release profiles" is used here because the release rates were no longer constant as in the first example, but it decreases with time based on the law of diffusion. The controlled release profiles were to mimic the release of nisin from polymer films, in which the release rates were governed by the diffusion of nisin through the polymer films. In this study, the diffusivity D of nisin in polymer was used to quantify the release profile of nisin.

Figure 2 compares growth kinetics of *M. luteus* under the conditions of absence of antimicrobial, instant addition, and control release. The growth curve of the slowest release profile ($D = 1.53 \times 10^{-12}$ cm$^2$/s) did not cause a decrease in cell number. For growth curves of faster release profiles ($D = 6.12 \times 10^{-12}$ cm$^2$/s or above), complete inhibition of *M. luteus* was observed for at least 48 hours. Thus the minimum D value required to effectively inhibit microbial growth is about $6.12 \times 10^{-12}$ cm$^2$/s which corresponds to total amount of nisin released (0.227 μmol) or final concentration in the media ($1.14 \times 10^{-3}$ μmol/mL) after 48 hours. 0.227 μmol is equal to 15% of the amount used for the best result (1.49 μmol) obtained from instant addition. Since the growth curve of instant addition shows that inhibition was not sustained after 12 hours, the delivery of antimicrobial was more effective using controlled release than instant addition.

This observation is more striking when considering controlled release may use only 15% nisin to achieve better results than instant addition of 100% nisin. The results show that the release profiles used in this study are highly effective.

The fast initial rates of these profiles are necessary to provide lethal stress to kill or injure the cells, while the subsequent slower rates with persistent release of small amounts of nisin are sufficient to suppress recovery of the injured surviving cells. Thus the combination of initial fast rate and subsequent slower rate provides good overall microbial inhibition.

Further support of the CRP concept can be found elsewhere (*4*, *5*).

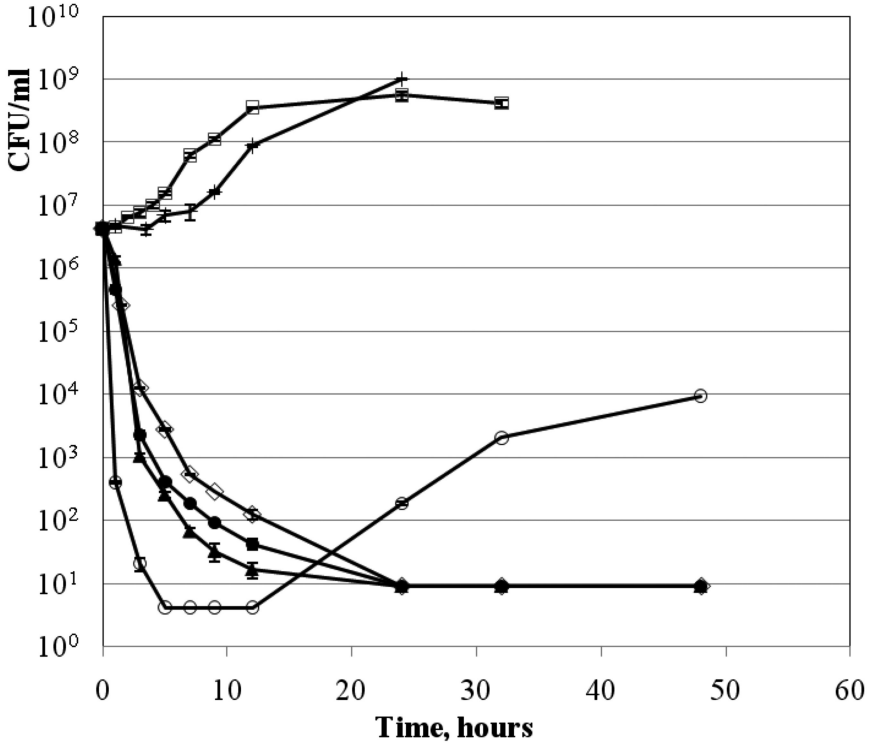


*Figure 2. Effect of controlled release profile of nisin on growth of M.luteus in 200 mL TSB at 30°C. (□) cultures in absence of nisin (control), (○) growth of M. luetus with instant addition of 7.45x10$^{-3}$ µmol/mL of nisin; (▲) growth for diffusivity of 1.53x10$^{-10}$ cm$^2$/s, (●) growth for diffusivity of 3.83x10$^{-11}$ cm$^2$/s, (◊) growth for diffusivity of 6.13x10$^{-12}$ cm$^2$/s, (+) growth for diffusivity of 1.53x10$^{-12}$ cm$^2$/s. Standard error was calculated based on plate count from 8 plates.*

## Conceptual Framework for CRP Development

CRP is as an emerging technology being developed by our and other laboratories around the world (*6*). The major challenge for CRP is to deliberately control the release of active compounds at rates suitable for a wide range of food products and specific degradation reactions, since there is a lack of fundamental understanding of the factors governing the release of active compounds from

packaging materials. A systematic approach based on the conceptual framework in Figure 3 has been developed to elucidate the relationships between the important variables in CRP systems. Four groups of variables are identified in this conceptual framework. The first three groups ('process variables', 'structure variables', 'property variables') are related to 'package research' and development. The process variables are those that can be manipulated directly by the designer to develop CRP packages. The structure variables and property variables are those package variables that cannot be manipulated directly; however, once the process-structure-property relationships are established, desirable package properties such as release behavior of active compound can be obtained by properly manipulating the process variables. The fourth group of variables ('food variables') is related to 'food research' to determine the 'target release rate' necessary to develop CRP systems.

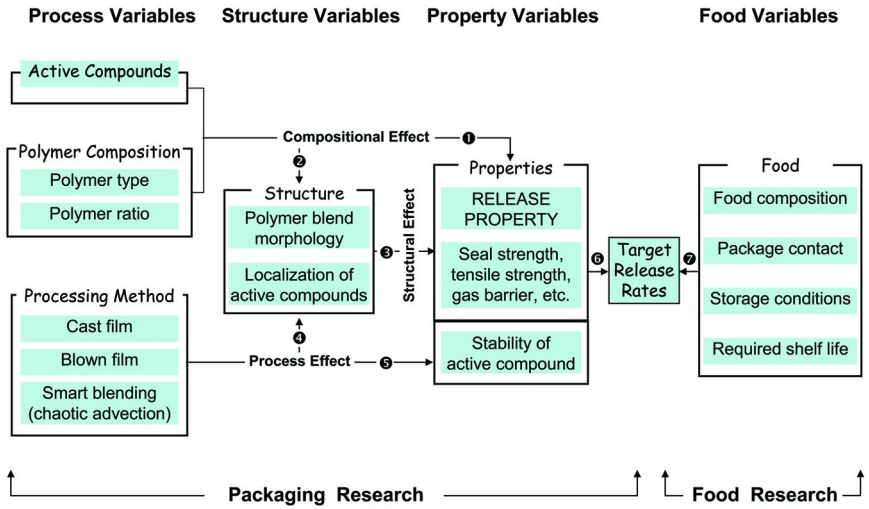


*Figure 3. Conceptual framework for development of CRP.*

The conceptual framework is a research roadmap for the systematic study of these variables and the relationships between composition, processing, structure, and properties. For example, proposed in this framework are three types of effects contributing to the observed properties: compositional effects, processing effects, and structural effects. As shown in Figure 3, the 'compositional effects' depend on the active compounds and polymer composition (polymer type and polymer ratio), which can affect properties either directly (via pathway **1**) or indirectly through its influence on the film structure (via pathways **2** and **3**). The 'processing effects' depend on the processing method, which can affect the structure and properties (via pathways **4** and **3**) and the stability of active compound (via pathway **5**). The 'structural effects' depend on compositional effects and processing effects, which can affect the release and other physical properties (via pathway **3**), although probably not the stability of active compound.

## Process Variables

The process variables have three sub-groups relating to active compounds, polymer composition, and processing methods, which can be manipulated directly by the designer to achieve a package with the desired properties.

### *Active Compounds*

The first consideration is to select effective food grade active compounds suitable for the application; for example, antimicrobials are used to inhibit microbial growth, and antioxidants are used to retard oxidation. Other possible active compounds include enzymes, flavors, nutraceuticals, etc. Sometimes two or more active compounds may be used to provide the desired results. Natural compounds (e.g., tocopherol extracted from nature sources and thymol extracted from essential oils) are preferred over synthetic compounds. Two or more active compounds may be used when necessary.

The second consideration is whether the active compound is volatile or nonvolatile, because the active compound must make contact with the food before it becomes effective. Volatile compounds such as sesamol or butylated hydroxytoluene (BHT) are necessary for products that do not have direct food package contact, such as breakfast cereal in a plastic bag. They are first released from the package, then vaporized into the headspace, and finally condense onto the food surface. Non-volatile compounds such as tocopherol and nisin may be used for products that have direct food package contact, such as pouches containing meat and gravy.

The third consideration is the release kinetics of active compound, which depends on the interactions between the active compound, the package, and the food. The effectiveness of active compounds can be greatly influenced by the quantity released to the food and the rate at which this takes place.

### *Polymer Composition*

'Polymer composition' refers to the film composition and its structure. A simple film may have a single layer consisting of a single polymer. A sophisticated film may have more than one layer, with each layer consisting of two or more polymers in which the ratio of polymers may be varied. The combination of different layers and polymers allows the production of films with different release rates suitable for the wide range of foods in the market.

The evaluation of estimated diffusion coefficients using single polymer types assisted the realization that a range of release rates could be achieved by combining two or more individual polymers at different ratios in a polymer blend. A wide selection of release rates can be obtained by varying the polymer type and polymer ratio.

*Processing Methods*

The methods of producing CRP films can greatly affect film structure and properties. The most common commercial processes of producing packaging films are the cast film and blown film processes, which involve melting a polymer resin, extruding the polymer melt through a die, and stretching and cooling the polymer melt into a film. Depending on processing conditions such as the feed rate, screw speed, barrier temperature, and extruder configuration, CRP films with significantly different properties may be obtained. When two or more polymers are used to form a polymer blend film, an innovative processing method known as smart blending technology based on the principle of chaotic advection may be used to produce polymer blend films with different film morphologies (*7*). The different film morphologies can greatly influence the release of active compounds and thus a wide range of release rates may be obtained for different food applications. Lamination, coextrusion, solution casting, and coating are also processing methods that can significantly influence release kinetics and other film properties.

**Structure Variables**

Structure variables can be controlled by the composition effects (via pathway **2**) and processing effects (via pathway **4**).

*Polymer Blend Morphology*

When two or more polymers are used to produce a blend film, the film morphology becomes an important variable. The term polymer blend morphology is used here to describe polymer film structures at microscopic level, observable by scanning electronic microscope, and displaying distinguishable phases that are formed by two or more immiscible polymers.

*Package Structure*

Package structures are related to package design. For example, a design may involve a packaging material of a three-layer structure, the outer layer consisting of a gas barrier polymer, the middle layer containing the first active compounds, and the inner layer containing the second active compounds. By manipulating factors such as loading of active compounds and thickness of layer, different release rates can be obtained.

*Localization of Active Compounds*

When there are two or more immiscible phases in a polymer blend film, localization refers to distribution of active compound in these phases, which can greatly influence the release of active compounds. The release of a compound from a polymer film to a food simulant involves three steps: molecular diffusion within the polymer film toward to the film/food interface, mass transfer across the interface, and dispersion into the bulk food. In most cases, diffusion is the rate controlling step due to the high diffusion resistance in the polymer matrix. Diffusion of active compounds through two immiscible phases in CRP depends on many factors: size, shape, and distribution of active compounds in the polymer matrix; polymer morphologies including density, crystallinity, tortuosity, degree of cross linking and branching, and glass transition temperature; molecular interactions of the compounds and host polymers; thermodynamic properties such as polarities and solubility; and the nature of the food or food simulant in the package. With high loadings, the active compound may also reside in the interfacial zone. In addition, sorption of solvent molecules by the film may result in an increase in free volume and swelling of the polymer film, leading to an increase in diffusion coefficient of the active compound in the film. This swelling may be both an advantage and a disadvantage. A better understanding of the factors affecting diffusion will provide many ways to tailor the release of active compounds for a wide range of food packaging applications

## Property Variables

The desired properties can be directly obtained by varying the polymer composition (pathway **1**), by varying the structure through composition and processing (pathway **3**), or by varying the processing method and conditions (pathway **5**).

*Release Properties*

The ability to release active compounds is the most important property of CRP packages. The release rate of antimicrobials or antioxidant should properly match the microbial or oxidation kinetics of the food and the shelf life requirement. Since different foods have different requirements, it is necessary to have the ability to produce CRP materials of different release rates for a wide range of food products. The release of active compounds such as tocopherol and nisin may be studied using the single-sided diffusion cell developed in our earlier studies (*8*). Release of active compound may be measured as a function of time and temperature. Partition coefficients of active compound between food simulants and polymer films may be determined, and 'overall diffusion coefficients' of active compound in the film may be calculated using the Fickian or non-Fickian models as appropriate (*9*).

*Other Film Properties*

Other important properties for CRP film include heat seal ability, ability to be laminated, tensile strength, and gas permeability. The active compounds may act as plasticizers that decrease mechanical properties and gas permeability, usually slightly since concentrations of active compound are low. These decreases are not a concern in situations when CRP is used as a functional layer in a multilayer structure, with other layers (such as an aluminum foil) that provide strength support and gas barrier.

**Food Variables**

Food variables include food composition, food/package contact area, storage condition, shelf life requirement, and other factors. For example, food composition determines whether antimicrobials or antioxidants or both are required for food stability; whether the food is in solid or liquid form determines whether a volatile or a non-volatile active compound is suitable; storage conditions and shelf life requirements determine how much and how fast active compounds need to be released. The study of food variables involves what the conceptual framework describes as 'food research'. Scientific research is needed to study microbial and oxidation kinetics of food as a function of slow or controlled release of active compounds since most data of microbial and oxidation kinetics in the literature are conducted under the condition of instant addition. For more sophisticated CRP systems, studies of kinetics for more than one active compound may be needed.

**Target Release Rate**

'Target release rate' is a new concept to connect the packaging research and food research in the conceptual framework (pathways **6** and **7** in Figure 3). The successful development of CPR food packages requires the collaboration of a packaging engineer and a food scientist. The packaging engineer is responsible for producing the package, and the food scientist is responsible for making sure that the package serves the purpose of extending the shelf life of the food. To design the package, the packaging engineer needs to know the target release rate of active compounds. The food scientist needs to provide this target release rate based on the knowledge gained from the food research in the conceptual framework. After the target release rate is provided, the packaging engineer can then design and produce the package based on the knowledge gained from the packaging research in the conceptual framework

The target rate release describes how fast and how much active compound should be released for a particular food application. The rate of release is not constant since the release of an active compound from a packaging film is usually controlled by diffusion of the active compound in the film, characterized by fast release initially followed by progressively slower release as time passes. Determination of the target release rate is challenging since it involves many factors and considerations (*10*).

## Potential Food Applications

Controlled release packaging has great potential to improve food quality and safety. CRP using antimicrobials may be used for short or intermediate term microbial inhibition for highly perishable foods such as fresh meats, seafood, fruits and vegetables. CRP using antioxidants may be used for long-term retardation of lipid oxidation for shelf stable foods such as ready-to-eat meals containing fatty components susceptible to oxidation. Usually, fast release rates are suitable for antimicrobials, while slow release rates are suitable for antioxidants. Benefits of CRP in food application may include: 1) Slow release of antimicrobial and antioxidants can provide sustained protection against microbial growth and lipid oxidation; 2) Antimicrobial and antioxidants can be released from packaging material onto food surface, where most contamination and lipid oxidation occur; 3) Less amount of chemical preservatives may be needed if CRP is used. However, there are also some limitations regarding the application of CRP technology. For example, some antimicrobial and antioxidants are heat sensitive. Therefore, there might be limited choices of antimicrobial and antioxidants can be used for CRP if the packaging film is made by extrusion.

## Safety Considerations

Two safety considerations are important for the development of CRP systems. First, there should be no significant amounts of harmful degradation products resulting from the incorporation of the active compound into the polymer materials. For example, if the active compound is incorporated into a polymer film using the extrusion process, the high temperature and shear of the extrusion process may cause the active compound to degrade. Second, the presence of the active compound should not facilitate the migration of those additives already in the packaging material.

Lang used GC-MS and LC-MS to investigate the possible formation of degradation products resulting from the incorporation of tocopherol into polyethylene films under severe extrusion processing conditions (*11*). The GC-MS analysis found only polymer additives, with no volatile degradation products formed from tocopherol. The LC-MS analysis provided tentative identification of two tocopherol dimers and tocoquinone—these are previously documented degradation products of tocopherol which have not shown to have harmful effects. Overall, the study concluded that there was no significant safety issue of incorporating tocopherol into polyethylene films using the extrusion process.

A CRP material is also considered as a food contact substance (FCS) and should comply with the FDA regulations before commercialization (*12*).

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Chapter 14

# Effect of High Pressure Processing on Migration Characteristics in Polymer Films

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The use of polymer-based flexible packaging materials has allowed application of high pressure processing (HPP) to pre-packaged food products. Many of these materials have been shown to withstand different HPP conditions without significant loss of physical and mechanical properties. There are, however, still substantial gaps in scientific information surrounding the effect of pressure on food and packaging interactions. If HPP promotes migration of additives and other residual contaminants into foods in direct contact with the materials, it could possibly become a concern to public health. This chapter reviews the results of published research concerning (1) changes in morphological properties of the polymeric packaging materials under high pressure conditions and (2) HPP effects upon chemical migration from packaging materials and sorption of food constituents (flavor scalping) into packaging.

## Introduction

High pressure processing (HPP) is readily gaining prominence worldwide as an alternative method of food preservation due to its benefits of improved quality, freshness and nutrient retention. It consists of applying high pressure (typically in the 300-800 MPa range over a period of several minutes) to food to greatly reduce the number of microorganisms and also to deactivate enzymes by mechanical

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action. The use of polymer-based flexible packaging materials has allowed application of HPP to pre-packaged food products. Many of these materials have been shown to withstand different HPP conditions without significant loss of physical and mechanical properties (*1*, *2*). There are, however, still substantial gaps in scientific information surrounding the effect of pressure on food and packaging interactions. Further advancement in the industrial implementation has expanded to HPP at elevated temperatures (90–120 °C) to allow the achievement of microbial spore inactivation. If HPP promotes migration of additives and other residual contaminants into foods in direct contact with the materials, it could possibly become a concern to public health.

This chapter briefly describes: (i) principles of HPP, (ii) changes in morphological properties of the polymeric packaging materials under high pressure conditions, and (iii) HPP effects upon chemical migration from packaging materials and sorption of food constituents (flavor scalping) into packaging.

## Principle of High Pressure Processing (HPP)

There are two basic principles that describe the effect of high pressure. First, Le Chatelier's principle states that any phenomenon (phase transition, change in molecular configuration, or chemical reaction) which results in a decrease in total volume is enhanced by pressure (*3*). The second principle, known as the isostatic or Pascal principle indicates that pressure is transmitted in a uniform and quasi-instantaneous manner, independent of the size and geometry of the food or food packaging (*4*). The effectiveness of HPP is greatly influenced by the physical and mechanical properties of the packaging material because foods to be processed are generally packaged prior to treatment with HPP. The packaging material must be able to withstand the high pressure and severe stress regime associated with the treatment process, while maintaining sealing integrity and its barrier properties (*5*). At least one interface of the package should be flexible enough to compensate for the collapse of the head space and for the possible volume reduction of the food inside the package (*6*, *7*). Thus, rigid metal, glass, or plastic containers cannot be used. The headspace must also be minimized while sealing the package, in order to ensure efficient utilization of the package as well as space within the pressure vessel. This also minimizes the time taken to reach the target pressure (*8*).

High pressure processing can be carried out either by batch or by semi-continuous in-line systems. The proper selection of equipment depends on the characteristics of the food product to be processed. Solid food products or food with large solid particles can only be treated in batch mode. Liquids, slurries, or other pumpable products have the additional option of semi-continuous production (*9*). Currently, most high pressure machines in industrial plants used for food processing function as a batch process, whereby the product pre-packed in flexible containers is placed in a high pressure chamber, the vessel is closed, filled with pressure-transmitting medium, and pressurized either by pumping pressure-transmission medium into the vessel or by reducing the volume of the pressure chamber using a piston. Once the desired pressure is achieved, the

pump or piston is stopped, the valves are closed and the pressure is maintained without any further energy input. After the required time has elapsed, the system is depressurized, the vessel opened and the product unloaded.

Figure 1 schematically shows temperature and pressure profiles during HPP. The typical HPP process cycle includes preheating time, come-up time, holding time, and come-down time, followed by a period of cooling. In general, high pressure pasteurization is performed at an initial temperature of 25 °C, while sterilization is conducted at a higher initial temperature which, depending on the food, is in the range of 60–90 °C (*10*). Since the adiabatic pressurization process determines a monotonic increase of the temperature, the actual treatment temperature is dependent on factors such as initial temperature, final pressure, and product composition. The temperature increase of food materials under pressure is dependent on factors such as final pressure, product composition, and initial temperature. The temperature of water increases about 3 °C for every 100 MPa pressure increase at room temperature (25 °C). On the other hand, fats and oils have a heat of compression value of 8–9 °C/100 MPa, and proteins and carbohydrates have intermediate heat of compression values (*11–13*).

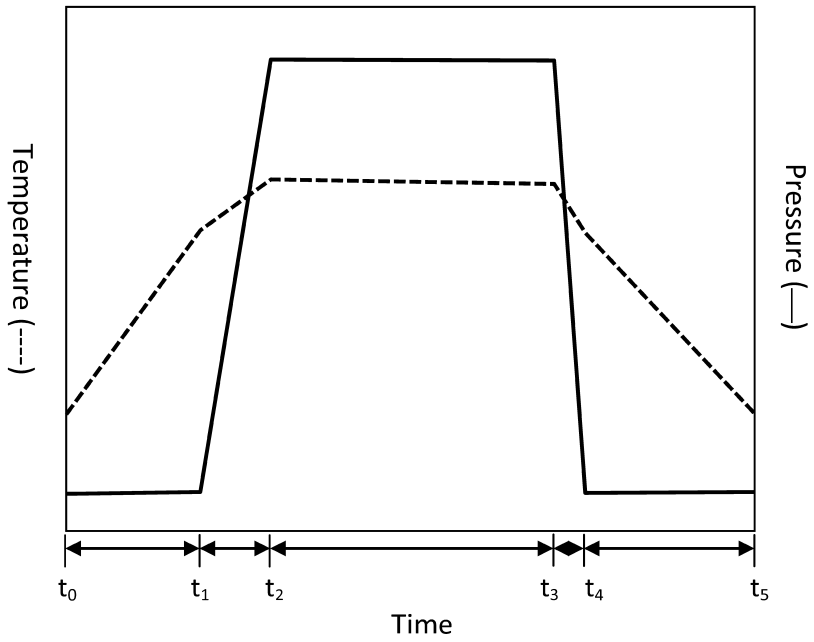


*Figure 1. Typical pressure-temperature response of food materials undergoing high-pressure processing. Pre-heating time, $t_0$-$t_1$; come-up time, $t_1$-$t_2$; holding time, $t_2$-$t_3$; come-down time, $t_3$-$t_4$; cooling time, $t_4$-$t_5$.*

## Chemical Structure and Morphology of Polymer Materials during HPP

HPP can have effects upon the main morphological properties of polymeric packaging materials such as the melting temperature ($T_m$) of crystalline regions, the glass transition temperature ($T_g$) of the amorphous regions, and the related changes in the amount of free volume of the material. Such morphological changes in the polymer can result in different migration behavior within the packaging. Crystalline regions of polymers have increased order of the macromolecular chains and reduced void spaces (free volume) relative to their amorphous regions. Highly crystalline materials of the same polymer type generally have an increased barrier to permeating gases and vapors, greater stiffness, and lower optical transparency.

Several researchers showed that a pressure enhancement promotes the polymers $T_g$ towards higher temperatures at higher pressures (*14*, *15*). Below their $T_g$, amorphous polymers (or amorphous regions within semicrystalline materials) are usually hard and brittle because of the low mobility of their molecular chains. Increasing the temperature induces molecular motion resulting in the typical rubber-elastic properties. The transition temperatures depend on the crystal growth rate and the number of crystals present, which are both a function of the local temperature. Crystal growth only occurs for temperatures below the $T_m$ and above the $T_g$ because higher temperatures destroy the molecular arrangement and below the $T_g$, the movement of molecular chains is frozen. Nevertheless, secondary crystallization can proceed even below $T_g$, in the time scale of months and years. With increasing pressure, the crystal growth rate distribution curve is shifted to higher temperatures without changing the shape of the curve or the degree of crystallinity of polymers (*16–18*). $T_g$ generally increases by 10 to 30 °C per 100 MPa depending on the polymer (*19*). Figure 2 illustrates the effect of pressure on $T_g$ for several polymer films commonly used for food packaging applications. These are experimental results obtained by using a high pressure mercury dilatometer (*20*, *21*). Although the maximum pressure accessible was 200 MPa, which is well below the maximum pressure normally used in HPP, these data provide a useful indication on how $T_g$ increases with pressure. Quantitative and qualitative differences are evident among different polymers.

A stronger pressure effect concerning the shift of $T_m$ to higher temperatures has also been observed in semi-crystalline polymers (*17*, *22*). Figure 3 illustrates the pressure dependence of the melting temperature for some commercial polymeric films widely used for food packaging applications, such as LDPE, LLDPE, PP, PLA, and PET (*16*, *20*). It is evident that pressure, up to a maximum of 200 MPa, promotes an upward shift of the $T_m$. Results from recent studies (*23*) using differential scanning calorimetry (DSC) analysis also showed that the $T_m$ of LDPE films used for food packaging increased with increasing pressure intensity from 200 to 800 MPa. Such important findings demonstrate the increase promoted by pressure on melting temperature could allow the use of the material for high pressure sterilization that would otherwise melt at those temperatures if high pressure was not applied. Mauricio-Iglesias and others (*24*) reported that LLDPE can undergo HPP treatments at temperatures as high as 110 °C without occurrence of melting.

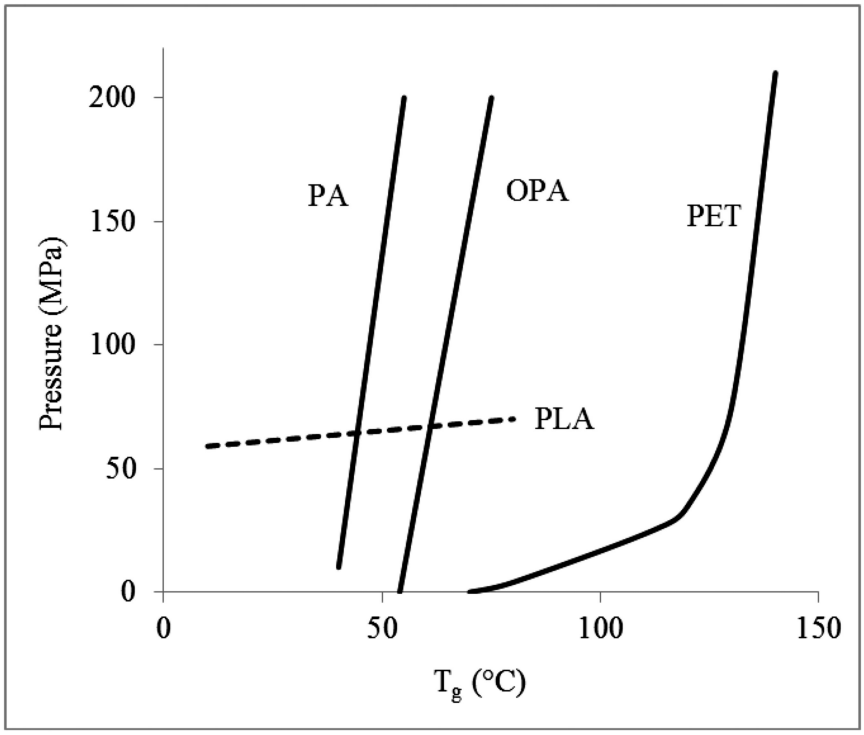


*Figure 2. Dependence of $T_g$ of PA, OPA, PLA and PET on pressure as detected from high pressure dilatometric experiments. Data from Sansone (20) (—) and Grassia et al. (21) (----).*

It is known that the crystalline region has a much smaller temperature and pressure sensitivity than that of the amorphous region (*25*). Most polymers utilized in food packaging applications are semi-crystalline, having both crystalline and amorphous regions. Only the amorphous regions can be penetrated by the diffusing substance. In amorphous polymers, the molecular chains are mobile, thus forming transient 'free volumes' that are used by diffusing particles to travel in a tortuous path within the polymer. A rise in temperature increases the free volume of the polymer, whereas a rise in pressure decreases these same void spaces (*26*). Therefore, it is expected that the complex temperature-pressure histories can promote changes of density of the sole amorphous regions of semi-crystalline polymers in the same way as it would occur in a completely amorphous polymer. In particular, changes in density are related to changes in free volume of the polymer that, in turn, affect diffusivity and solubility of low molecular weight (MW) compounds within the polymer (*15*). Grassia and others (*21*) used a high pressure volumetric dilatometer and tested density change of

PLA under high pressure histories up to 500 MPa analogous to those actually imposed during HP sterilization (90 °C for 5 min) and pasteurization (36 °C for 5 min) food processing conditions. Results from the studies showed that the degree of crystallinity did not change in the temperature and pressure ranges of interest. Polymer density increased with pressure, but there were no significant changes in density before and after HPP treatments, which implies that this effect recedes as the pressure is released unless irreversible structural changes occur in the polymeric material. Schmerder and others (*27*) reported that the 47% amorphous fraction of Nylon 6 measured by DSC was unchanged before and after pressure treatments up to 200 MPa at 20–60 °C. Results of DSC experiments using LDPE films also showed that the crystallinity changes were not detectible after HPP at 200, 400, 600, and 800 MPa for 5 and 10 min at 25 and 75 °C (*23*). They observed that HPP reduced the volume of the polymer by compressing its amorphous regions when measured using X-ray diffraction method.

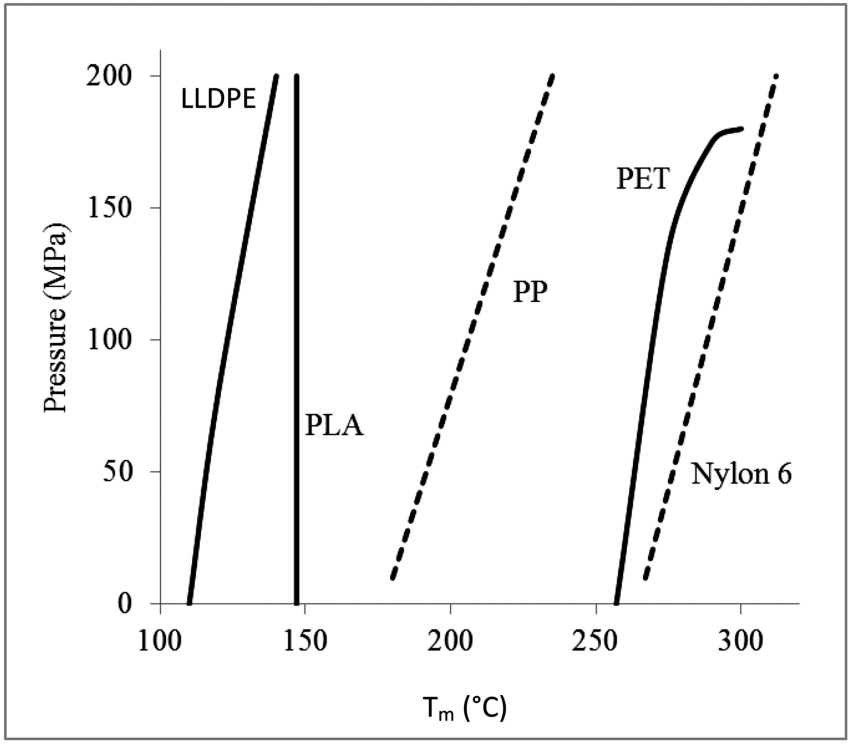


*Figure 3. Dependence of $T_m$ of LLDPE, PLA, PP, PET, and Nylon 6 on pressure as detected from high pressure dilatometric experiments. Data from He and Zoller (16) (----) and Sansone (20) (—).*

When there are low MW substances inside the package or in the HPP vessel fluid, the behavior with pressure could be more complex because the dissolution and sorption of the low MW compound within the polymer itself could promote the plasticization of the material, resulting in a decrease of $T_g$ and overall crystallinity (*15*). To further complicate the matter, the sorption of low MW compounds is, in turn, affected by both the treatment temperature and pressure. This issue is particularly relevant since the combination of the effects by pressure and by the action of sorbed low MW compounds could promote an increase of brittleness of the originally rubbery polymers or a plasticization to the rubber state of originally glassy polymers (*28*). Recent studies from X-ray diffraction measurement by Dhawan and others (*29*) show that there was a significant decrease in overall crystallinity of multilayer EVOH films after HPP sterilization treatments caused by the disruption of the crystalline structure due to the plasticization of the EVOH layer in contact with water under high pressure. The decrease in crystallinity of the film lead to a reduced orderliness in the polymeric chains, which caused an easier path for the gas to travel through the polymer matrix. As a result, reduced oxygen and water vapor barrier properties and eventual quality deterioration of the food were observed (*23*).

## Global Migration

Concerning chemical migration from packaging materials into food as a result of high pressure processing, the first study found in the literature was from Ochiai and Nakagawa in 1992 (*30*). They evaluated total migration on five different types of laminated plastic pouches filled with four food simulating liquids (FSL) including water, 4% acetic acid, 20% ethyl alcohol and n-heptane. Extraction tests after pressurization at 400 MPa for 30 min at 20 °C or 60 °C indicated that HPP treatment did not significantly affect migration.

Even though the HPP conditions employed were different, many researchers have observed similar results with other packages. Mertens (*6*) determined the effect of HPP on migration of two multilayer films (LLDPE/EVA/EVOH/EVA/LLDPE and PET/AL/PP). Sheets of film were treated at 400 MPa and 60 °C for 30 min and their overall migration into olive oil was compared with those for films that had received the same thermal treatment at atmospheric pressure. The results showed that HP does not result in a significant increase of the overall migration in both films. Global migration varied from 4.6 and 2.6 mg/dm$^2$ before treatment to 3.5 and 2.9 mg/dm$^2$ after treatment. Pastorelli (*31*) measured total migration from PE/EVOH/PE and PET/AL/PP multilayer packaging materials using different simulants (water, 5% acetic acid, 20% ethyl alcohol and n-heptane) after treatment at 400 MPa for 30 min at 25 °C. They reported that overall migration of migrants or by-products were not significant. These materials complied with the requirement that plastic materials in contact with foodstuffs should not transfer migrants in quantities over the EU legislation limit of 10 mg/dm$^2$ of surface area to food (*32*). Lambert and others (*33*) also

studied the effect of high pressure on migration characteristics in food packages in contact with different simulants (water, 3% acetic acid, 15% ethyl alcohol, and olive oil) for 10 days at 40 °C. The overall migration was analyzed on six different types of multilayer structures subjected to high pressure pasteurization (500 MPa, 30 min, 20 °C) such as PA/PE, PET/PVDC/LDPE, PA/PE-SY, and PA/PP/LDPE. All the values of global migration for each package treated with the four simulants were below 10 mg/dm$^2$ and unchanged before and after the HPP treatment. The results show quite clearly that the selected packages are not affected by high-pressure treatment and could be used for high-pressure food processing. They highlighted that global migration of the packages does not seem to be correlated with the thickness of the film, the nature of the materials, or the fabrication process.

In contrast, several researchers have reported significant changes in global migration after HPP treatments. Dobias and others (*34*) examined migration characteristics of seven single material films (50-µm PE, 70-µm PE, anti-fog PP, CPP, BOPP, 60-µm SY, 70-µm SY) as well as seven laminates (100-µm PA/PE, 90-µm PA/PE, 80-µm PA/PE, LDPE/EVOH/LDPE/APET/PET/ APET, PE/PA/EVOH/PE, PET/PE/EVOH/PE, LDPE/PA/LDPE) suitable for high pressure preservation of food or commonly used as food contact layers. Strips of polymeric materials were processed at 600 MPa for 60 min at room temperature with distilled water as a pressure medium, and stored in 95% ethanol for 10 days at 40 °C and in isooctane for 2 days at 20 °C. Considering levels of overall migration into both fatty food simulants, relatively significant changes due to high pressure were determined for most of the tested samples. After the HPP treatment, global migration into 95% ethanol decreased by 16-79% for 50-µm PE, anti-fog PP, 60-µm SY, 80-µm PA/PE, and LDPE/EVOH/LDPE/APET/PET/ APET, but increased by 5-107% for other polymer materials. Likewise, global migration into isooctane decreased by 10% for 50-µm PE, but increased by 5–300% for anti-fog PP, BOPP, 60-µm SY, and 70-µm SY materials compared after HPP. For all of the laminates, global migration into isooctane increased as much as 102% compared to untreated control. These results confirmed the migration properties of tested packing films were substantially influenced by high pressure treatment.

Recently, Galotto et al. (*35*) investigated the effect of HPP on the total migration into water (aqueous food simulant) and olive oil (fatty food simulant) from four packaging materials (PE/EVOH/PE, mPET/PE, PET/PE and PP-SiOx). Pouches made from these films were filled with food simulants, sealed, and then processed at a pressure of 400 MPa for 30 min at 20 or 60 °C. Pouches kept at atmospheric pressure were used as controls. They observed that HPP decreased the total migration into water and the values for all the HPP treated pouches were below 0.5 mg/dm$^2$. On the other hand, the total migration into oil after HPP treatment was significantly higher compared to the control. This was because when in contact with oil during the HPP, the oil absorbed by the pouches acts as a plasticizer and, thus, films undergo significant changes in structure such as swelling, delamination, pinhole formation, and cracking. In general the total migration from the HPP treated pouches was lower at 60 °C than at 20 °C.

## Specific Migration

Limited effects due to HPP have been observed for the specific migration of several organic compounds from packaging materials. Goetz and Weisser (*36*) developed an *in situ* method by using a high pressure permeation cell and measured permeation of an aromatic organic compound, p-cymene (0.25% v/v), through packaging materials during high pressure treatments at 23 °C and 50 MPa. They observed a slight decrease of p-cymene permeation rate with increasing pressure in LDPE/HDPE/LDPE and PET/Al foil/LDPE multi-layer films, which was ascribed to a shift of glass transition of the polymers to higher temperatures. The extent of permeation and migration was found to depend on pressure and time, and some reversible structural changes in the polymers were also detected.

Schauwecker et al. (*7*) investigated migration of 1,2-propanediol (PG), a pressure fluid often used in batch HPP, through selected films exposed to high pressure. Pouches made from 142-µm thick PET/PA/AL/PP and 75-µm thick PA/EVOH/PE films were filled with 95% ethanol and then sealed. The packages were processed at 400, 600 and 827 MPa at 30, 50 and 75 °C for l0 min and stored at 40 °C for 10 days. Controls were processed at the same temperatures and duration at atmospheric pressure. No detectable PG migration was found in the PET/PA/AL/PP either before or after pressure treatment. However, PG migration in EVOH pouches significantly decreased, which may be attributed to a reduction in the void spaces within the material. At 75 and 50 °C, the PG migration was significantly higher than at 30 °C.

Schmerder et al. (*27*) examined the influence of high hydrostatic pressure (60-400 MPa) on the permeation of an aromatic organic compound, raspberry ketone (4-(4-hydroxyphenylbutan-2-one)), in 10% ethanol through Nylon 6 films at temperatures of 20, 40, and 60 °C. The permeated amounts of raspberry ketone during high pressure and after high pressure were measured by a 'bag-in-bag' method (*37*) and by an *in situ* permeation cell technique (*36*), respectively. Permeation was lowered by increasing pressure at all temperatures. At 23 °C, the increasing pressure correlated with the decreasing permeation and diffusion coefficients. Pressure and temperature acted antagonistically to each other. The decrease in permeation at 200 MPa was compensated for by a temperature increase of 20 °C. Measurement of the amorphous part of Nylon 6 by DSC suggested that crystallinity was not affected by increases in pressure. Therefore, it appears that the reduced permeation under high pressure is caused by hindered diffusion through the decreased 'free volume' in the polymer matrix. After release of pressure, the original permeation coefficients were recovered due to relaxation of the polymer chains, which suggests that the structural changes that occur in the material are reversible (*21*).

The specific migration of an actual packaging additive, Irganox 1076, from PP pouches containing either 10% or 95% ethanol was first studied by Caner and Harte (*38*). HPP was carried out for 5 and 10 min at temperatures of 40 and 60 °C at 800 MPa. Following processing, whether under HPP or 1 atmosphere, all samples were stored at 23 °C for up to 20 days. Results from the study showed that migration into foods is likely, especially if there is a long contact period. No significant difference in the migration level of Irganox 1076 was observed after HPP treatment

into either food simulant compared to the controls. Migration from the PP film into 95% ethanol was significantly greater than into the 10% ethanol. An increase in temperature during HPP was shown to yield to an increase in Irganox migration but they did not investigate if the effect of temperature was lowered or accentuated by pressure. Overall, the data indicated that migration from a monolayer PP material did not significantly increase as a result of HPP.

Mauricio-Iglesias et al. (*39*) investigated the migration of Irganox 1076 and Uvitex OB, used as an antioxidant and ultraviolet light absorber, respectively, from LLDPE and PLA films in contact with four FSL (water, 3% acetic acid, 15% ethanol, olive oil). Migration from sample film strips was assessed after two HPP treatments intended for pasteurization (800 MPa for 5 min at 40 °C) and a sterilization treatment (800 MPa for 5 min at 115 °C) and were compared with conventional pasteurization and sterilization, respectively. While LLDPE and PLA samples are not traditionally able to withstand the thermal sterilization process, no change in migration behavior or the sample's visual appearance was detected after high pressure sterilization; this is due to the shift of polymer $T_m$ to higher temperatures. For pasteurized samples, there were no differences between conventionally pasteurized and HPP treated samples. In the case of PLA, migration of Uvitex OB was very low or not detectable for all the samples studied.

Yoo et al. (*40*) also evaluated the influence of HPP on the migration of Irganox 1076 from LDPE films into 10 and 95% ethanol. Pouches manufactured from the LDPE films were exposed to pressures of 600 and 800 MPa for 5 min at 25 and 75 °C, and subsequently stored at 25 °C for up to 40 days. The results showed little difference in the migration of Irganox 1076 in 10% ethanol for all treatment conditions and all storage times up to 40 days. Higher levels of Irganox migration were observed from LDPE films into 95% ethanol for HPP treated samples, but the differences were attributed to processing temperature rather than pressure. Based on the 'free volume' theory, it would be expected that the smallest molecular volume migrants would be most affected by high pressure processing. Therefore, Irganox 1076 with its large molecular weight (531 g/mol) would be a poor surrogate compound to test the effect of HPP.

Concerning migration of volatile compounds, only a few studies were reported. Rivas-Canedo and others (*41–43*) ran a series of experiments and determined the impact of HPP on migration in three different food matrices subjected to the multilayer packaging material (LDPE/EVA/VDC). In fresh meats no significant migration of compounds from the plastic material was observed (*41*). Furthermore, compound concentrations absorbed from the packaging material into dry-cured Serrano ham were reduced by the application of high pressure (*42*). It was, however, remarkable that the level of migration of compounds from the plastic material, especially branched-chain alkane and benzene, was significantly higher when pressure was applied to dry-fermented sausage 'salchichón' (*43*). They speculated that the enhanced migration found in 'salchichón' is most likely due to the higher fat content of salchichón in comparison with other meat products. In general, the migration rate of a substance into the food matrix depends on the polarity and solubility of both the polymer and the food product (*44*). It has been proved that compound migration from polymers into meat products increases with the lipid content (*45*).

The lack of comprehensive studies of additive migration from the packaging material used to contain the food after treatment by HPP and the ambivalent results of such migration experiments has led the FDA to initiate research on the migration properties of materials after HPP to address the perceived gap in the scientific knowledgebase. Recently, Zhao (*46*) in collaboration with FDA conducted a systematic approach to measure the migration of surrogate compounds into 10% ethanol and Miglyol 812 from CPP films during and after HPP (71 °C and 700 MPa) and equivalent thermal processing (TP) for 5 min, and during subsequent storage at 25 °C for up to 10 days. Zhao successfully developed and applied a comparable TP system at atmospheric conditions to mimic temperature profiles of HPP. Four low MW surrogate compounds including chloroform, toluene, methyl salicylate, and phenyl cyclohexane were selected based on molecular weight, polarity, and volatility. Results from the study showed that HPP significantly decreased the migration of those surrogates into both Miglyol and 10% ethanol when compared to TP. After 5 min of HPP, migration of the surrogates into Miglyol was less than 55%, while migration reached 100% for all compounds after 5 min of TP. In 10% ethanol, total migration after TP and HPP were 9–51% and 8–23%, respectively, which indicated that HPP significantly decreased the migration rate of the selected surrogates from CPP film into 10% ethanol. The extended storage study was performed at 25 °C for up to 10 days. Overall migration of surrogates into 10% ethanol after HPP and TP were lower than those into Miglyol. Results from the extended storage study indicated that after initial 8–48 hours of storage, the differences in percent migration of the selected surrogates into both Miglyol and 10% ethanol were not significant among HPP treated, TP treated, and/or untreated film samples. In general, the data are in accordance with the previous observations made on the effect of HPP on structural and morphological changes in polymers— (1) the shift of the $T_g$ region of the polymer to higher temperatures, (2) the compression of void spaces within the material, and/or (3) quick recovery of polymers to its original state—and thus, migration after HPP treatment proceeds as expected at normal atmospheric pressure. The glass transition temperatures measured by DMA dynamic mechanical analysis for untreated and HPP/TP treated PP films showed no significant difference, which supports the concept that physical changes of CPP film that may occur during HPP are reversible.

## Sorption of Food Constituents (Flavor Scalping)

All the experimental results reported so far in the literature highlight that the application of high pressure decreases the free volume of the polymer resulting in a decrease of sorption and diffusivity during treatment. Masuda and others (*47*) investigated the sorption of d-limonene into different flexible plastic films as a result of treatment at 400 MPa and 20 °C for 10 min. An increase of d-limonene sorption was observed in LDPE, CPP, and EVA film samples after HPP. However, the sorption into high barrier materials such as

EVOH, PET, and Nylon were not greatly affected by HPP. Ludwig et al. (*48*) evaluated interactions between ethanolic solutions of *p*-cymene and acetophenone and different polymer packaging materials at 500 MPa and 25 °C for up to 60 hours. The p-cymene solution, filled into LDPE bags, lost 30% of its aroma concentration after 24 h at 500 MPa compared to a 60% loss at atmospheric pressure. Using LDPE/HDPE/LDPE bags, aromatic compound losses were 20% and 30%, respectively. In PET/AL/LDPE bags impermeable to p-cymene, sorption was confined to the inner LDPE layer and caused higher loss in the non-pressurized sample. Acetophenone has not been found to interact with those materials during pressure treatment, while at atmospheric pressure sorption loss in aroma concentration was nearly 70% after 60 hours.

Kubel et al. (*37*) investigated the effect of HPP on the sorption of aroma compounds, *p*-cymene and acetophenone by flexible polymeric films (LDPE/HDPE/LDPE, PET/AL/LDPE and HDPE). Internal pouches (70 × 12 mm) and external pouches (205 × 15 mm) were prepared. The internal pouches were filled with 2 mL of p-cymene in 10% ethanol (0.25% v/v) or acetophenone in 33% ethanol (0.33% v/v). Each internal pouch was placed into an external pouch containing 3 mL of ethanol solutions and then heat-sealed. After pressure treatment in the range of 0.1 to 450 MPa, sorption rates were measured as a function of pressure level using UV spectroscopy. It was observed that the concentration of *p*-cymene and acetophenone were lower in the high-pressure treated films than under atmospheric pressure. The researchers suggested that the transition of the films to the glassy state at higher pressures was the reason for the decrease in the sorption of the aroma compounds.

Caner et al. (*49*) compared the sorption behavior of selected polymer films after HPP treatment. Pouches made from the films were filled with an aqueous solution of 10% ethanol or 3% acetic acid containing 165 ppm d-limonene. The pouches were heat-sealed and then processed at 800 MPa for 10 min at 60 °C. They observed that d-limonene concentration, in both PP and PE/PA/EVOH/PE films and the food simulants, was not significantly affected by HPP. However, the met-PET/EVA/LLDPE film showed lower d-limonene sorption as compared to the untreated control pouches. Sorption was also affected by the acidity of the FLS. Using acetic acid to lower the pH altered the solubility of d-limonene in all the tested materials.

Recently, researchers have shown a significant effect of the HPP/high temperature sterilization on scalping, in some cases contrary to the results reported in the aforementioned studies. Mauricio-Iglesias et al. (*24*) assessed the impact of HPP treatments on scalping of four aroma compounds (2-hexanone, ethyl butanoate, ethyl hexanoate, d-limonene) in LDPE and PLA films. HPP treatments intending to perform pasteurization (800 MPa, 40 °C) and sterilization (800 MPa, 115 °C) for 5 min were carried out on film samples in contact with four FSL (water, 3% acetic acid, 15% ethanol and olive oil) enriched with aroma compounds. For LDPE, HPP pasteurization led to a slight increase in total amount of sorption in water, but no significant differences were found in other FSL. LDPE melted during the conventional sterilization, whereas it withstood the HPP sterilization treatment. For scalping in PLA, temperature turned out to be a critical factor, especially if the temperature of the treatment is higher than

the $T_g$ of PLA. The amorphous phase of PLA is in the glassy state during HPP pasteurization, but certainly in the rubbery state during HPP sterilization. They reported that HPP pasteurization led to a significant decrease in sorption, but the HPP sterilization notably increased the scalping for PLA when compared to controls (0.1 MPa, 40 °C; 0.1 MPa, 115 °C) for 7 min. However, their thermal processing temperature and time at atmosphere used as control may not reflect the true history of temperature that occurs during HPP. The effect of temperature on sorption becomes more substantial as temperature increases, but they did not investigate if the effect of temperature was lowered or accentuated by the processing time.

## Conclusion

The application of HPP coupled with increasing temperature can possibly alter physicochemical properties of polymeric flexible structures for food packaging. HPP conditions can result in a decrease of density of the amorphous regions in semi-crystalline polymers, a shift of melting and glass transitions to higher temperatures, and a change of morphology of the crystalline and amorphous domains in polymer films induced by pressure or plasticization due to absorption of low MW compounds. As the pressure is released, the packaging material ideally recovers its original dimensions. Therefore, HPP is expected to have limited effects on food and packaging interactions in terms of migration and scalping.

Presently, limited experimental studies are available which have been focused on the effect of HPP on morphology and, consequently, on the migration properties of the packaging materials. In general, most research studies reported so far are in accordance with the observations discussed, and thus, showed a significant decrease or no-change in migration levels during and after HPP. In contrast, several researchers have reported a significant increase of global migration into food stimulants after HPP in several homogeneous and multi-laminate packaging materials. The discrepancy may result from several factors, such as different polymer compositions, neglected temperature effect of HPP, different chemical properties of migrants, and lack of a standard procedure for migration studies under HPP (*46*). Therefore, it is suggested to further evaluate different polymer materials and structures, specifically suited to provide the extra performance required by HPP. Additional focused studies will provide a clear understanding of the physicochemical behavior of food packaging polymers under HPP conditions as well as the migration/scalping behavior under these conditions. Further research will ultimately give regulatory agencies and the food industry a sound scientific basis to evaluate food contact substances which are processed by high pressure processing, thereby, ensuring consumer safety.

## Addendum: Nomenclature of Packaging Structures

| | |
|---|---|
| AL | Aluminum |
| APET | Amorphous polyethylene terephthalate |
| CPP | Cast polypropylene |
| BOPP | Biaxially oriented polypropylene |
| EVA | Ethylene vinyl acetate |
| EVOH | Ethylene-vinyl alcohol copolymer |
| HDPE | High density polyethylene |
| LDPE | Low density polyethylene |
| LLDPE | Linear low density polyethylene |
| mPET | Metallized polyethylene terephthalate |
| Nylon | Polyamide |
| OPA | Bi-oriented polyamide |
| PA | Polyamide |
| PE | Polyethylene |
| PET | Polyethylene terephthalate |
| PP | Polypropylene |
| PLA | Polylactic acid |
| PVDC | Polyvinylidene chloride |
| SiOx | Silicon oxide |
| SY | Surlyn ionomer |

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## Chapter 15

# Bisphenol A in Japanese Canned Foods

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Bisphenol A (BPA), a suspected endocrine disrupter, is used mainly as a monomer in the production of polycarbonate and epoxy resins. Metal cans for food are usually coated with BPA-based epoxy resins. Therefore, the residual BPA in the can coatings has a possibility to migrate into canned food when the canned food is heated over 105°C during cooking and/or sterilization. We surveyed BPA Levels in 100 domestic, and 60 imported canned foods purchased in Japan. In the domestic canned foods, the highest BPA level was 30 ng/g in hashed beef stew, and the average was 3.7 ng/g. On the contrary, the imported canned foods contained much higher BPA levels. The maximum BPA level was found 390 ng/g in demiglace sauce, followed by 340 ng/g in white sauce, 320 ng/g in gratin sauce and blue crab. The average was 57 ng/g, which was 15 times higher than that found in the domestic cans. The BPA levels in the domestic canned foods showed a significant reduction in comparing to the levels found in the imported cans or reported in other surveys. This drastic reduction is likely due to the use of "BPA reduced cans" which Japanese can manufacturers have developed in the past decade.

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## Introduction

Bisphenol A (2,2-bis(4-hydroxyphenyl) propane, BPA) is a suspected endocrine disrupter producing estrogenic effects (*1*). Its chemical structure is shown in Figure 1. It is mainly used as a monomer in the production of polycarbonate (PC) and epoxy resins. Because of its use, food contact articles made from PC or epoxy resins usually contain trace levels of free BPA, which has a possibility to migrate into foods.

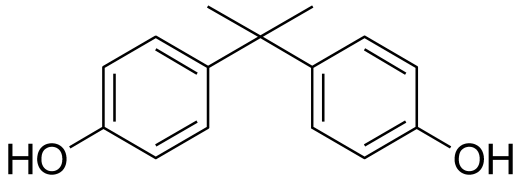


*Figure 1. Chemical structure of bisphenol A.*

PC products contain BPA as an unreacted monomer and also a degradation product of PC. The BPA residues were found to be 5-80 μg/g in dishes and 18-37 μg/g in baby bottles in Japan, though their migration levels were mainly below limit of detection (*2*). However, Japanese consumers refused to buy PC tableware and baby bottles, then the Japanese manufacturers stopped their production in about year 2000. Therefore, the market share for PC baby bottles was significantly reduced to less than 1% on the Japanese market, and polyphenylsulfone and polyethersulfone became the substitutes for PC. The Japanese government has not prohibited PC baby bottles yet, but Canada, the EU, China and several other countries have.

Epoxy resin is a thermosetting copolymer formed from the reaction of an epoxide resin with a polyamide hardener. The most common epoxy resin is the diglycidylether of BPA produced from epichlorhydrin and BPA. Epoxy has a wide range of applications including paints, coating and adhesives. For food contact purposes, it is used as a coating of tablewares and metal cans, and as an adhesive for laminate films. Most of epoxy resins contain residual BPA as an unreacted monomer.

Metal cans for food contact are commonly coated on the surface by epoxy resin to protect both cans and foods, because cans can be eroded by foods, and foods then are contaminated by the released metal ion. Epoxy resin is very stable under its glass transition temperature (ca. 105°C), but above 105°C its physical property changed and begins to release free BPA to moisture contacted with it. Therefore, when food is packed in a can and heated during cooking and/or sterilization over 105°C, BPA will readily migrate into the food from the can coatings. The migration levels in canned foods are extremely higher than that from polycarbonate products. The main source of human exposure to BPA is from the diet, especially the canned foods (*3*, *4*).

**Table I. Frequency, Maximum and Average of BPA Levels in Canned Foods Reported in Literatures**

| *Food group* | *Japan (Ca. 2000)*[3-7] | | | *U.K. (2002)*[8] | | | *New Zealand (2005)*[9] | | | *Canada (2009)*[10] | | | *Belgium (2010)*[11] | | | *U.S.A. (2011)*[12] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Freq. (%)* | *Max. (ng/g)* | *Ave. (ng/g)* | *Freq. (%)* | *Max. (ng/g)* | *Ave. (ng/g)* | *Freq. (%)* | *Max. (ng/g)* | *Ave. (ng/g)* | *Freq. (%)* | *Max. (ng/g)* | *Ave. (ng/g)* | *Freq. (%)* | *Max. (ng/g)* | *Ave. (ng/g)* | *Freq. (%)* | *Max. (ng/g)* | *Ave. (ng/g)* |
| Fish | 67 | 97 | 36 | 90 | 44 | 21 | 50 | 109 | 28 | 100 | 534 | 137 | 100 | 169 | 75 | 100 | 22 | 12 |
| Meat | 91 | 602 | 139 | 100 | 422 | 108 | 33 | 98 | 28 | − | − | − | 100 | 27 | 27 | − | − | − |
| Vegetable | 78 | 95 | 32 | 100 | 48 | 27 | 65 | 24 | 13 | 100 | 92 | 20 | 100 | 116 | 42 | 92 | 730[a] | 88 |
| Fruit | 11 | 7 | 1 | 100 | 41 | 29 | 0 | < 10 | 0 | − | − | − | 100 | 20 | 12 | 64 | 19 | 5 |
| Other food | 67 | 86 | 33 | 53 | 41 | 12 | 25 | 21 | 8 | 94 | 189 | 68 | 100 | 73 | 35 | 100 | 790[b] | 72 |
| Coffee, tea | 58 | 213 | 26 | − | − | − | − | − | − | − | − | − | 100 | 1 | 0.7 | − | − | − |
| Other drink | 0 | <2 | 0 | 0 | < 7 | 0 | 0 | < 10 | 0 | − | − | − | 90 | 8 | 1 | − | − | − |
| All | 46 | 602 | 27 | 63 | 422 | 27 | 31 | 109 | 14 | 96 | 534 | 72 | 86 | 169 | 16 | 91 | 790 | 60 |

Freq: % of BPA-detected samples/total samples analyzed, Max.: maximum BPA level, Ave.: average BPA level. [a] Green beans. [b] Refried beans. Reproduced with permission from reference (*16*). Copyright 2014 Taylor & Francis Group.

**Table II. Residue and Migration Level of BPA in the BPA Reduced Cans and Conventional Cans**

| *Sample* | *Bpa Reduced Can* | | *Conventional Can* | |
|---|---|---|---|---|
| | *Residue (Mg/Can)* | *Migration (Ng/Ml)* | *Residue (Mg/Can)* | *Migration (Ng/Ml)* |
| A | 0.5 | 3 | 15.6 | 82 |
| B | 1.3 | 6 | 29.5 | 124 |
| C | 1.3 | 4 | 12.1 | 35 |
| Average | 1.0 | 4.3 | 19.1 | 80.3 |

Extraction method of residue BPA: Coating were remove off by a knife and extracted with dichloromethane. Migration conditions: Water at 121°C for 30 min.

## Previous Reports on BPA Levels in Canned Foods

There are many reports on BPA levels in canned foods, such as from Japan in about 2000 (*5–9*), the United Kingdom (U.K.) (*10*), New Zealand (N.Z.) (*11*), Canada (*12*), Belgium (*13*) and the United States of America (U.S.A.) (*14*). Table I shows the maximum and average BPA levels in canned foods reported in these papers. The maximum BPA level was found 790 ng/g in refried beans from the U.S.A, followed by 730 ng/g in green beans also from the U.S.A. In every report, fish, meat, vegetable and other foods sometimes contained BPA levels above 100 ng/g. Japanese canned coffee and teas also showed high BPA levels in about 2000. The averages of BPA levels in all kinds of canned foods from these countries were between 14 and 72 ng/g. In comparison, the average BPA levels in the foods from Canada and U.S.A. were higher than those from other countries.

In 1990s, the Japanese can manufacturers tried to develop “BPA reduced cans”, which are coated with low-BPA epoxy or covered with a PET film that replaced the epoxy coating. Based on the self-regulation of “The Can Manufacturers Institute of Japan”, the migration limits of BPA are 10 ng/mL for food cans and 5 ng/mL for drink cans into food simulants at 121°C for 30 min. We analysed the content and migration levels of BPA in BPA reduced cans compared with conventional cans produced by three different manufacturers. As the result, we confirmed that the BPA content in their coating decreased and the migration levels were below 6 ng/mL as shown in Table II (*15*).

## Present Survey of BPA Levels in Japanese Canned Foods

Our present survey was performed to determine the BPA levels in domestic and imported canned foods on Japanese market and to verify the effect of “BPA reduced cans” (*16*).

## Samples

In the survey, we purchased 100 domestic canned foods (produced in Japan) and 60 imported canned foods from the markets in Tokyo in 2011 to 2012. They were classified seven food groups: fish, meat, vegetable, fruit, other food, coffee and tea, and other drink. The fish cans were cooked fishes such as tuna, sardine, salmon and mackerel in oil or in tasted water. The meat cans were corned beef, sausage, luncheon meat, cooked chicken and horse meat. The vegetable cans were boiled in water such as sweet corn, green peas, asparagus, and mushroom. The fruit cans were mainly cooked fruits in syrup, except coconut milk. Other food cans included various types of soup, sauce, stew, and curry. Canned coffee and tea are very popular in Japan and these production amounts are more than 90% of the Japanese canned foods. Other drink cans included soda, juice, beer and liquor. The imported drink cans are very small portion in Japanese market; therefore, we did not include imported drinks in this survey.

## Test Method

A homogenized sample was added with an internal standard (BPA-$d_{16}$) and extracted with methanol. The extract was defatted with hexane and purified with 5% NaCl, 1 mol/L NaOH, 6 mol/L HCl and dichloromethane if needed. Then the extract was ethylated with diethyl sulfate and 1 mol/L KOH in ethanol at 70°C for 1 h, dried, and diluted with acetone to make a test solution. The test solution was then analyzed by GC/MS with addition of a syringe spike (pyrene-$d_{10}$).

The GC/MS conditions were as follows; GC/MS equipment: 6890 Series PLUS, 5973 mass selective detector (Agilent Technologies, Wilmington, DE); column: Inertcap 17ms, 0.25 mm x 30 m, 0.25 μm (GL Science, Tokyo, Japan); column temperature: 100°C (1 min) - 20°C/min - 250°C - 10°C/min - 290°C (5.5min); inlet temperature: 250°C; interface temperature: 290°C; carrier gas: helium, 1 mL/min; detection ion (*m/z*): 269* and 284 (BPA), 280* and 298 (BPA-$d_{16}$), 212* (pyrene-$d_{10}$) (*: for quantification)

## Results

The top ten of the highest BPA levels in the domestic and imported canned foods are shown in Table III. In the domestic cans, the maximum BPA level was 30 ng/g in hashed beef stew, followed by 21 ng/g in boiled scallop, 18 ng/g in corned beef, 17 ng/g in sardine with miso, and 16 ng/g in bonito with bean. On the contrary, the imported canned foods contained much higher BPA levels. The maximum BPA level was found 390 ng/g in demiglace sauce, followed by 340 ng/g in white sauce, 320 ng/g in gratin sauce and blue crab, 240 ng/g in tomato soup, and 200 ng/g in coconut milk. In general, the other food and fish cans contained higher BPA levels than other kind of canned foods. Particularly, the imported canned foods sometimes contained BPA at a level greater than 100 ng/g.

**Table III. Top 10 of BPA Levels in the Domestic and Imported Canned Foods**

| *Domestic Canned Foods (Japan)* | | | *Imported Canned Foods* | | | |
|---|---|---|---|---|---|---|
| *Food group* | *Food* | *BPA (ng/g)* | *Food Group* | *Food* | *BPA (ng/g)* | *Origin* |
| Other food | Hashed beef stew | 30 | Other food | Demiglace sauce | 390 | N.Z. |
| Fish | Boiled scallop | 21 | Other food | White sauce | 340 | N.Z. |
| Meat | Corned beef | 18 | Other food | Gratin sauce | 320 | N.Z. |
| Fish | Sardine with miso | 17 | Fish | Blue crab | 320 | Thailand |
| Fish | Bonito with bean | 16 | Other food | Tomato soup | 240 | U.S.A |
| Fish | Tuna in oil | 13 | Fruit | Coconut milk | 200 | Thailand |
| Fish | Boiled salmon | 12 | Fish | Oil sardine | 150 | Spain |
| Meat | Cooked chicken | 12 | Other food | Onion gratin soup | 150 | U.S.A |
| Meat | Grilled chicken | 12 | Fish | Tuna in oil | 120 | Vietnam |
| Vegetable | Boiled asparagus | 11 | Other food | Minestrone soup | 110 | U.S.A |

**Table IV. Summary of BPA Survey in the Domestic and Imported Canned Foods**

| *Food Group* | *LOQ (ng/g)* | *Domestic Canned Food* | | | | *Imported Canned Food* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *Sample number* | *Freq. (%)* | *Max. (ng/g)* | *Ave. (ng/g)* | *Sample number* | *Freq. (%)* | *Max. (ng/g)* | *Ave. (ng/g)* |
| Fish | 5 | 19 | 68 | 21 | 7.9 | 10 | 80 | 320 | 76 |
| Meat | 5 | 12 | 58 | 18 | 6.8 | 10 | 100 | 25 | 14 |
| Vegetable | 5 | 13 | 38 | 11 | 4.1 | 18 | 89 | 85 | 35 |
| Fruit | 5 | 8 | 0 | < 5 | 0 | 10 | 10 | 200 | 20 |
| Other food | 5 | 12 | 8 | 30 | 2.5 | 12 | 100 | 390 | 139 |
| Coffee, tea | 1 | 21 | 43 | 4 | 1.1 | – | – | – | – |
| Other drink | 1 | 15 | 0 | <1 | 0 | – | – | – | – |
| All | – | 100 | 35 | 30 | 3.4 | 60 | 78 | 390 | 57 |
| All* | – | 64 | 43 | 30 | 4.9 | – | – | – | – |

* All samples except coffee, tea and other drinks.

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Table IV shows the summary of the BPA survey results in the domestic and imported canned foods. The limit of quantification (LOQ) is 5 ng/g in foods and 1 ng/g in drinks. Frequency is the percentage of the number of BPA-detected samples to the total number of samples analyzed in each food group. In the domestic can, the fish, meat and vegetable were detected to contain BPA at a frequency between 38 and 68%. The detection frequency for coffee and tea was about 43%, though all of their BPA levels were below 5 ng/g LOQ. The maximum BPA level was 30 ng/g, and the average ranged from less than LOQ to 7.9 ng/g in each food group and 3.4 ng/g for all samples.

On the other hand, in the imported cans, 100% of meat and other food, 89% of vegetable and 80% of fish cans contained BPA above the LOQ. The frequency of BPA-detected samples to all samples was 78%, which was two times higher than that of the domestic cans. The averages of BPA levels in each food group were much higher than those of the domestic cans. The average of BPA levels in all imported cans was 57 ng/g, which was about 12 times higher than 4.9 ng/g found in the domestic cans, except for drinks. Clearly, the domestic cans contained BPA at much lower levels than the imported cans did.

## Comparison of BPA Levels with the Previous Surveys

Figure 2 shows a comparison of the maximum and average BPA levels in the canned foods that were found in the present survey (*16*) and those reported in the previous surveys (*5–14*). The maximum BPA levels in the previous surveys ranged from 109 ng/g (from N.Z.) to 790 ng/g (from the U.S.A.) and the average value of the range was about 400 ng/g, which is about the same level as the maximum BPA level found in the imported cans in the present survey. However, the maximum BPA level in the domestic cans was 30 ng/g, which was significantly lower than that in the imported canned foods as reported in the previous surveys. The maximum BPA level in domestic canned foods was about one-third of that from New Zealand and one-26$^{th}$ of that from the U.S.A. The average BPA levels found in the previous surveys ranged from 14 to 72 ng/g. The average BPA level for all imported cans in the present survey was 57 ng/g, which was at the same level as that from the U.S.A. However, the average BPA level for the domestic cans was only 3.4 ng/g, which was significantly lower than that from the other cans, e.g., one-fourth of that from New Zealand, and one-21$^{st}$ of that from Canada.

The BPA levels in the domestic canned foods were significantly lower than those found in the imported canned foods and in the previous survey results. This drastic reduction of the BPA levels in Japanese domestic cans is likely due to the use of “BPA reduced cans”.

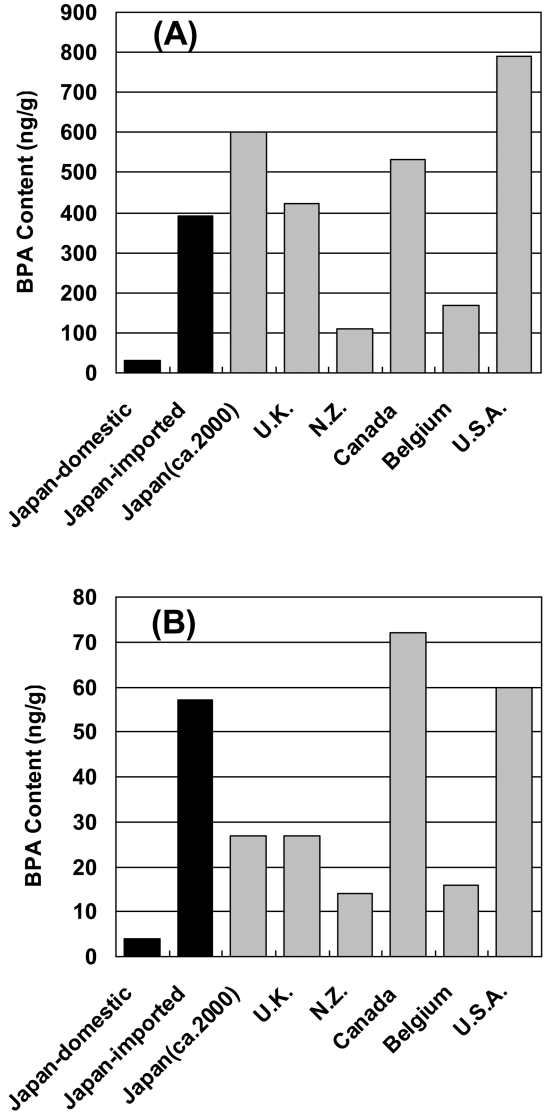


*Figure 2. Comparison of maximum and average BPA Levels in canned foods found in the present survey and reported in other surveys: (A) Maximum BPA content, (B) Average BPA content. Reproduced with permission from reference (16). Copyright 2014 Taylor & Francis Group.*

**Table V. Estimated BPA Intake from Canned Foods in Japan**

| *Food group* | *Present (2011-12)* | | | | | *Past (1999-2001)* | |
|---|---|---|---|---|---|---|---|
| | *Domestic Can* | | *Imported Can* | | *Total can* | *Total Can* | |
| | *Food intake (g/p/d)* | *BPA intake (ng/p/d)* | *Food intake (g/p/d)* | *BPA intake (ng/p/d)* | *BPA intake (ng/p/d)* | *Food intake (g/p/d)* | *BPA intake (ng/p/d)* |
| Fish | 2.2 | 17 | 0.9 | 68 | 85 | 3.1 | 112 |
| Meat | 0.2 | 1 | 1.4 | 20 | 21 | 1.2 | 167 |
| Vegetable | 1.1 | 5 | 6.3 | 221 | 226 | 8.6 | 275 |
| Fruit | 0.8 | 0 | 5.3 | 106 | 106 | 7.6 | 12 |
| Other food | 1.0 | 3 | 1.0 | 139 | 142 | 3.5 | 116 |
| Coffee, tea | 58.1 | 64 | 0 | 0 | 64 | 77.4 | 2012 |
| Other drink | − | 0 | − | 0 | 0 | − | 0 |
| All | 63.4 | 90 | 14.9 | 554 | 644 | 101.4 | 2694 |

p: person, d: day Reproduced with permission from reference (*16*). Copyright 2014 Taylor & Francis Group.

## Estimated BPA Intake from Canned Foods in Japan

The estimated BPA intake from canned foods in Japan was separately calculated from the domestic canned foods and the imported canned foods, then, both were added together to obtain the total intake. Because the Japanese consume not only domestic canned foods but also imported canned foods in not a small part. The calculated BPA intake was the summation of the canned food intake in each food group multiplied by their average BPA levels. The canned food intake was calculated from the annual production, export and import amount of canned foods in 2010 (*17*) for the present survey, and that in 2001 (*18*) for the past survey (ca.2000), divided by the Japanese population which was 128 million in 2010 and 127 million in 2001, and divided by 365 days per year. We could not get the annual production amount of canned alcohol and the export and import amount of other foods and canned alcoholic drinks. The import amount of other foods was presumed to be the same as that of domestic canned foods. Other drinks include alcohol did not contain BPA; therefore, the BPA intake is relatively zero.

The estimated BPA intake from canned foods in Japan is shown in Table V. From the domestic canned foods, the BPA intake was 90 ng/person/day. The main source of BPA was coffee and teas because their intake was 58.1 g/person/day or about 92% of canned food consumption. But their average was only 1.1 ng/g and their effect was limited. On the other hand, from the imported canned foods, the BPA intake was 554 ng/person/day, which was six times higher than the domestic cans. The main sources of higher exposure to BPA were meat and vegetable cans, because their food intakes and the average BPA levels for both were higher. Therefore, from the sum of the domestic and imported canned foods, the estimated BPA intake was 664 ng/person/day. In Japan about 2000, the food intake was calculated based on the annual production minus the exported amount plus the imported amount. The estimated BPA intake was 2694 ng/person/day. It indicates that the present BPA intake is significantly affected by the imported cans; however, it is only one-fourth of the previous BPA exposure level.

## Conclusions

The BPA levels in Japanese domestic canned foods have been significantly reduced as compared to those found in the imported canned foods from other countries, and in the canned foods from the past Japan. This drastic reduction in the BPA levels is likely due to the "BPA reduced cans" which Japanese can manufacturers have developed since late 1990s. The survey results show that an estimated BPA intake from the domestic cans is about 90 ng/person/day. However, the BPA intake from the imported cans is 554 ng/person/day, thus total intake is 644 ng/person/day. These results indicate that the BPA intake can be reduced by the can manufacturing techniques.

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Chapter 16

# Food Law Compliance of Poly(ethylene Terephthalate) (PET) Food Packaging Materials

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Poly(ethylene terephthalate) (PET) is widely used as a packaging material for all kinds of foodstuff. Low diffusivity of the polymer combined with the limited number of additives and their low concentrations lead to very limited mass transfer (migration) of monomers, catalysts, or impurities from the PET polymer into food. This mass transfer (migration) for monomers, oligomers, catalysts, additives and non-intentionally added substances (NIAS) is discussed within this study.

Based on the data given in the scientific literature it could be concluded that overall migration tests as well as specific migration tests for monomers and catalysts, like ethylene glycol, diethylene glycol, terephthalic acid, *iso*-phthalic acid and antimony are superfluous, because their migration limits cannot be exceeded, even if worst case conditions and swelling simulants like 95% ethanol are applied. A more suitable procedure for evaluation of the food law compliance of PET is the determination of migration relevant substances in PET and calculation of their migration by use of migration models. In addition, the analytical screening for low molecular weight migrants (like NIAS) gives an additional safety factor, because such NIAS were not determined using the conventional migration testing procedures.

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## Introduction

The use of poly(ethylene terephthalate) (PET) in packaging applications is increasing year by year. In 2012 about 15 Mi. t (million tons) of PET were produced world-wide for PET packaging materials. In 2019, the predicted amount will be about 19 Mi. t. Reasons for this trend are the good material properties of the PET. PET is a light-weight packaging material, is transparent and is nearly unbreakable in comparison to glass. In addition, PET can be recycled into new PET bottles up to a recycled content of nearly 100% (*1*, *2*). The good recyclability of PET is related to one of the most important properties of PET as a packaging material: a high inertness. This high inertness leads to very low interactions between PET packaging and foodstuff. In addition, PET contains only a small number and amount of additives. Therefore, this leads to very limited mass transfer (migration) of monomers, catalysts, or impurities from the PET polymer into food.

The migration of substances from the packaging material into food (or simulants) depends on the contact conditions, e.g. storage time, temperature as well as contact area. In addition, diffusion and partition coefficients play also an important role. As mentioned above, the high inertness of PET leads to low migration into food. Therefore, experimental studies attempting to determine the migration of monomers and additives through PET failed in most cases due to insufficient detection limits of the analytical test methods employed and the short testing timeframes (*3*, *4*). PET is, therefore, a candidate for new food law evaluation and migration testing concepts. Especially the application of migration modeling seems to be a useful tool for compliance evaluation purposes of PET packaging materials.

The purpose of this chapter is to give an overview over potential migrants from PET, their typical use levels in PET and concentrations in food (simulants) in relation to the current migration limits set by the U.S. Food and Drug Administration (FDA), and the European Food Safety Authority (EFSA) as well as the prediction of the migration by using migration modeling.

## Requirements on PET Bottles According to European Food Law

As for any other packaging material, the mass transfer (migration) of monomers or additives from PET into foodstuffs is restricted. According to Article 3 of the European Framework Regulation 1935/2004 for food packaging materials (*5*), "materials and articles shall be manufactured in compliance with good manufacturing practice so that, under normal or foreseeable conditions of use, they do not transfer their constituents to food in quantities which could: (a) endanger human health, or (b) bring about an unacceptable change in the composition of the food, or (c) bring about a deterioration in the organoleptic characteristics thereof". EU Regulation 1935/2004 gives, however, only a general statement about safety issues on packaging materials. More specific details like a positive list of all compounds, which can be used for manufacturing of food

packaging materials as well as specific migration limits are given in Regulation 10/2011 (*6*) and amendments. In general, considering the mass transfer of substances from packaging materials, distinguished distinction is made between overall migration and specific migration. Overall migration can be considered as a measure for the inertness of the packaging material, determined as a gravimetric parameter, whereas specific migration limits are given for individual monomers or additives regarding their toxicological profiles. The overall migration limit for any packaging material is 10 mg $dm^{-2}$. The specific migration limits for PET monomers, some additives and catalysts according to European legislation are summarized in Table 1.

**Table 1. Specific Migration Limits (SML) for PET Monomers, Some Additives, and Catalysts According to European Law (*6*)**

| *Substance* | *SML [mg $l^{-1}$]* |
|---|---|
| Terephthalic acid | 7.5 |
| *iso*-Phthalic acid | 5 |
| Mono- and diethylene glycol, inclusive stearic acid glycol ester | 30 |
| Acetaldehyde | 6 |
| Antimony | 0.04 |
| 2-Aminobenzamide (anthranilamide) | 0.05 |

## Requirements on PET Bottles According to U.S. Food Law

Packaging materials as well as other substances, which migrate from the packaging materials into food, are defined in the U.S. as indirect food additives or food contact substances. The requirements for food contact materials are laid down in Chapter 21 of the Code of Federal Regulations (denoted as 21 CFR). The general requirements are given in 21 CFR 174.5 such as good manufacturing practice (GMP) or the organoleptic inertness of a packaging material. Specific requirements for all "polyethylene phthalate polymers" inclusive of their raw materials are given in 21 CFR 177.1630 (Polyethylene phthalate polymers). These requirements are summarized in Table 2. In contrast to European law, the compliance of PET articles in the U.S. is shown by the use of short term extraction tests. In addition, the recent Food Contact Notification system of the FDA also has other polyesters related to PET.

**Table 2. Requirements for "Polyethylene Phthalate Polymers" According to 21 CFR 177.1630 f-j**

| *Conditions of use* | *Specifications* |
|---|---|
| (f) Plastics used for packaging, transporting, or holding food, excluding alcoholic beverages, at temperatures not to exceed 250 °F. | chloroform- soluble extractives not to exceed 0.5 mg $in^{-2}$ of food contact surface exposed to<br>(i) distilled water at 250 °F for 2 h<br>(ii) *n*-heptane at 150 °F for 2 h |
| (g) Plastics used for packaging, transporting, or holding alcoholic beverages that do not exceed 50% (v/v) alcohol | chloroform- soluble extractives not to exceed 0.5 mg $in^{-2}$ of food contact surface exposed to<br>(i) distilled water at 250 °F for 2 h<br>(ii) *n*-heptane at 150 °F for 2 h<br>(iii) 50% ethanol at 120 °F for 24 h |
| (h) Plastics are used to contain foods during oven baking or oven cooking at temperatures above 250 °F | chloroform- soluble extractives not to exceed 0.02 mg $in^{-2}$ of food contact surface exposed to<br>(i) distilled water at 250 °F for 2 h<br>(ii) *n*-heptane at 150 °F for 2 h |
| (j) Plastics used for packaging, transporting, or holding alcoholic beverages that do not exceed 95% (v/v) alcohol | chloroform- soluble extractives not to exceed 0.5 mg $in^{-2}$ of food contact surface exposed to<br>(i) distilled water at 250 °F for 2 h<br>(ii) *n*-heptane at 150 °F for 2 h<br><br>chloroform- soluble extractives not to exceed 0.005 mg $in^{-2}$ of food contact surface exposed to 95% ethanol at 120 °F for 24 h for containers with greater than 500 ml capacity<br><br>chloroform- soluble extractives not to exceed 0.05 mg $in^{-2}$ of food contact surface exposed to 95% ethanol at 120 °F for 24 h for containers with less than or equal to 500 ml capacity |

## Migration Modeling as a Tool for Evaluation

Migration modeling is an alternative to experimental migration tests. Migration from polymers into food (or simulants) typically follows Fickian laws of diffusion. The mathematical equations for the diffusion in polymers were published by Crank (*7*, *8*). Equation 1 is the exact mathematical equation for the prediction of the mass transfer (migration) from a packaging material into food (or simulants). The parameter m/A is the area related mass transfer from the polymer into the foodstuff (typically expressed in μg $cm^{-2}$). The concentration of

the migrant in the polymer before the migration experiment is $c_{P,0}$ (in mg kg$^{-1}$). The diffusion coefficient of the migrant in the polymer is $D_P$ (in cm$^2$ s$^{-1}$) and t is the storage time (in s). $\rho_P$ (g cm$^{-3}$) is the density of the polymer and the thickness of the packaging material is $d_P$ (in cm). $q_n$ values are the non-zero positive roots of $tan\ q_n = \alpha\ q_n$. The dimensionless factor α (Equation 2) contains the partition coefficients $K_{P,F}$ (dimensionless) and the volumes of the packaging and foodstuff ($V_P$ and $V_F$ in cm$^3$) (*7*). $K_{P,F}$ can be calculated from the ratio of the concentration of the migrants in the packaging polymer $c_{P,\infty}$ and the food(simulant) $c_{F,\infty}$ at equilibrium (t → ∞, Equation 3).

$$\text{Equation 1: } \frac{m}{A} = c_{P,0}\rho_P d_P \left(\frac{\alpha}{1+\alpha}\right)\left[1 - \sum_{n=1}^{\infty} \frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2 q_n^2} e^{\left(-D_P t \frac{q_n^2}{d_P^2}\right)}\right]$$

$$\text{Equation 2: } \alpha = \frac{V_F}{K_{P,F}\, V_P}$$

$$\text{Equation 3: } K_{P,F} = \frac{c_{P,\infty}}{c_{F,\infty}}$$

The most important piece of information necessary for the prediction of the migration is the diffusion coefficient $D_P$ of a potential migrant in PET polymer. However, the diffusion coefficient is at first glance not known for every migrant and temperature. Therefore, predictive models for diffusion coefficients have been developed. Typically these predicted diffusion coefficients are over-estimates when compared to experimentally determined values. However, the parameters for the migration modeling should be not too conservative. Experimentally determined diffusion coefficients play, therefore, a major role in the realistic estimation of the migration as well as to calibrate the prediction methods for the diffusion coefficients in PET.

Within this Chapter, as a first approach, the specific migration was calculated according to the current scientific recognized predictive model (Equation 4) (*9*). This model (referred to as the $A_P$ model) predicts the diffusion coefficient in a "worst case" scenario, which means that the predicted value is in any case higher than the experimentally determined value. Due to this worst-case character, the migration value has a certain safety factor. The value of this safety factor is, however, unknown and might be different for different migrants. Within this model the diffusion coefficients were predicted from the molecular weight M of the migrant according to Equation 4. The temperature dependency of $A_P$ is given in Equation 5. The factor $A'_P$ is a polymer specific parameter and T is the temperature (in K). Within Equation 5 the parameter τ is some kind of activation energy of diffusion of potential migrants. The current predictive model for diffusion coefficients in PET is using the polymer specific parameter $A'_P$ = 3.1 and the activation energy of τ = 1577 K for the prediction of the migration from PET at below the glass transition temperature. Above the glass transition temperature $A'_P$ = 6.4 should be used (*9*).

Equation 4: $$D_P = 10^4 e^{A'_P - 0.1351\,M^{2/3} + 0.003\,M - \frac{10454}{T}}$$

Equation 5: $$A_P = A'_P - \frac{\tau}{T}$$

For a more realistic calculation of the migration within this study, the diffusion coefficient was also predicted according to another method, which is based on experimentally determined activation energies of diffusion (*10*) (Equation 6). In contrast to the AP model, the diffusion coefficient from this model (referred to as the realistic model) is not over-estimative. This method has, therefore, no potential safety factor if the predicted diffusion coefficients are used for migration evaluation. Equation 6 is based on the molecular volume V of the migrant instead of the molecular weight M of the migrant. T is the temperature (in K). The parameters "a" to "d" are PET specific parameters (a = 1.93 $10^{-3}$ $K^{-1}$, b = 2.37 $10^{-6}$ $cm^{2\ -1}$, c = 11.1 $Å^3$ and d = 1.50 $10^{-4}$ $K^{-1}$) (*10*).

Equation 6: $$D_P = b\left(\frac{V}{c}\right)^{\frac{a - \frac{1}{T}}{d}}$$

The calculation of the migration from the PET bottles was performed using the AKTS SML software version 4.54 (AKTS AG Siders, Switzerland). The program uses finite element analysis. The mathematical procedure and the main equations are published (*11*). The applied diffusion coefficients were predicted according to Equation 4 and Equation 6. All applied diffusion coefficients are given in Table 6. In all cases, the migration was calculated for a package with 1 l volume and a surface area of 600 $cm^2$. The partition coefficient was in all cases K = 1, which assumes good solubility of the migrant in the simulant (worst-case).

Migration modeling can be applied only for substances with known molecular weight or molecular volume. Therefore within this study, only the specific migration according to European law was modeled. Overall migration for EU or the short term extraction tests required according to the U.S. food law were not calculated.

## Overall Migration

The overall migration was previously determined by Störmer et al. (*12*). In their study, the overall migration from 36 PET bottles was determined in the food simulants 3% acetic acid, 10% ethanol and 95% ethanol after storage for 10 d at 40 °C. The investigated PET bottles were provided from the mineral water filling companies and include refillable bottles, non-refillable bottles, recyclate containing bottles as well as bottles with the acetaldehyde scavenger anthranilamide (*12*). As a result, the overall migration in all cases was below 0.5 mg $dm^{-2}$. These results are in good agreement with data published by Ashby (*13*) where the overall migration was below 0.6 mg $dm^{-2}$ for food simulants water, 3% acetic acid, 15% ethanol and 50% ethanol after 40 °C for 10 days.

It is interesting to note that the overall migration into 50% or 95% ethanol is not significantly higher than the overall migration into 3% acetic acid or water. The food simulant 95% ethanol is a well-known swelling solvent towards PET, which should increase the migration significantly (*14*). Therefore, the overall migration values are not related to PET monomers, oligomers or additives, because the migration of these substances should be significantly higher if migration is determined into swelling food simulants in comparison to non-swelling conditions, like 3% acetic acid. The experimentally determined overall migration values from these two studies (*12*, *13*) can be considered as the analytical uncertainty at the analytical detection limit.

As a conclusion, the overall migration limit of 10 mg $dm^{-2}$ cannot be exceeded under normal storage conditions and shelf lives even if extractive or swelling conditions are applied, e.g. by use of 95% ethanol as food simulant. In consequence, experimental overall migration tests in any of the above mentioned food simulants are superfluous. Furthermore, overall migration is not a suitable instrument for the evaluation of PET, because the method is too unspecific and the analytical uncertainty is too high in order to deliver insight into the food law compliance evaluation of PET packaging materials.

## Specific Migration of Monomers, Additives, and Catalysts

In the following, the specific migration of monomers, additives and catalysts were summarized from literature studies. The relevant specific migration limits for PET relevant monomers, additives and the antimony catalyst are given Table 1. The diffusion coefficients used for the prediction of the specific migration are summarized in Table 6. In the first step, the current over-estimative $A_P$ model should be used for compliance evaluation. This model has a sufficient safety margin due to the over-estimative character of the modeling parameters and is generally accepted by authorities. If the predicted migration with the $A_P$ model under realistic storage conditions is below the specific migration limit, or in the case of NIAS below 10 µg $l^{-1}$, the food contact material may be considered as safe. On the other hand, if the predicted migration limit is at the boundary or above, a more realistic migration model should be applied instead of using the very conservative $A_P$ model. It is noteworthy that storage conditions like 10 d at 60 °C as recommended in the EU Regulation 10/2011 (*6*) are not necessary when migration models are used for compliance evaluation. The 10 d at 60 °C migration storage conditions were introduced in Regulation 10/2011 as a pure kinetic accelerator, and under the assumption that no swelling occurs. Accelerated testing conditions like 10 d at 60 °C are solely introduced to increase the diffusion in experimental migration tests and therefore to shorten the storage time for the experimental tests. For the prediction using migration models, realistic storage conditions, e.g. room temperature storage up to the shelf life of the PET packaged food, are more useful for the compliance evaluation. In some cases high temperatures like 60 °C together with high ethanol solutions (e.g. 50% or 95% ethanol) lead to highly over-estimative migration values. It is well-known that

high ethanol solutions like 50% or 95% ethanol act as swelling agents towards PET, which increases the migration significantly (*15*, *16*).

### Ethylene Glycol and Diethylene Glycol

Ethylene glycol and diethylene glycol have a specific migration limit of 30 mg $l^{-1}$ as a sum parameter. The residual concentrations of these monomers found in PET bottles are typically below 100 mg $kg^{-1}$. Using the $A_P$ model (Equation 4 with $A'_P = 3.1$, $\tau = 1577$ K), the migration into food of 0.23 mg $l^{-1}$ (38 µg $dm^{-2}$) was predicted for ethylene glycol after storage for 10 d at 60 °C. This predicted migration is more than a factor of 100 below of the specific migration limit. Experimental data of ethylene glycol into 3% acetic acid are in the range of (or below) 0.1 mg $l^{-1}$ after storage for 6 months at 32 °C (*17*), which is in good agreement with the above mentioned, predicted concentrations. In conclusion, experimental migration tests for ethylene glycol are superfluous because the specific migration limit cannot be exceeded under normal storage conditions.

The molecular weight of diethylene glycol is significantly higher than that of ethylene glycol and as such, its migration is expected to be lower than ethylene glycol. In addition, typically diethylene glycol is not detectable in PET containers. Therefore, the contribution of diethylene glycol on the specific migration from PET is negligible. Experimental migration data for diethylene glycol are not available in the scientific literature.

### 1,4-Bis(hydroxymethyl) Cyclohexane

1,4-*Bis*-(hydroxymethyl) cyclohexane is used as a co-monomer in PET to reduce the crystallinity of PET. It is listed in the European Regulation 10/2011 (*6*) without a specific migration limit. Maximum residual concentrations have not been published in the scientific literature. However, typically this substance is not detectable in PET containers. Assuming a concentration of 10 µg $kg^{-1}$ as the analytical detection limit, the concentration in food after storage for 10 d at 60 °C is predicted according to the $A_P$ model (Equation 4 with $A'_P = 3.1$, $\tau = 1577$ K) to be 11.6 µg $l^{-1}$ (1.9 µg $dm^{-2}$). More realistic prediction of the diffusion coefficient according to Equation 6 leads to a migration level of 1.18 µg $l^{-1}$ (0.2 µg $dm^{-2}$). The migration of 1,4-*bis*-(hydroxymethyl) cyclohexane is therefore not an issue for experimental migration tests.

### Terephthalic Acid, iso-Phthalic Acid, and Terephthalic Acid Dimethylester

Terephthalic acid, *iso*-phthalic acid and terephthalic acid dimethylester are typically used as monomers or co-monomers for the polymerization of PET. The residual concentration of terephthalic acid was determined to be 30 mg per kg PET as a maximum (*18*). Using this concentration for the prediction of the migration according to the $A_P$ model (Equation 4 with $A'_P = 3.1$, $\tau = 1577$ K), the migration of terephthalic acid into PET packed food is calculated to be 30 µg $l^{-1}$ (5 µg $dm^{-2}$) after storage for 10 d at 60 °C. This predicted "worst case" concentration in food is far below the specific migration limit of 7.5 mg $l^{-1}$ (Table 1), and is in good

agreement with experimental data. For example, Kim and Lee (*4*) found that the migration of terephthalic acid was below of 0.94 μg $dm^{-2}$ after storage for 10 days at 40 °C. In another study, Park et al. (*3*) investigated the migration of the PET monomers terephthalic acid, *iso*-phthalic acid and terephthalic acid dimethyl ester. Within this study, 56 PET containers and trays were investigated for the specific migration of these monomers into water, 4% acetic acid, 20% ethanol, and *n*-heptane at 60 °C for up to storage times of 30 d. No migration was found in all cases at a detection limit of 0.1 μg $l^{-1}$. Ashby (*13*) determined the specific migration of terephthalic acid and *iso*-phthalic acid into 3% acetic acid, 15% ethanol, 50% ethanol, vodka, and olive oil after storage for 10 d at 40 °C. As a result, the specific migration of terephthalic acid and *iso*-phthalic acid was found to be below the analytical detection limits of 10 μg $l^{-1}$ and 50 μg $l^{-1}$, respectively. In vodka, 50% ethanol, and olive oil, the specific migration of terephthalic acid was determined to be 20 to 30 μg $l^{-1}$, whereas the concentration of *iso*-phthalic acid was below the analytical detection limit. In conclusion, the specific migration of PET monomers like terephthalic acid, *iso*-phthalic acid and terephthalic acid dimethylester is negligible under storage conditions.

**Antimony**

Antimony glycoxylate complexes are used as catalysts in the polymerization of PET. The catalyst remains in the polymer after polymerization. The typical concentration of antimony is between 180 mg $kg^{-1}$ and 290 mg $kg^{-1}$. The mean concentration of antimony in PET bottles and preforms in Europe was 224 ±32 mg $kg^{-1}$ (*19*). It is interesting to note that the concentration of antimony in PET is relatively high compared to the residual concentrations of the monomers such as ethylene glycol or terephthalic acid (see above). On the other hand, its specific migration limit is significantly lower than that for these monomers (see Table 1). As a consequence, the conservative over-estimative $A_P$ model (Equation 4) predicts antimony concentrations after storage for 10 d at 60 °C to exceed the specific migration limit of 40 μg $l^{-1}$. On the other hand, experimentally determined antimony concentrations under ambient temperature conditions were determined to be 1 μg $l^{-1}$ at a maximum (*19*). These low specific migration values can be confirmed if the more realistic predictive model is applied (Equation 6). Using experimentally determined activation energies of diffusion for the calculation (*19*, *20*), the predicted migration of antimony is in the range of about 1 μg $l^{-1}$ after storage for one year at room temperature ($c_{P,0}$ = 300 mg $kg^{-1}$). This value is in good agreement with the experimentally determined values of samples drawn from the EU market (*19*). After storage of 10 d at 60 °C, the migration is in the range of 10 μg $l^{-1}$ which is still below the specific migration limit of 40 μg $l^{-1}$ set by the EU (see Table 1).

**2-Aminobenzoic Acid Amide**

2-Aminobenzoic acid amide (anthranilamide) is used in PET bottles as a scavenger for acetaldehyde during preform manufacturing (*21*). Acetaldehyde reacts with anthranilamide to generate a cyclic compound with a significantly

higher molecular weight (*22*). The specific migration limit of anthranilamide is 50 μg $l^{-1}$ (see Table 1). The maximum concentration of anthranilamide in PET bottles for natural mineral water is approximately 350 mg $kg^{-1}$. Assuming this concentration is a maximum concentration in PET bottles, the specific migration after storage for 10 d at 60 °C is predicted to be 82 μg $l^{-1}$ by use of the realistic diffusion coefficients (Equation 6). This value exceeds the specific migration limit. However, such elevated storage conditions (10 d at 60 °C) are introduced in order to speed up experimental migration tests. Using migration modeling for compliance evaluation, the application of the elevated storage conditions is not useful. Instead, the realistic storage conditions should be applied. For example, applying the same storage conditions as mentioned above, the specific migration for anthranilamide into food after one year at 25 °C is only 29 μg $l^{-1}$. Anthranilamide meets the specific migration limit under room storage conditions. However, due to the lack of a safety factor when using realistic migration modeling, the concentration of anthranilamide in the PET bottle wall should be controlled and the bottle wall concentration should not exceed 350 mg $kg^{-1}$. Otherwise the specific migration limit might be exceeded at the end of the shelf life of the PET packed mineral water.

## Specific Migration of NIAS

In addition to the monomers and additives, which are intentionally added during polymerization or processing of PET or during production of the PET packaging material, some non-intentionally added substances (NIAS) can be found in PET. In most cases, NIAS are impurities in the packaging material like traces of solvents or degradation or reaction side-products of additives or the polymer itself (*23*). Typically, NIAS are evaluated with a specific migration limit of 10 μg $l^{-1}$. Compared to the specific migration limits of monomers and additives given in Table 1, the 10 μg $l^{-1}$evaluation criteria is very low. Therefore, NIAS play an important role in the food law compliance evaluation of PET bottles.

### Acetaldehyde

One of the most important NIAS in PET is acetaldehyde. Acetaldehyde is a degradation product of the vinyl end groups of the PET polymer chain. Moisture hydrolyses the ester bond in these vinyl end groups during PET preform manufacturing and generates acetaldehyde as a volatile reaction product. Because traces of moisture are ubiquitous, acetaldehyde can be determined in every PET packaging material. Typically, its concentrations in PET bottles are between 1 mg $kg^{-1}$ and 10 mg $kg^{-1}$. From a food law compliance point of view, acetaldehyde is very easy to evaluate. Assuming a PET bottle weight of 25 g with a concentration of acetaldehyde of 10 mg per kg PET, and total mass transfer into 1 l of natural mineral water (or any other foodstuff), the concentration of acetaldehyde in food is 250 μg $l^{-1}$. This migration value is a factor of 24 below the EU's specific migration limit for acetaldehyde (6000 μg $l^{-1}$) Therefore it can be concluded that the specific migration limit of acetaldehyde in food generally cannot be exceeded.

However, acetaldehyde has a low organoleptic threshold concentration in water which is between 10 µg $l^{-1}$ (retronasal) and 25 µg $l^{-1}$ (orthonasal) (*24*). Therefore, such low concentrations of acetaldehyde in natural mineral water lead to an off-taste and the packaging material is not in compliance with Article 3 of the European Framework Regulation 1935/2004 or the U.S. Federal Food, Drug, and Cosmetic Act. In soft drinks, fruit juices and beer, however, the natural concentration of acetaldehyde in these beverages is much higher than the above mentioned threshold limits for acetaldehyde in water (*25*).

Therefore, migration of acetaldehyde into food is only relevant for natural mineral water due to possible organoleptic complaints. The prediction of the acetaldehyde food concentrations from those in the PET bottle wall at certain storage conditions might be useful for compliance evaluation as well as production control.

Figure 1 shows the correlation between the area related migration of acetaldehyde and the bottle wall concentration for PET packed natural mineral water after storage for 10 d at 40 °C (an unpublished study using 58 bottles of different natural mineral water collected from the German market). From this correlation, the concentration of acetaldehyde in natural mineral water can be predicted if the concentration of acetaldehyde in the PET bottle wall is known. Determination of acetaldehyde in PET preforms is a routine testing parameter in PET bottle manufacturing, so the concentrations of acetaldehyde in the preform or bottle wall are easily available. Moreover, the diffusion coefficient for acetaldehyde in PET at 40 °C has been determined experimentally to be 2.7 $10^{-11}$ $cm^2$ $s^{-1}$ (*26*). Therefore, Equation 1 can be used for the calculation of the acetaldehyde migration (dashed line in Figure 1). As a result, the predicted migration of acetaldehyde from PET using the above mentioned diffusion coefficient at 40 °C is in good agreement with the experimentally determined values of acetaldehyde in carbonated natural mineral water. Only four bottles show slightly higher migration values than the predicted values. The bottles used to establish the correlation given in Figure 1 were supplied by mineral water filling companies and the time-point of filling is not known exactly in every case. Therefore the higher concentration of acetaldehyde might be due to the undefined pre-storage at room temperature before storage for 10 d at 40 °C. This undefined pre-storage increases the concentration of acetaldehyde in natural mineral water. It is interesting to note that nearly all of the carbonated natural mineral water bottles show migration levels that are close to the predicted migration levels using the published diffusion coefficient, whereas the non-carbonated natural mineral water samples significantly deviate from this correlation. For non-carbonated mineral water samples, the bottle wall concentration does not correlate with the concentration of acetaldehyde in mineral water. The acetaldehyde concentration in non-carbonated mineral water is in any case lower than the predicted concentration in water. This indicates that acetaldehyde is not stable enough in non-carbonated mineral water. Most probably acetaldehyde is degraded in non-carbonated natural mineral water by microorganisms (*27*). Due to the bacteriostatic effect of carbon dioxide this degradation does not occur in carbonated water and the concentration of acetaldehyde in carbonated mineral water is therefore higher compared to non-carbonated natural mineral water.

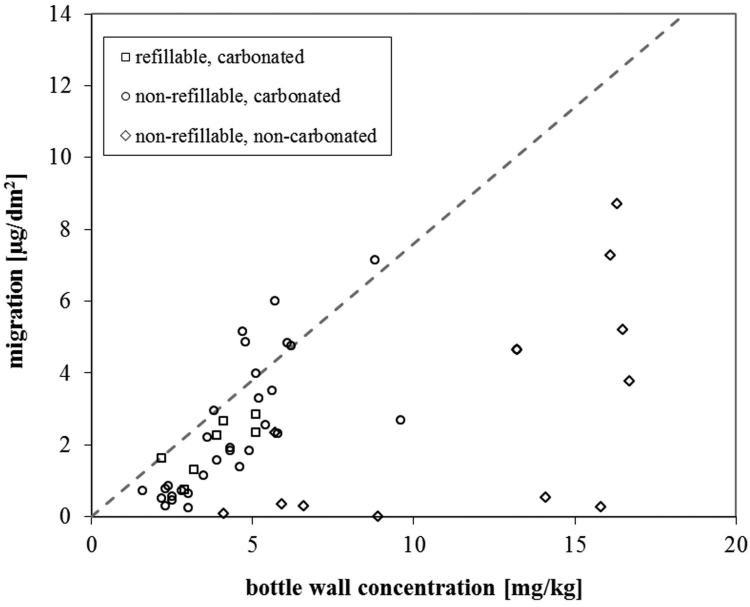


*Figure 1. Correlation between the bottle wall concentration of acetaldehyde (in mg kg$^{-1}$) and the migration (in µg dm$^2$) after storage for 10 d at 40 °C; dashed line: predicted from diffusion coefficient of 2.7 10$^{-11}$ cm$^2$ s$^{-1}$(26).*

**Formaldehyde**

Formaldehyde is also a degradation product of PET. Traces of formaldehyde might also be generated during preform and bottle manufacturing. The mechanism of this reaction is, however, not published in the scientific literature. According to EU Regulation 10/2011, the specific migration limit of formaldehyde is 15 mg l$^{-1}$. The concentration of formaldehyde in PET bottles is typically lower than those of acetaldehyde (*28*). For example, concentrations of formaldehyde in PET pellets were below the analytical detection limit. On the other hand, formaldehyde concentrations in PET bottles of 1.7 ±0.7 mg kg$^{-1}$ (bottles from Japan, n = 20), 0.7 ±0.4 mg kg$^{-1}$ (bottles from Europe, n = 13), and 0.8 ±0.4 mg kg$^{-1}$ (bottles from America, n = 5) were determined (*28*). The higher concentrations in PET bottles compared to PET pellets indicates that formaldehyde is generated during bottle manufacturing. From the concentrations given above, it can be concluded that the ratio between the determined formaldehyde and acetaldehyde concentrations was approximately 1:10.

In all water samples bottled in Japan, levels of formaldehyde were determined to be in the range of 10.1 to 27.9 µg l$^{-1}$ (*28*). On the other hand, of eleven European bottled water samples, eight did not contain formaldehyde, while the remaining three had detectable levels of 7.4 to 13.7 µg l$^{-1}$. In three North American bottled water samples, two contained formaldehyde (13.6 and 19.5 µg l$^{-1}$), and one did not (*27*). In PET sheets, the formaldehyde concentrations were lower than those found

in PET bottles. All values published in the scientific literature for formaldehyde in mineral water were far below of the specific migration limit of 15 mg $l^{-1}$ given by the European legislation.

**2-Methyl-1,3-dioxolane**

2-Methyl-1,3-dioxolane is also a substance which could be determined in every PET bottle. This substance is the reaction product of acetaldehyde and ethylene glycol (*29*). It is generated during re-extrusion of the PET pellets to PET preforms. The highest concentrations of 2-methyl-1,3-dioxolane are therefore found in PET preforms. Typically, the concentrations found for 2-methyl-1,3-dioxolane are below those found for acetaldehyde. In general, the reaction of aldehydes with alcohol functional groups is a reversible reaction with the equilibrium depending on the water concentrations. During PET preform manufacturing, the water concentration is very low, so that the equilibrium is at the 2-methyl-1,3-dioxolane side. On the other hand, the high concentration of water in food (or simulants) shifts the equilibrium concentrations towards acetaldehyde and ethylene glycol. Therefore, 2-methyl-1,3-dioxolane is not stable in aqueous simulants (or mineral water) and typically cannot be determined in natural mineral water samples.

It is interesting to note that 2-methyl-1,3-dioxolane is masking acetaldehyde in PET and makes acetaldehyde "invisible" for acetaldehyde scavengers. For example, anthranilamide does not react with 2-methyl-1,3-dioxolane. The levels of 2-methyl-1,3-dioxolane remain constant even if anthranilamide is used for the production of PET preforms. After migration into mineral water, however, 2-methyl-1,3-dioxolane re-generates acetaldehyde. This might be the reason why acetaldehyde scavengers like anthranilamide do not reduce the acetaldehyde concentrations in natural mineral water to zero levels even if high scavenger amounts were used for PET preform manufacturing.

**Cyclic Trimer of PET and Other Oligomers**

During PET polymerization and processing, oligomers were generated in the PET melt. Due to the high molecular weight of the PET oligomers, the specific migration under room temperature conditions is negligible. However, the migration of PET oligomers can be determined if high temperatures are applied, e.g. microwave cooking or baking conditions. The migration of oligomers from PET oven trays into food (lasagna, sausages, French fries, etc.) was determined at 204 °C (*30*) at contact times between 30 min and 80 min according to the recipes. The concentrations of PET oligomers were 0.10 mg $kg^{-1}$ and 1.74 mg $kg^{-1}$. Microwave trays were also tested between 1.5 min and 15 min at moderate microwave power (600 W). The concentrations of PET oligomers were between 0.02 mg $kg^{-1}$ and 2.73 mg $kg^{-1}$. Of these two test conditions, the highest values were found when using baking conditions, e.g. 175 °C for 120 min or 150 °C for 30 min. The migration of PET oligomers into olive oil was determined to be 17.0 mg $kg^{-1}$ and 12.0 mg $kg^{-1}$, respectively (*31*). In another study, quantities of PET

oligomers migrated from aluminized PET susceptor films into microwave cooked foods ranged from less than 0.012 mg $kg^{-1}$ to approximately 7 mg $kg^{-1}$ (*32*).

The migration of cyclic oligomers into PET packed beverages was much lower, because the measure levels were from beverages stored under normal storage conditions. These concentrations were determined to be below the analytical detection limit of 0.05 mg $kg^{-1}$ and 0.29 mg $kg^{-1}$ (*30*). It should be noted, that the cyclic trimer of PET is present at significantly higher concentrations in PET than other oligomers like dimers or tetramers. Therefore the values mentioned above are mostly related to the cyclic trimer of PET.

The diffusion coefficients of the cyclic trimer of PET at high temperatures were determined experimentally to 2.9 $10^{-9}$ $cm^2$ $s^{-1}$ (176 °C), 6.6 $10^{-10}$ $cm^2$ $s^{-1}$ (146 °C) and 1.2 $10^{-12}$ $cm^2$ $s^{-1}$ (115 °C) (*33*). The values are in agreement with the predicted diffusion coefficients of 1.4 $10^{-9}$ $cm^2$ $s^{-1}$ (176 °C), 2.5 $10^{-11}$ $cm^2$ $s^{-1}$ (146 °C) and 2.0 $10^{-13}$ $cm^2$ $s^{-1}$ (115 °C) according to Equation 6.

**Phthalate and Adipate Esters**

Contrary to several publications in the scientific literature, phthalate and adipate esters were not used as plasticizers or additives in the manufacturing of PET or PET bottles. However, trace amounts in concentrations below 1 mg $kg^{-1}$ might be found in PET bottles. These trace amounts are due to contamination during manufacturing or transport. Due to the high molecular weights of the substances and the low concentrations in PET, the migration thereof is negligible.

**Limonene**

Limonene is a contaminant in PET bottles which is related to the use of post-consumer PET recyclate. It can be determined in trace amounts in PET bottles, which were produced with a certain amount of post-consumer recyclates. However, the applied recycling processes reduce the concentrations of limonene in PET recyclates to values below the analytical detection limits of the applied screening methods (see Topic on Post-consumer Recycled PET in Direct Food Contact). However, trace amounts at the lower μg $kg^{-1}$ level can be found in some post-consumer PET recyclates.

Limonene acts also as an aroma compound and some mineral water filling companies control the amount of limonene in PET recyclates in order to prevent organoleptic complaints. Figure 2 shows the correlation between the bottle wall concentration of limonene and the storage time at room temperature and 40 °C. The bottle wall concentrations shown in these correlations correspond to a migration of 35 μg $l^{-1}$, which is the taste threshold of limonene in water (*34*). For the calculation, realistic PET diffusion coefficients were used (Equation 6). As a result, the maximum concentrations of limonene in the PET bottle wall corresponding with the taste threshold limit were calculated to be 1240 mg $kg^{-1}$ for storage at room temperature and 300 mg $kg^{-1}$ for storage at 40 °C. For both values, a storage time of 1 year was assumed. At shorter storage times, the maximum concentrations of limonene in the bottle wall are even significantly higher. In conclusion, the taste threshold of limonene cannot be reached from

recyclate containing PET bottles. However, due to the fact, that limonene is detectable in nearly every post-consumer recyclate; limonene can be used as an internal indicator for the cleaning efficiency of the recycling process.

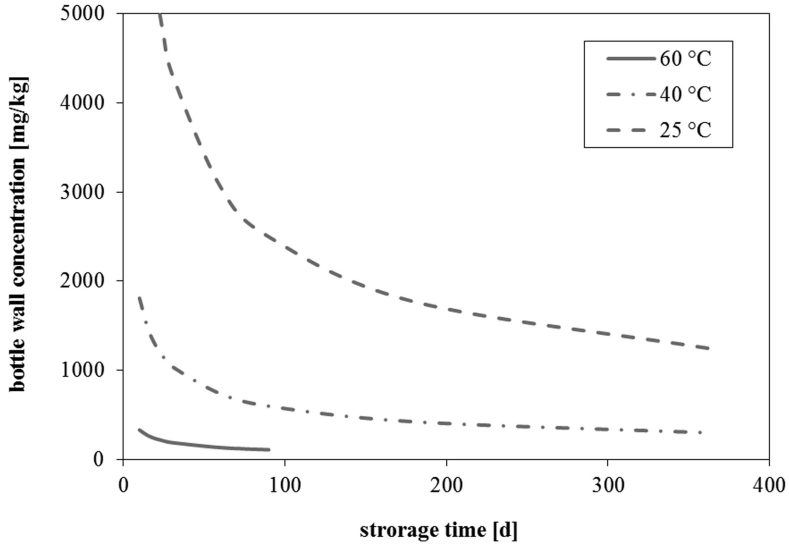


*Figure 2. Calculated bottle wall concentrations of limonene corresponding to a migration of 35 µg l-1 (taste threshold limit of limonene in water) as a function of the storage time and temperature calculated according with realistic diffusion coefficients (Equation 6).*

**Toluene, Xylenes, and Other Solvents**

In some PET bottles traces of solvents like toluene or xylenes (three isomers) are found. These substances are not listed in European Regulation 10/2011 (*6*). The sources of such solvents are not clear, but it seems that these solvents are impurities of monomers, additives or processing aids. Due to the fact that solvents are not regulated by Regulation 10/2011, a 10 µg $l^{-1}$ migration limit typically used for NIAS should be used for evaluation.

The concentrations of toluene and *para*-xylene are typically below 1 mg $kg^{-1}$. Assuming such a concentration the migration of toluene after storage of 365 d at 25 °C is 1.3 µg $l^{-1}$ (calculated with $A'_P = 3.1$ and $\tau = 1577$ K) (*9*). Using realistic diffusion coefficients (Equation 6) the specific migration of toluene is 0.24 µg $l^{-1}$. Since *para*-xylene has a slightly higher molecular weight than toluene, its diffusion coefficients are lower, and as a consequence the migration is lower than that calculated for toluene. In conclusion, trace amounts of toluene and *para*-xylene in PET are typically not a problem.

Another solvent, tetrahydrofuran, can be determined in some PET bottles. Most probably tetrahydrofuran is the residual solvent of the colorants, which is not completely removed during colorant manufacturing. In some cases, the

concentration is higher than that found for toluene and *para*-xylene. However, tetrahydrofuran has a specific migration limit of 0.6 mg $l^{-1}$ which is relatively high so that the trace amounts typically found in colored PET bottles are not relevant for compliances evaluation.

### Cyclopentanone and iso-Phthaldialdehyde

In some barrier bottles with polyamides the substances cyclopentanone and *iso*-phthaldialdehyde could be determined. Polyamide is used as a barrier material to oxygen. If the polyamide layer is in the core layer of multilayer bottles, the migration of both substances is negligible, because the substances are behind a functional barrier of PET. If the polyamide, however, is introduced into the PET bottle as a blend, migration for cyclopentanone is much higher. For example, cyclopentanone at a typical concentration of 15 mg $kg^{-1}$ results in a migration of 20 µg $l^{-1}$ after storage of 365 d at 25 °C as predicted using the $A_P$ model ($A'_P$ = 3.1 and $\tau$ = 1577 K) (Equation 4). This value exceeds the 10 µg $l^{-1}$ limit which is typically used as an evaluation criterion for NIAS. Using the realistic approach for the prediction of the diffusion coefficient of cyclopentanone (Equation 6), a concentration of 6.7 µg $l^{-1}$ is calculated under the same side conditions. This value meets the 10 µg $l^{-1}$ criterion.

## Permeation of Label Components through the PET Bottle Wall

Contamination of food might also occur by permeation of substances from the environment through the PET bottles wall. For PET bottles and trays, such substances might be located in the adhesives of the labels. In order to evaluate the amount of permeated substances from the adhesives, migration modeling can be applied. For this purpose, the permeation of potential migrants from the adhesive was calculated in a two layer system. The first layer was assumed to be a 50 µm thick adhesive film. The second layer represented the PET bottle of variable layer thickness, which contacts the food assuming that 1 l of food is surrounded by a packaging surface of 6 $dm^2$. The packaging is assumed to be fully covered with adhesive (6 $dm^2$), which can be considered as the worst-case. It was also assumed that the adhesive components have good solubility in the food (partition coefficient K = 1). For PET, the parameters $A'_P$ = 3.1 and $\tau$ = 1577 K were used for the calculations (*9*). For the adhesive layer, higher diffusivity was assumed with values of $A'_P$ = 11.5 and $\tau$ = 0 K which is used for a high diffusive polymer like low density polyethylene (LDPE) (*9*). The calculations were carried out using model substances of defined molecular weight, namely independent of a given adhesive system. The permeation was calculated at 25 °C and 40 °C, respectively. In each case, a contact time of 365 d was assumed. The initial concentration of the adhesive components in the PET bottle wall was set to 1000 mg $kg^{-1}$. As the permeation of the adhesive components is a linear function of the initial concentration, the permeation value calculated for an initial concentration of 1000 mg $kg^{-1}$ can be used for other initial concentrations by multiplying by the relevant conversion factor.

The calculated results for the permeation of label adhesive components through the PET layer are shown in Table 3 and Table 4 as a function of the layer thickness and the molecular weight of the permeating substance. As expected, the smallest molecules show the highest migration. At room temperature (25 °C) there was no breakthrough of adhesive components even after 365 d, provided the thickness of the PET bottle wall is at least 100 μm. After storage for 365 d at 40 °C, there was no significant permeation of adhesive components into the food for a PET wall thickness of 250 μm. The typical wall thickness of PET bottles is 300 μm. It can therefore be concluded that at room temperature or 40 °C, no adhesive components can permeate through the bottle wall into the food. As the permeation was calculated for model components (based on their molecular weight), the permeation is independent of a given adhesive system.

Assuming realistic diffusion coefficients predicted from Equation 6 the lag time of small molecules (e.g. acetone) are in the range of about 40 years at room temperature for a 300 μm PET bottle wall. Higher molecular weight compounds will have correspondingly higher lag times. In summary, the calculations show that permeation of label adhesive components through PET bottle walls can be neglected under normal storage conditions.

## Post-Consumer Recycled PET in Direct Food Contact

Post-consumer recycled PET might contain substances which are atypical for PET packaging materials. The potential migrants originate from food (e.g. flavor components), from the recycling processes (e.g. degradation products from the polymer) or from misuse of the PET containers for storage of household or garden chemicals (e.g. solvents). Therefore, the evaluation of post-consumer PET recyclates is in principle similar to the evaluation of NIAS in PET packaging materials. However, the identities are not known and concentration ranges of potential migrants in post-consumer recycled PET are completely different. In post-consumer recyclates, the concentrations of NIAS range from trace impurities, in the case of flavor compounds, up to the percentage range in the case of misused PET bottles for storage of solvents. On the other hand, it is very rare event that a misused PET bottle enters the recycling input stream. As such, the highly contaminated, misused individual PET bottle is likely mixed with thousands of non-misused PET bottles, which dilutes the concentrations of hazardous chemicals in the whole recycling input stream. Within a European project, the concentrations of three substances not typically found in foods and present due to possible improper bottle usage in washed post-consumer PET flakes were determined to be 1.4 mg $kg^{-1}$ to 2.7 mg $kg^{-1}$ (*35*). The average concentration of substances typically found in foods, such as the aroma component limonene, was on average 2.9 mg $kg^{-1}$ over all the tested post-consumer recyclates. The concentrations of post-consumer substances like aroma compounds or chemicals from misused PET bottles are in a similar concentration range to other NIAS found in PET (see Topic on Specific Migration of NIAS).

**Table 3. Calculated Permeation of Components through PET Layers of Different Thickness at a Temperature of 25 °C and after a Storage Time of 365 d ($A'_P$ = 3.1, $\tau$ = 1577, K = 1, $c_{p,0}$ = 1000 mg kg$^{-1}$)**

| *Molecular weight [g mol$^{-1}$]* | *Concentration in the food [µg l$^{-1}$] for different PET bottle wall thicknesses* | | | | | |
|---|---|---|---|---|---|---|
| | *50 µm* | *100 µm* | *150 µm* | *200 µm* | *250 µm* | *300 µm* |
| 50 | 80.5 | 0.243 | 9.4 10$^{-8}$ | n.c. | n.c. | n.c. |
| 100 | 2.45 | 3.9 10$^{-9}$ | n.c. | n.c. | n.c. | n.c. |
| 150 | 9.7 10$^{-6}$ | n.c. | n.c. | n.c. | n.c. | n.c. |
| 200 | n.c. | n.c. | n.c. | n.c. | n.c. | n.c. |
| 250 | n.c. | n.c. | n.c. | n.c. | n.c. | n.c. |
| 300 | n.c. | n.c. | n.c. | n.c. | n.c. | n.c. |
| 400 | n.c. | n.c. | n.c. | n.c. | n.c. | n.c. |
| 500 | n.c. | n.c. | n.c. | n.c. | n.c. | n.c. |

n.c.: not calculated because very small values are obtained.

**Table 4. Calculated Permeation of Components through PET Layers of Different Thickness at a Temperature of 40 °C and after a Storage Time of 365 d ($A'_P$ = 3.1, $\tau$ = 1577, K = 1, $c_{p,0}$ = 1000 mg kg$^{-1}$)**

| *Molecular weight [g mol$^{-1}$]* | *Concentration in the food [µg l$^{-1}$] for different PET bottle wall thicknesses* | | | | | |
|---|---|---|---|---|---|---|
| | *50 µm* | *100 µm* | *150 µm* | *200 µm* | *250 µm* | *300 µm* |
| 50 | 1480 | 429 | 877 | 119 | 1.06 | 6.2 10$^{-5}$ |
| 100 | 551 | 451 | 1.35 | 1.4 10$^{-5}$ | 4.8 10$^{-8}$ | 5.1 10$^{-11}$ |
| 150 | 148 | 1.39 | 1.4 10$^{-6}$ | 1.3 10$^{-10}$ | n.c. | n.c. |
| 200 | 23.5 | 5.9 10$^{-6}$ | n.c. | n.c. | n.c. | n.c. |
| 250 | 1.69 | n.c. | n.c. | n.c. | n.c. | n.c. |
| 300 | 3.7 10$^{-5}$ | n.c. | n.c. | n.c. | n.c. | n.c. |
| 400 | 3.7 10$^{-11}$ | n.c. | n.c. | n.c. | n.c. | n.c. |
| 500 | n.c. | n.c. | n.c. | n.c. | n.c. | n.c. |

n.c.: not calculated because very small values are obtained.

On the other hand, the concentrations of atypical substances are significantly higher in samples before recycling (e.g. washing of the ground PET bottles) because the recycling process significantly decreases the concentrations of atypical substances. Therefore, the evaluation principle of post-consumer PET recyclates in direct food contact is essentially based on the cleaning efficiency of the recycling process. The cleaning efficiency is determined by a so-called Challenge Test (*36*). A Challenge Test is carried out on PET that has been deliberately contaminated with model contaminants to simulate misuse of PET bottles, such as for the storage of solvents or household chemicals. The artificially contaminated or "challenged" plastic is then subjected to the recycling process. The difference between the initial contamination and the residual contamination for each surrogate in the end product is a measure of the cleaning efficiency of the recycling process.

**U.S. FDA Evaluation**

The FDA suggests that dietary exposures to contaminants from recycled food contact articles that result in a dietary concentration of 0.5 ppb or less are generally of negligible risk (*37*). With help of so-called consumption factors (CF), which is the proportion of PET to the total of food packaging materials, this dietary exposure can be converted into migration limits. For recycled PET used for food contact, for instance, the FDA system applies a CF = 0.05 as the currently valid consumption factor for each post-consumer plastic, and therefore the migration limit of PET recyclate containing food packaging is 10 μg $l^{-1}$ for each individual surrogate. Migration modelling can be used to convert this migration limit into a maximum bottle wall concentration for any substance occurring in post-consumer plastics, including substances from virgin polymers. Table 5 summarizes the maximum bottle wall concentrations for several surrogates which correspond to a migration value of 10 μg $l^{-1}$. The time-temperature conditions for the calculations were: storage time 10 d, storage temperature 40 °C, volume of food 1 l, surface area 600 $cm^2$, partition coefficient K = 1 (for good solubility).

It is noteworthy that the FDA has not defined a specific migration model for use in the calculations. The $A_P$ model is accepted by the FDA, but also other scientific recognized methods for the prediction of the diffusion coefficients might be accepted. Therefore, the maximum residual concentrations in Table 5were determined with the currently accepted migration model using $A'_P = 3.1$ ($\tau = 1577$ K) (*9*) and with the "realistic" method (*10*). As target compounds for the migration calculations, the model compounds (surrogates) typically recommended for a challenge test were used (*36*). The concentrations given in Table 5 are considered as maximum concentrations in the PET bottle wall produced from contaminated and recycled PET in a challenge test. If the concentrations of the surrogates are below the concentrations as given in Table 5, then the recyclate containing PET bottle is considered in compliance with U.S. FDA regulations.

**Table 5. Maximum Residual Concentrations $c_0$ Corresponding to a Migration Limit Equal to or Smaller than 10 µg $l^{-1}$ Estimated from Diffusion Models (*9*) Calculated with K = 1, 10 d at 40 °C, Volume 1 l, Surface Area 600 $cm^2$)**

| *Substance* | *Molecular weight [g $mol^{-1}$]* | *Molecular volume [$Å^3$]* | *Calculated maximum residual concentration $c_{P,0}$ [mg $kg^{-1}$] corresponding to a migration of 10 µg $l^{-1}$* | |
|---|---|---|---|---|
| | | | *$A'_P$ = 3.1, $\tau$ = 1577 K (9)* | *variable activation energy $E_A$ (10)* |
| Toluene | 92 | 100.6 | 18.3 | 78.6 |
| Chlorobenzene | 113 | 97.6 | 21.8 | 69.0 |
| Chloroform | 119 | 70.1 | 22.7 | 17.3 |
| Methyl salicylate | 152 | 136.5 | 29.7 | 287 |
| Phenyl cyclohexane | 160 | 174.0 | 30.7 | 792 |
| Benzophenone | 182 | 174.4 | 35.2 | 800 |
| Methyl stearate | 298 | 342.6 | 69.1 | 13700 |
| fictive substance | 400 | / | 113.5 | / |
| fictive substance | 500 | / | 177.3 | / |

**EFSA Evaluation**

The EFSA evaluation (*38*) is based on cleaning efficiencies of the applied recycling processes. The cleaning efficiencies are determined by use of a challenge test with artificial contaminated PET flakes. EFSA assumes a concentration of 3 mg $kg^{-1}$ per substance in the washed PET flakes as a worst-case scenario. This concentration follows the findings and conclusions of the above mentioned European research project (*35*). The next step involved linking this worst-case concentration to the required cleaning efficiency of the recycling process. On the basis of a standardized initial concentration of 3 mg $kg^{-1}$, the residual concentration in the recyclate ($c_{res}$) was specified. This means that the residual concentration after the recycling process is independent of the initial concentration actually used in the challenge test. This simplifies the evaluation and facilitates comparison of different recycling processes. The residual concentration after recycling $c_{res}$ is therefore specific to a (model) substance and a recycling process.

However, a concentration of 3 mg $kg^{-1}$ per substance in the washed flakes is unsuitable for a challenge test, because the very high cleaning efficiency of a super-clean PET recycling process means that the analytical detection limits would quickly be reached and the cleaning efficiencies cannot be determined. EFSA hence stipulates that the challenge test is carried out with (considerably) higher concentrations. This allows the challenge test to be carried out under optimum conditions. However, the cleaning efficiency, namely the key parameter for calculating $c_{res}$, would then not be available. If the initial concentration of the (artificial) contaminants was too high, this could lead to artifacts because the recycling processes can no longer be operated under routine conditions. For the EFSA evaluation process, the higher initial concentrations for the challenge test are then normalized to the standardized initial concentration of 3 mg $kg^{-1}$ using a factor for the respective (model) substance. The final concentrations from the challenge test then naturally have to be corrected by the same factor. In the last step, the normalized residual concentration $c_{res}$ is compared to a (calculated) concentration ($c_{mod}$).

The concentration $c_{mod}$ represents the expected migration of a (model) substance into the food by the end of the shelf-life of the packaged food. In the case of bottle-to-bottle recycling, it is mainly recyclable bottles for mineral water and soft drinks. Consequently, EFSA has set a storage time of 365 d at 25 °C as the boundary usage conditions. In contrast to the U.S. FDA, the EFSA specified the predictive model parameters for calculating $c_{mod}$, namely $A'_P = 3.1$ and $\tau = 1577$ K (*9*). The calculation for $c_{mod}$ also assumes that 1 l drink is packaged in packaging having a surface area of 6 $dm^2$. It is also assumed that the PET bottle is made of 100% recyclate and that the migrant has good solubility in the drink (partition coefficient K = 1).

EFSA adopts an exposure scenario for toxicological evaluation of post-consumer PET recyclates. In principle, it is impossible to individually evaluate all possible substances that could come into contact with the PET bottles during their first usage or if there is misuse of the bottles. This is why the evaluation can only be undertaken by considering “worst-case” scenarios. In adopting a pragmatic approach, EFSA assumes that exposure to 0.0025 µg of a substance per kg body weight per day is not harmful to human health. This threshold is low enough to cover all toxicological effects. This also ensures that even unknown contaminants are dealt with conservatively. For the exposure scenario, EFSA considers an infant having a body weight of 5 kg. It is assumed that this infant drinks 0.75 l water from a PET bottle made of 100% recyclate. This means that the migration into the food by the end of the shelf-life may not exceed 0.017 µg $l^{-1}$. From this concentration one can calculate a concentration in the PET bottle wall under the aforementioned use conditions. This value corresponds to the (calculated) concentration $c_{mod}$. Due to the fact that the underlying $A_P$ migration model for calculating the corresponding maximum concentration in the PET bottle wall ($c_{mod}$) overestimates the migration by at least a factor of 5, EFSA rounds the value of 0.017 µg $l^{-1}$ to 0.1 µg $l^{-1}$., This means that the migration of a substance from a PET bottle containing recyclate by the end of its shelf-life may be present at a maximum concentration of 0.1 µg $l^{-1}$. If at the end of the shelf-life the concentration $c_{res}$ of each (model) substance is smaller than $c_{mod}$, then the

recycling process is deemed to be safe. Due to the different amounts of food people eat relative to their body weight, older children (toddlers) and adults have higher migration values of 0.15 µg $l^{-1}$ and 0.75 µg $l^{-1}$, respectively, compared to infants. These higher values also mean proportionally lower minimum cleaning efficiencies be required. As mineral water producers clearly cannot ensure that only adults consume water from recyclate-containing PET bottles, only the lowest limit value (for infants) actually applies.

Due to the fact that migration is dependent on the molecular weight of the migrant, the required minimum cleaning efficiency can be calculated for different (model) substances. Conversely, the maximum bottle wall concentration can be calculated assuming migration of 0.1 µg $l^{-1}$ after storage for 365 d at 25 °C. The calculated maximum bottle wall concentrations ($c_{mod}$) as well as the relationship between the cleaning efficiency and the molecular weight of the migrating substance are shown in Figure 3.

## Conclusions

From the data and examples compiled within this review the following main conclusions for virgin and safely recycled PET migration testing can be drawn:

- Experimental overall migration tests are superfluous because the overall migration limit cannot be exceeded, even if worst case conditions and swelling simulants like 95% ethanol are applied.

- Experimental specific migration tests for the monomers and catalysts like ethylene glycol, diethylene glycol, terephthalic acid, *iso*-phthalic acid and antimony are also superfluous, because the specific migration limits cannot be exceeded.

- The $A_P$ model with $A'_P = 3.1$ ($\tau = 1577$ K) is extremely over-estimative for molecules with molecular weight of >100 g $mol^{-1}$ and in some cases not useful for compliance evaluation of PET food contact articles. The currently accepted $A_P$ model should be exchanged by a more realistic, but still (slightly) over-estimative migration model.

- Low molecular weight NIAS might exceed a mass transfer of 10 µg $l^{-1}$ from the packaging material into food (simulants). Therefore such compounds should be investigated with a suitable screening method in the PET food contact material.

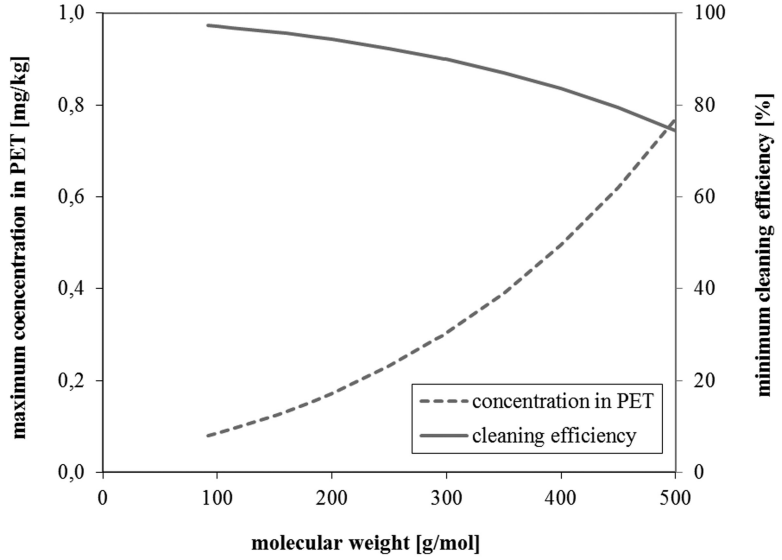


*Figure 3. Correlation of the calculated maximum bottle wall concentrations ($c_{mod}$), the corresponding minimum cleaning efficiency and the molecular weight of the migrating substance.*

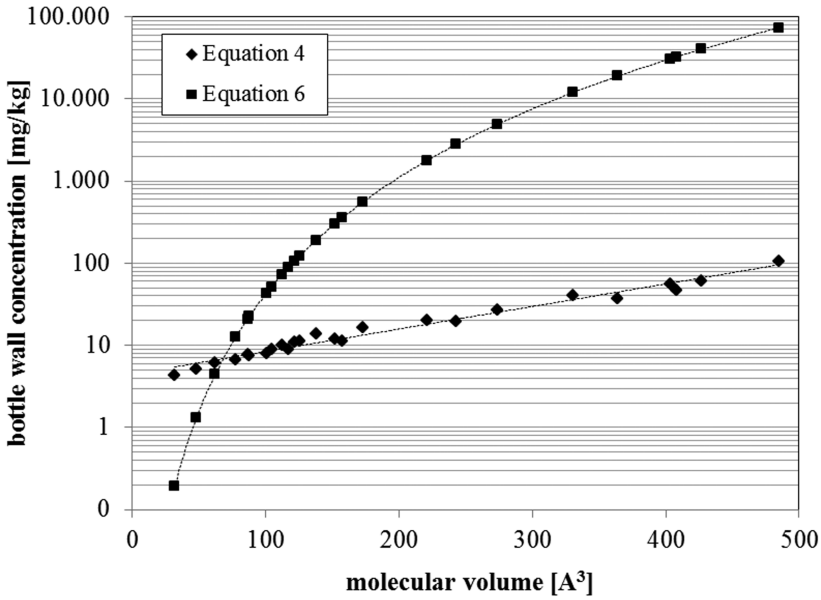


*Figure 4. Correlation between the bottle wall concentration (in mg kg$^{-1}$) which corresponds to a migration of 10 µg l$^{-1}$ after storage for 365 d at 25 °C and the molecular volume of the migrant (diffusion coefficients from Table 6).*

**Table 6. Diffusion Coefficients Predicted According to the Current $A_P$ Model (*9*) and the Realistic Approach (*10*)**

| *Migrant* | *Prediction model* | *Diffusion coefficient [$cm^2$ $s^{-1}$]* | | |
|---|---|---|---|---|
| | | *25 °C* | *40 °C* | *60 °C* |
| Acetaldehyde | $A_P$ = 3.1, τ = 1577 K | $1.4\ 10^{-13}$ | $9.7\ 10^{-13}$ | $9.7\ 10^{-12}$ |
| | realistic | $2.1\ 10^{-12}$ | $1.0\ 10^{-11}$ | $6.7\ 10^{-11}$ |
| 2-Aminobenzonitrile | $A_P$ = 3.1, τ = 1577 K | $3.7\ 10^{-14}$ | $2.5\ 10^{-13}$ | $2.5\ 10^{-12}$ |
| | realistic | $6.9\ 10^{-16}$ | $8.2\ 10^{-15}$ | $1.6\ 10^{-13}$ |
| Anthranilamide | $A_P$ = 3.1, τ = 1577 K | $2.8\ 10^{-14}$ | $1.9\ 10^{-13}$ | $1.9\ 10^{-12}$ |
| | realistic | $2.4\ 10^{-16}$ | $3.2\ 10^{-15}$ | $7.1\ 10^{-14}$ |
| Bisphenol A | $A_P$ = 3.1, τ = 1577 K | $8.5\ 10^{-15}$ | $5.9\ 10^{-14}$ | $5.9\ 10^{-13}$ |
| | realistic | $1.1\ 10^{-18}$ | $2.7\ 10^{-17}$ | $1.2\ 10^{-15}$ |
| Cyclopentanone | $A_P$ = 3.1, τ = 1577 K | $6.4\ 10^{-14}$ | $4.4\ 10^{-13}$ | $4.4\ 10^{-12}$ |
| | realistic | $7.0\ 10^{-15}$ | $6.4\ 10^{-14}$ | $9.0\ 10^{-13}$ |
| Diethylene glycol | $A_P$ = 3.1, τ = 1577 K | $4.4\ 10^{-14}$ | $3.0\ 10^{-13}$ | $3.1\ 10^{-12}$ |
| | realistic | $1.3\ 10^{-15}$ | $1.5\ 10^{-14}$ | $2.6\ 10^{-13}$ |
| Ethylene glycol | $A_P$ = 3.1, τ = 1577 K | $9.6\ 10^{-14}$ | $6.7\ 10^{-13}$ | $6.7\ 10^{-12}$ |
| | realistic | $1.9\ 10^{-13}$ | $1.2\ 10^{-12}$ | $1.1\ 10^{-11}$ |
| Formaldehyde | $A_P$ = 3.1, τ = 1577 K | $2.0\ 10^{-13}$ | $1.4\ 10^{-12}$ | $1.4\ 10^{-11}$ |
| | realistic | $1.2\ 10^{-10}$ | $3.3\ 10^{-10}$ | $1.3\ 10^{-9}$ |
| 1,4-bis(Hydroxymethyl) | $A_P$ = 3.1, τ = 1577 K | $2.5\ 10^{-14}$ | $1.7\ 10^{-13}$ | $1.7\ 10^{-12}$ |

| *Migrant* | *Prediction model* | *Diffusion coefficient [cm² s⁻¹]* | | |
|---|---|---|---|---|
| | | *25 °C* | *40 °C* | *60 °C* |
| cyclohexane | realistic | $3.8\ 10^{-17}$ | $6.3\ 10^{-16}$ | $1.8\ 10^{-14}$ |
| *iso*-Phthaldialdehyde | $A_P = 3.1$, $\tau = 1577$ K | $2.9\ 10^{-14}$ | $2.0\ 10^{-13}$ | $2.0\ 10^{-12}$ |
| | realistic | $3.1\ 10^{-16}$ | $4.1\ 10^{-15}$ | $8.7\ 10^{-14}$ |
| Limonene | $A_P = 3.1$, $\tau = 1577$ K | $2.8\ 10^{-14}$ | $1.9\ 10^{-13}$ | $1.9\ 10^{-12}$ |
| | realistic | $2.8\ 10^{-17}$ | $4.8\ 10^{-16}$ | $1.4\ 10^{-14}$ |
| 2-Methyl-1,3-dioxolane | $A_P = 3.1$, $\tau = 1577$ K | $6.0\ 10^{-14}$ | $4.1\ 10^{-13}$ | $4.1\ 10^{-12}$ |
| | realistic | $8.0\ 10^{-15}$ | $7.2\ 10^{-14}$ | $1.0\ 10^{-14}$ |
| PET cyclic trimer | $A_P = 3.1$, $\tau = 1577$ K | $3.2\ 10^{-16}$ | $2.2\ 10^{-15}$ | $2.2\ 10^{-14}$ |
| | realistic | $6.4\ 10^{-22}$ | $3.7\ 10^{-20}$ | $4.6\ 10^{-18}$ |
| Terephthalic acid and *iso*-phthalic acid | $A_P = 3.1$, $\tau = 1577$ K | $1.8\ 10^{-14}$ | $1.3\ 10^{-13}$ | $1.3\ 10^{-12}$ |
| | realistic | $9.6\ 10^{-17}$ | $1.4\ 10^{-15}$ | $3.6\ 10^{-14}$ |
| Terephthalic acid dimethyl ester | $A_P = 3.1$, $\tau = 1577$ K | $1.3\ 10^{-14}$ | $8.8\ 10^{-14}$ | $8.9\ 10^{-13}$ |
| | realistic | $1.1\ 10^{-17}$ | $2.1\ 10^{-16}$ | $7.2\ 10^{-15}$ |
| Tetrahydrofurane | $A_P = 3.1$, $\tau = 1577$ K | $7.9\ 10^{-14}$ | $5.5\ 10^{-13}$ | $5.5\ 10^{-12}$ |
| | realistic | $2.2\ 10^{-14}$ | $1.8\ 10^{-13}$ | $2.1\ 10^{-12}$ |
| Toluene | $A_P = 3.1$, $\tau = 1577$ K | $5.6\ 10^{-14}$ | $3.8\ 10^{-13}$ | $3.9\ 10^{-12}$ |
| | realistic | $2.0\ 10^{-15}$ | $2.1\ 10^{-14}$ | $3.5\ 10^{-13}$ |

*Continued on next page.*

**Table 6. (Continued). Diffusion Coefficients Predicted According to the Current $A_P$ Model (*9*) and the Realistic Approach (*10*)**

| *Migrant* | *Prediction model* | *Diffusion coefficient [$cm^2$ $s^{-1}$]* | | |
|---|---|---|---|---|
| | | *25 °C* | *40 °C* | *60 °C* |
| *para*-Xylene | $A_P = 3.1$, $\tau = 1577$ K | $4.4\ 10^{-14}$ | $3.0\ 10^{-13}$ | $3.1\ 10^{-12}$ |
| | realistic | $4.6\ 10^{-16}$ | $5.7\ 10^{-15}$ | $1.2\ 10^{-13}$ |

The bottle wall concentrations, which correspond to a migration of 10µg $l^{-1}$ are given in Figure 4. These concentrations were calculated for a PET container with a volume of 1.0 l and a surface area of 600 $cm^2$ under the storage condition of were 365 d at 25 °C, which typically represents the maximum shelf life of PET bottled mineral water and most of all other PET packed food. The diffusion coefficients used for the prediction of the bottle wall concentrations are given in Table 6. Figure 4 shows that the current migration model based on $A_P$ values (*9*) is significantly over-estimating the migration for higher molecular weight compounds. For example, the realistic diffusion coefficients for molecular weight of 300 to 500 g $mol^{-1}$ are a factor of $10^5$ to $10^6$ lower than that was predicted using the currently accepted $A_P$ model. Small molecules like solvents, however, are under-estimated. The main reason for under-estimation for small molecules like acetaldehyde in the $A_P$ model is that due to the use of the fixed activation energy of diffusion for all migrants. For most migrants, an activation energy of diffusion of 100 kJ $mol^{-1}$ is a conservative parameter, which leads to an over-estimation of the migration. However, for a very small molecule, e.g. acetaldehyde, the activation energy of diffusion is significantly as low as 100 kJ $mol^{-1}$ (*26*).

As an overall conclusion, PET is a very inert polymer in comparison to other packaging materials with very low interactions between food and packaging. The above given procedure for compliance evaluation which is based on the determination of migration relevant substances in PET and the calculation of the migration by use of realistic migration models is much faster and also cheaper than the experimental migration tests. In addition, the screening for low molecular weight migrants (NIAS) gives an additional safety factor, because NIAS were not determined using the conventional migration testing procedures.

## Acknowledgments

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Chapter 17

# Cellulose Nanocrystals: A Potential Nanofiller for Food Packaging Applications

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This chapter focuses on emerging technologies developed for the fabrication of cellulose nanocrystal (CNC) based composite films for food packaging applications. Due to its strong reinforcing effect, CNC is a promising smart material for application in several fields such as healthcare, biomedical engineering, packaging etc. These bionanoparticles possess several attractive properties such as biodegradability, non-toxicity and bio-based origin. Fabrication of CNC based polymeric films via industrially viable approaches is a challenging task. Therefore, novel strategies for surface modification and innovative fabrication techniques need to be developed to effectively disperse CNCs into polymeric matrices. CNCs, due to their bio-origin and renewability, are emerging nanomaterials for the 21st century, whose demand will continue to grow in the near future. In response, robust, cost effective, high volume industrial scale production processes for CNCs are required to meet the growing demand.

## Introduction

Cellulose is a unique, abundantly available biomaterial which possesses several favorable properties such as renewability, biodegradability and non-toxicity. It is a polysaccharide consisting of repeating β-D-glucopyranose

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units with three hydroxyl groups per anhydrous glucose unit giving it a high functionality. Cellulose has widely been used either in pure form or as derivatives for fabrication of wide array of products in several industries such as food, pharmaceutical etc (*1*). Aqueous colloidal solution of cellulose micelles was first obtained by Ranby in 1950s through sulfuric acid treatment (*2*). TEM images of dried powdered nanocellulose having needle shaped morphology were first reported by Mukherjee et al. in 1953 (*3*). The terminology "nanocellulose" was first coined by Turbak, Snyder and Sandberg in the late 1970s at the ITT Rayonier Lab in Whippany, New Jersey, USA to describe the gel-like product formed by wood pulp homogenization at high temperature and high pressure (*1*, *4*). In the early 1980s, several patents were granted to ITT Rayonier detailing the preparation and application of these cellulosic nanomaterials (*1*).

Acid hydrolysis of cellulose fibers has been found to produce rod-like cellulose particles of nanometer dimensions, called cellulose nanocrystals (CNCs). Several terms are employed in the literature when referring to CNCs such as nanowhiskers, nanocrystals, nanoparticles, micro crystallites or nanofibers. These rod-like cellulose nanoparticles possess many unique morphological aspects such as nanoscale dimension, high aspect ratio, high surface area as well as favorable properties such as high specific strength, unique optical transparency etc. Due to its attractive physicochemical and structural properties, CNC has received considerable interest from academia and industry. Therefore, at present, significant research is being undertaken on CNC based technologies.

When incorporated into polymer matrix, CNCs can improve the mechanical properties of the neat polymer. Moreover, CNCs can also intercalate into the polymer matrix, resulting in the improvement of the water vapor and oxygen barrier properties. This may be attributed to the creation of torturous pathways by the dense polymer-CNC network that hinders the diffusion of small gas molecules. Biopolymers and biodegradable polymers (biopolymers that are biodegradable) are currently being explored as alternatives to conventional polymers which usually possess high water and/or gas permeability which may be undesirable for several applications. Incorporation of CNCs into such biopolymers is expected to significantly improve their gas barrier properties without altering the biodegradability. In fact, CNCs have been reported to decrease the oxygen permeability of biopolymers such as poly (lactic acid) (PLA) significantly which makes CNCs a potential filler for use in packaging applications (*5*).

In the twentieth century, there have been significant advancements in the packaging industry and a rapid increase in plastic use has been observed, especially in food packaging applications. During the storage of raw or minimally processed food for long periods of time, there is a risk of biofilm formation due to microbial contamination, oxidation, surface dehydration etc. The safety and quality of polymer based packaged food may be compromised not only by significant permeation of oxygen, water vapor and other gases, but also by migration of potentially toxic chemicals from the packaging material to the food product. Biodegradable polymers are defined as those that undergo mineralization by microbial chain scission under specific conditions in terms of pH, temperature, humidity etc. Such environment friendly polymers can be synthesized from petrochemical precursors (e.g. polycaprolactone), obtained from bio-sources

such as corn, wood etc. (e.g. cellulose) or synthesized by bacterial fermentation (e.g. polyhydroxyalkanoates). Figure 1 provides a classification of biodegradable polymers based on their source of origin.

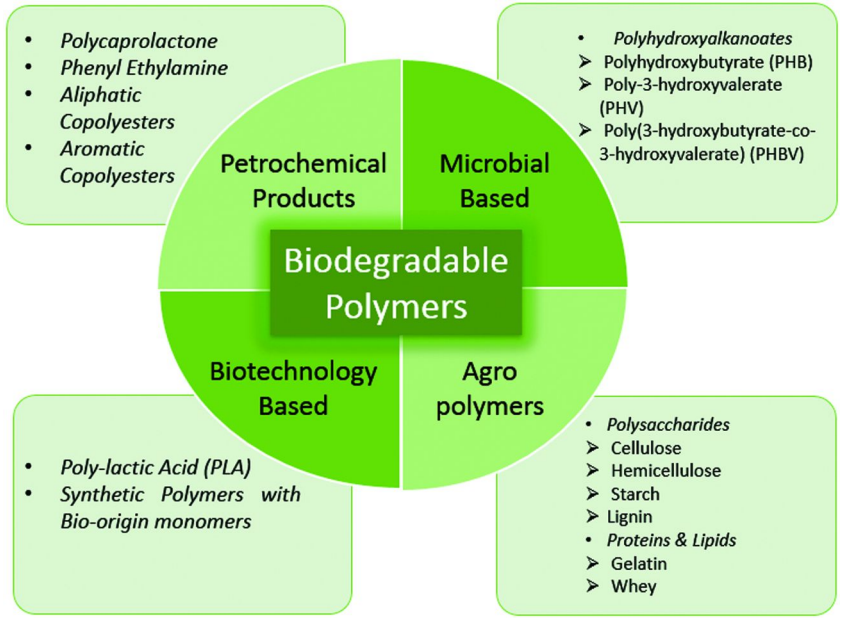


*Figure 1. Potential sources of degradable polymers.*

This chapter focuses on the recent developments in fabrication of biopolymer/CNC based (green) nanocomposites, and discusses the possibility of their application in food packaging. The chapter begins with a general discussion of biopolymer nanocomposites having potential food packaging applications. Next, various methods of acid based CNC synthesis and surface modification of CNCs are described. Thereafter, several biopolymer/CNC nanocomposites are discussed and properties of CNCs relevant to food packaging applications are detailed. Next, a technical discussion on different methods for CNC based film preparation and their potential for scale-up to industrial scale is discussed. Finally, different scale-up strategies and troubleshooting practices for CNC production are discussed keeping in mind that industrial scale production of the material will be necessary in the next few decades.

## Biopolymers for Food Packaging Applications

The success of conventional non-biodegradable polymer nanocomposites coupled with the problems associated with their proper disposal has stimulated new research on bio-based nanocomposites having a biodegradable polymer matrix. Innovations in the development of economically and ecologically

attractive green materials from biodegradable polymers and their widespread adoption will lead to preservation of fossil based raw materials. Moreover, use of such polymers will lead to complete biological degradation of bioplastics in due period of time through natural ecological cycle. So far, the most studied biodegradable polymers for the fabrication of nanocomposites are PLA, starch, cellulose, PHB, chitosan etc. Potential of these bio-based materials towards fabrication of bio-based nanocomposites for food packaging applications will be briefly discussed next.

**Starch-Based Nanocomposites**

Starch is a promising bio-based, renewable raw material because of its easy availability, low cost and complete biodegradability (*6*). When mixed with synthetic polymers, it has the ability to promote the degradation of the article. Starch does not form films with high mechanical strength and requires proper plasticization agent or chemical modifications. Glycerol and other low molecular weight poly- hydroxyl compounds, polyether, urea and water are common plasticizers for processing of starch. Thermomechanically extruded starch in the presence of plasticizers is referred to as thermoplastic starch (TPS). Starch suffers from several drawbacks such as hydrophobicity, change in water content and performance changes during processing (*7*). To overcome these limitations, several different film fabrication techniques, starch nanoparticle synthesis, and chemical modification of starch have been reported (*8–10*).

Starch based composites, in the form of films or bag, can be employed as packaging for fruits and vegetables, snacks, dry products or as adsorbent pads for meat exudation due to its hygroscopic nature. De Carvalho et al. were the first to provide insight into the preparation and characterization of thermo plasticized starch-kaolin composites by melt intercalation technique (*11*).

Starch and cellulose based poly(L-lactic acid) (PLLA) nanobiocomposites are of interest to researchers because of the improvement in degradation properties over neat PLLA (*12*). Park et al. (*13*) compared the thermal degradation of PLLA and PLLA/starch nanobiocomposites, and observed a shift in the thermal degradation range upon addition of starch. For neat PLLA, the onset of degradation was observed at 310°C and degradation was complete by 400°C. After addition of starch, the degradation temperature of PLLA/starch nanobiocomposite decreased to 220-230°C while near-complete degradation was observed between 280°C and 340°C. Further, increasing the starch content increased the moisture absorption capacity (6-8% compared to 1% for neat PLLA) and crystallinity indicating that starch acts as a nucleating agent. Changing the plasticizer used in PLLA/starch composite modifies the mechanical properties such as tensile strength, percentage elongation at break, and modulus of the nanobiocomposite (*14*).

Hydrophobicity of starch based films and their poor mechanical properties can also be improved by fabrication of nanobiocomposite of TPS and nanocellulose fibers (NCF) (*12*, *15*). Savadekar et al. (*16*) observed that the tensile strength of the base polymer film increased upon addition of NCF. Maximum tensile strength of the film was observed at 0.4% NCF loading. Significant decrease in water vapor

transmission rate (WVTR) was observed in 0.4% NCF/TPS films ($4.3 \times 10^{-4}$ g/h/sq m as compared to $7.8 \times 10^{-3}$ g/h/sq m for neat TPS films). Moreover, oxygen transmission rate (OTR) reduced by 93% in 0.4% NCF/TPS films compared to neat TPS films.

## Cellulose-Based Nanocomposites

Cellulose is one of the most promising natural raw material and constitutes the most abundant renewable polymer resource available today. Cellulosic materials when subjected to acid hydrolysis (Figure 2) yield defect-free, crystalline CNC residues. CNCs possess many favorable characteristics such as nanoscale dimension, high specific strength and modulus, high surface area, unique optical properties etc. These properties make them a promising material for various applications such as fabrication of polymer nanocomposite materials and films, drug delivery, protein immobilization and metallic reaction template (*17*). Polymer matrix gets transformed when pooled with cellulose nanocrystals. The resulting nanobiocomposite has enhanced mechanical, thermal, barrier and antibacterial properties along with greater ease of degradability.

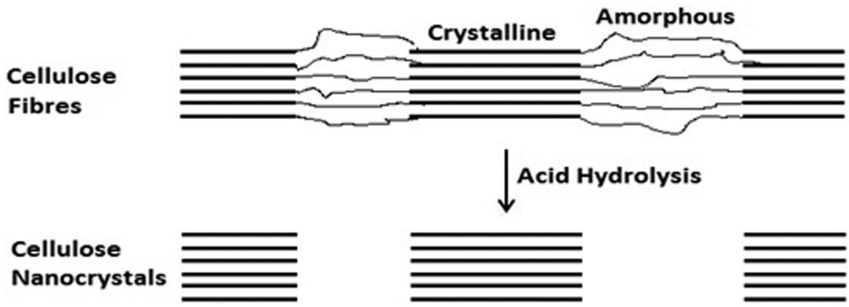


*Figure 2. Acid hydrolysis to form cellulose nanocrystals.*

CNCs improve the barrier properties (such as OTR and WVTR) as well as mechanical properties (such as Young's modulus and strength) of biodegradable polymers such as PLA (*18*, *19*). A detailed discussion on the effects of CNC reinforcement and barrier properties is provided later in the chapter.

Cellulose acetate (CA), cellulose acetate propionate (CAP), and cellulose acetate butyrate (CAB) are thermoplastics produced through esterification of cellulose (*20*). Among the different derivatives of cellulose, cellulose acetate (CA) is of particular interest because of its biodegradable nature, excellent optical clarity and high toughness. Cellulose ester powders derived from different raw materials such as cotton, recycled paper, wood cellulose and sugarcane in the presence of different plasticizers and additives are melt processed via extrusion to produce commercial pelletized cellulose plastics (*21*).

### Gelatin-Based Nanocomposites

Gelatin is prepared by the thermal denaturation and hydrolytic cleavage of collagen, isolated from the skin of animal and fish, and animal bones using very dilute acid (*22*). Gelatin contains a large number of glycine, proline and 4-hydroxyproline residues. Gelatin is a heterogeneous mixture of single- or multi-strand polypeptides with extended left handed proline helix conformations of 300-400 amino acids (*23*) with a typical structure –Ala–Gly–Pro–Arg–Gly–Glu–4Hyp–Gly–Pro–. Gelatin can be used as biopolymer in tissue engineering as well as in edible coatings as it reduces oxygen, moisture and oil migration, and can be loaded with antioxidant or antimicrobial agents. However, the major limitation of gelatin with regards to packaging applications is its poor mechanical strength (*22*).

Grapefruit seed extracts (GFSE) derived from the seed and pulp of grapefruit is a potential additive to gelatin based polymers because it is non–toxic and has been reported to increase shelf life by inhibiting the growth of food borne pathogens. Polymer films coated with GFSE layer using polyamide as binder have shown antimicrobial activity against a variety of microorganisms. These gelatin based GFSE composites find application in packaging of beef and fish products (*24*). Barley bran (BB), a byproduct of the barley powder manufacturing industry, can be used for protein film preparation because of its low cost. BB films coated with GFSE have potential application in the packing of salmon, since GFSE decreases the peroxide value and thiobarbituric acid content (*25*). Concentration of the GFSE added does not affect the quality of the food product (*25*). The thermoreversible nature at its melting point, which is close to body temperature, makes gelatin a good base material for protein films. However, its large scale production possibilities are debatable due to high costs.

## Cellulose Nanocrystals: Synthesis and Surface Modification

CNCs are fabricated through stringent acid hydrolysis techniques, in which amorphous parts are degraded, leaving behind only the crystalline section of nanometer dimensions. CNCs fabricated from different biomasses have different morphologies and yields. This is because of the different proportions of cellulose, hemicellulose and lignin contents in biomass and interfacial binding between them, which makes it difficult to separate out pure cellulose during pretreatment. There have been several pretreatment procedures reported to date which are listed in Figure 3. The pretreatment process yields relatively pure cellulose pulp with trace amounts of lignin and hemicellulose. As trace amounts of these impurities significantly hinder the CNC fabrication process, proper pretreatment procedure should be selected depending on the biomass type. Figure 4 shows a detailed schematic diagram of CNC production through different pretreatment routes. The different types of acids used for the fabrication of CNCs, significantly alters the stability of the colloidal suspension and physical properties. The choice of biomass source and hydrolyzing acid is important in the optimization of CNC synthesis.

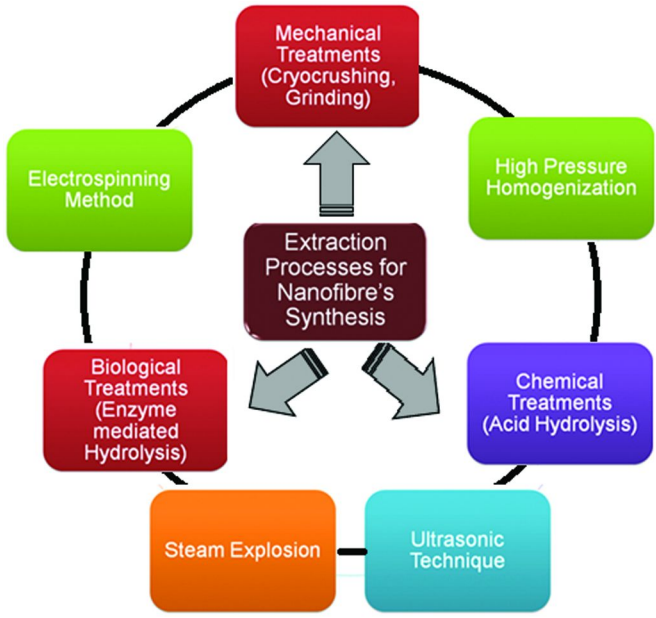


*Figure 3. Different biomass pretreatment methods for cellulose extraction.*

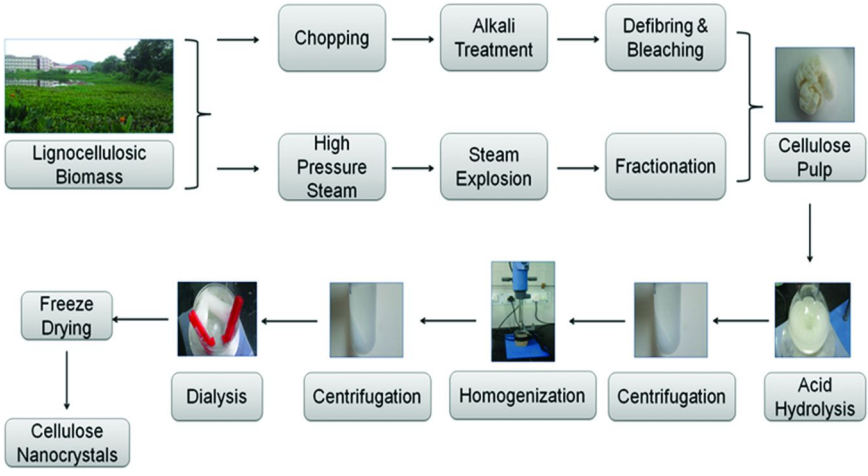


*Figure 4. Overview of the general pathway followed for CNC production.*

**Effect of Bio-Feedstock on Cellulose Hydrolysis**

Production of CNC is a complex process which is influenced by several process parameters viz. acid concentration, biomass content, presence of lignin and hemicellulose, process followed for biomass pretreatment, temperature and reaction time. Yield of CNC from cellulose hydrolysis strongly depends on the biomass type, impurities present as well as on the biological origin (*26*). The degree of crystallinity of cellulose in the biomass/microorganism varies widely with species and natural location and is a critical parameter for the determination of the dimension and yield of nanocellulose. Cellulose, especially from algal and bamboo source, is highly crystalline in nature which hinders the penetration of acid to deep crystalline regions leading to CNCs that are several micrometers long (*27*). Cellulose from cotton and wood, having lower crystallinity, yields much shorter dimensions of CNCs. Moreover, CNC generation from different biomass and waste products makes it a valuable nano-product with complete biodegradability and recyclability (*28*).

CNCs are most frequently produced at lab scale using filter papers with sulfuric acid (64 %) at room temperature with a yield of <50 % making it an expensive process (*29*). Sulfuric acid has been used most frequently for CNC production because of higher stability than hydrochloric or other acids (*30*). Several bio-feedstock sources for cellulose have been used to date for CNC synthesis ranging from microbial source, waste plant products, industrial wastes, bio-composts and other polysaccharides.

Brown et al. (*31*) reported the effect of geometry and dimension of enzyme complex embedded in the plasma membrane of green alga Oocystis on the size, shape and orientation of cellulose microfibrils synthesized by the complex. Read et al. (*28*) proposed the rosette model for cellulose based on microscopic examinations which revealed that elementary crystalline cellulose units are composed of 36 chains, having 3–4 nm diameter, corresponding to the crystallite size found in wood microfibrils or in particles from acid-hydrolyzed Avicel. The number of microfibrils laterally adhering to each other and the length of bound regions along the microfibrils may statistically vary in the cell wall, which after acid hydrolysis would result in a variety of particles with a wide distribution of widths. A statistical study (*26*) of cellulose hydrolysis was carried out taking several factors into consideration which showed that hydrolysis at harsh conditions leads to short crystallites with more charged sulfate half ester groups at the surface. Moreover, the study concluded that the energy of adhesion of two crystallites is proportional to the contact area of fibers; hence less contact leads to easier separation of thinner and shorter particles into nanocrystals. Candanedo et al. (*33*) characterized wood biomass into two types: softwood (length 3-4 mm & width ~35 μm) and hardwood (length 0.5-1.5 mm & width ~20 μm) depending upon the cell wall structure (*32*, *33*). They investigated the properties of CNCs fabricated from softwood (black spruce) and hardwood (eucalyptus) pulp taking into consideration the reaction time and acid-to-pulp ratio for hydrolysis. Atomic force microscopy (AFM) studies showed that microfibrils subjected to identical hydrolysis conditions produced nanocrystals of 5 nm diameter for both wood

species. The fabricated nanocrystals under different parametric conditions were identical, even when the initial microfibril microstructure was different, implying that the basic unit of wood cellulose organization is same for the two species (*33*).

Recent studies have focused on recycling industrial residue for CNC production to overcome the problems associated with utilizing renewable bio-based feedstock. Oksman et al. (*34*) used bio-residue from wood bioethanol production plant as a raw material for CNC production, since it contains almost 50% highly crystalline cellulose. The bioresidue was prehydrolyzed due to which CNC could be isolated by simple mechanical disintegration (high pressure homogenization or ultrasonication), leading to an economical process with an yield of ~48% (*34*, *35*). Although the fabricated CNCs had higher thermal stability, the tendency for agglomeration was higher as well because of reduction in surface charge density. This was due to the increased inter- and intra-molecular hydrogen bonding between CNCs which dominated the repulsive forces caused by sulfate groups. Teixeira et al. (*36*) used cassava bagasse, a by-product of the cassava starch industry, hydrolyzed under harsh acidic condition of 6.5 M sulfuric acid followed by ultrasonication for better dispersion. Colloidal suspension of nanocrystals thus obtained contained curved elongated particles (length ~360-1700 nm and diameter ~2-11 nm) and perfect rod-like nanoparticle morphology was absent. Such studies demonstrate that residue from industrial processes can be an excellent low cost source of raw material for the production of the CNCs.

Natural fibers derived from different plant products have crystalline structure, microfibril angle and cell dimension which differ drastically from one plant part to the other. Coconut fiber, an agro-industry byproduct which is a large source of cellulosic waste generated in bulk amount annually, remains an untapped potential resource. Coconut fibers have high toughness and are more durable compared to other natural fibers due to higher lignin content. A high lignin content also improves the dispersion of CNCs in hydrophobic polymers due to the presence of aromatic carbons in lignin chains (*37*). Prewashed coconut fibers are pretreated by the traditional alkaline treatment methods and further subjected to sulfuric acid hydrolysis with varying fiber to acid ratio (1:10) and time, under constant acid concentrations to obtain CNCs (*38*). Fahma et al. (*39*) analyzed the effect of different pretreatment procedures on extraction of CNCs from coconut husks and found benzene-ethanol and sodium hypochlorite-potassium hydroxide extraction as the best procedures to obtain cellulose with the highest crystallinity index, degree of polymerization and thermal stability. Increase in time and the number of runs for bleaching steps in pretreatment leads to a decrease in thermal stability of the synthesized CNCs after hydrolysis. It was found that trace residual lignin improves the thermal stability of CNCs as lignin decomposition starts from 200°C and persists up to 700°C; however mechanical and other relevant properties might deteriorate considerably. Both studies (*38*, *39*) showed that CNCs synthesized from coconut were ultrathin with diameters as low as ~5 nm, lengths ranging from 70 to 400 nm and an average aspect ratio of ~60 (*39*, *40*). Kenaf (*Hibiscus cannabinus*) fibers from the Malvaceae family are single, straight, branchless stalks with inner woody core and fibrous outer bark. These fibers have a reasonably high cellulose content of 30-60% (*41*). To obtain CNC,

the fibers were extensively prewashed and pretreated followed by hydrolysis with varying in reaction times. Increased reaction times lead to better disintegration and defibrillation of fibers but also lead to decrease in thermal stability. This may be attributed to an increase in the amount of short fragments of cellulose chains leading to larger number of chain ends which enhances decomposition (*42*). Thermal stability increased upon neutralization (~pH 7) of sulfate groups with sodium hydroxide. Thus it can be concluded that the negatively charged sulfate groups on CNCs act as active centers for cellulose chain degradation. CNC extraction from kenaf fibers was optimized at 65 wt.% sulfuric acid and a hydrolysis time of 40 min at 45°C (*43*).

**Effect of Different Acids on Cellulose Hydrolysis**

Acid hydrolysis of cellulose from different sources leads to degradation of amorphous segments and yields highly crystalline rod like colloidal suspension of nanometer dimension. Stable suspensions of colloidal CNCs were first fabricated by Ribi and Ranby in 1949 from wood and cotton cellulose (*2*). Mukherjee et al. studied the degradation of cellulose to CNCs through sulphuric acid treatment and captured the first electron microscopy images of CNCs which indicated dimensions of approximately 50-60 nm long and 5-10 nm wide (*44*). Several types of acids have been used by researchers to date, ranging from strong acid to weak bio-based acids coupled with ultrasonication and homogenization to increase the activity of acids to degrade amorphous regions of cellulose. Sulfuric acid, most commonly used acid for CNC extraction, provides highly stable aqueous suspensions, due to the esterification of surface hydroxyl groups to give charged sulfate groups, whereas hydrochloric acid leads to unstable CNC suspension, with minimal surface charge. One of the major drawback of sulfuric acid based fabrication of CNCs is that sulphonyl functional groups catalyze the degradation of the host polymer at higher temperature (*42*). Although hydrochloric acid based CNCs lead to increased thermal stability of the polymer/CNC nanocomposite, the stability of dispersion is poor (*45*). Hence, the application of the bio-based acids coupled with mechanical disintegration processes for CNC extraction is studied extensively.

2, 2, 6, 6-Tetramethyl-1-piperidinyloxy (TEMPO) has been extensively used as an oxidant for CNC fabrication. TEMPO acts as a free radical and selectively oxidizes the hydroxyl groups in cellulose chains. The oxidation process occurs at the surface of the microfibrils only; hence a combination of mechanical disintegration by ultrasonication and high pressure homogenization is required for high yield of CNCs (over 80%). However, to date the above method has been unsuccessful for the hydrolysis of non-wood fibers with high crystallinity and degree of polymerization (DP). TEMPO coupled with NaBr/NaClO oxidation carried out at pH 10 and room temperature leads to significant amount of sodium carboxylate group functionalization and small amount of aldehyde group formation at the C6 primary hydroxyl groups of cellulose. Trace amounts of aldehyde can be further converted to carboxyl groups by overnight treatment

with $NaClO_2$ (*46*). Analysis of DP values with Mark Houwink Sakurada (MHS) equation showed a decrease from 1200 to approximately 600 with increase in reaction time. Changing the oxidation system from NaBr/NaClO to TEMPO/NaClO/$NaClO_2$ resulted in lower DP, with no aldehyde byproducts and lower carboxylate group content (*47*). This is because the NaClO oxidizes TEMPO to N-oxoammonium ion which converts the C6 primary hydroxyl groups of cellulose to aldehyde under acidic or neutral conditions forming hydroxylamine. The NaClO oxidizes hydroxylamine to N-oxoammonium ion which further converts aldehyde to a carboxyl group (Figure 5). Thus, the depolymerization process of cellulose chains due to the $\beta$-elimination is avoided. The NaBr/NaClO system had a maximum carboxylate content of 1.7 mmol/g-pulp whereas the TEMPO/NaClO system had 0.8 mmol/g-pulp (*29*). Here, sodium chlorite acts as a primary oxidant, which immediately oxidizes aldehyde groups, formed as intermediate structures, to carboxyl groups under weakly acidic or neutral conditions. Similar trends were observed with biomass having higher percentage of cellulose crystal structure such as cotton linters, bleached kraft and sulfite pulps, bacterial cellulose, ramie fibers, bamboo pulps and other fiber based bio resources. $^{13}C$ solid state NMR study supports the finding that TEMPO based oxidants attack the glucosyl units in cellulose chains at C6 ends to convert them to anionic sodium carboxylate groups. The functionalized microfibrils are further converted to nanofibers by mechanical disintegration through application of ultrasonication and homogenization for short periods (*46*). The whitish cellulose slurry obtained after the disintegration process is converted to transparent dispersion by the formation of nanofibers of cellulose. Recent study showed that transparency increased with increase in carboxylate content (*46*, *48*). As expected, lower width of microfibrils leads to higher carboxylate content (*49*). When treatment was carried out on different biomass sources, nanofiber widths were almost constant whereas length distribution varied significantly. Such variation in length of CNCs could be attributed to variations in oxidation and disintegration process conditions. The TEMPO-oxidized CNC, when dispersed in water, had a highly stable negative potential of -80mV due to anionic charged carboxyl groups, without formation of bundles and aggregates (*29*). This may be due to strong electrostatic repulsion between the negatively charged CNC rods. Moreover, zeta potential, which is related to the surface density of carboxylate groups on the CNC surface, showed very little variation on changing the biomass source (*50*). However several properties such as transparency, viscosity and zeta-potential varied with the type of mechanical disintegration process such as magnetic stirring, homogenization and ultrasonic homogenization.

Recently, Espinosa et al. fabricated CNCs by hydrolysis of cotton using phosphoric acid (85%), which was added drop wise to cellulose slurry up to a predetermined concentration and further heated to 50°C/100°C. The maximum yield of CNC from phosphoric acid, determined to be ~80%, strongly depends on the reaction temperature and acid concentration. The fabricated CNCs had a width of ~30 nm and a length of ~300 nm, obtained at optimized conditions of 10.7 M phosphoric acid and 90 min reaction time at 100°C (*51*). Cellulose phosphorylation has also found potential application in biomedical technologies such as bone tissue engineering. For example, Fricain et al. (*52*) phosphorylated

microcrystalline cellulose with *ortho*-phosphoric acid in the presence of phosphorus pentoxide to generate cellulose hydrogels for biocompatibility and histological studies. Phosphorylated cellulose alongwith hydroxyapatite has also been used as an alternative to collagen fibers for biomimetic growth (*53*).

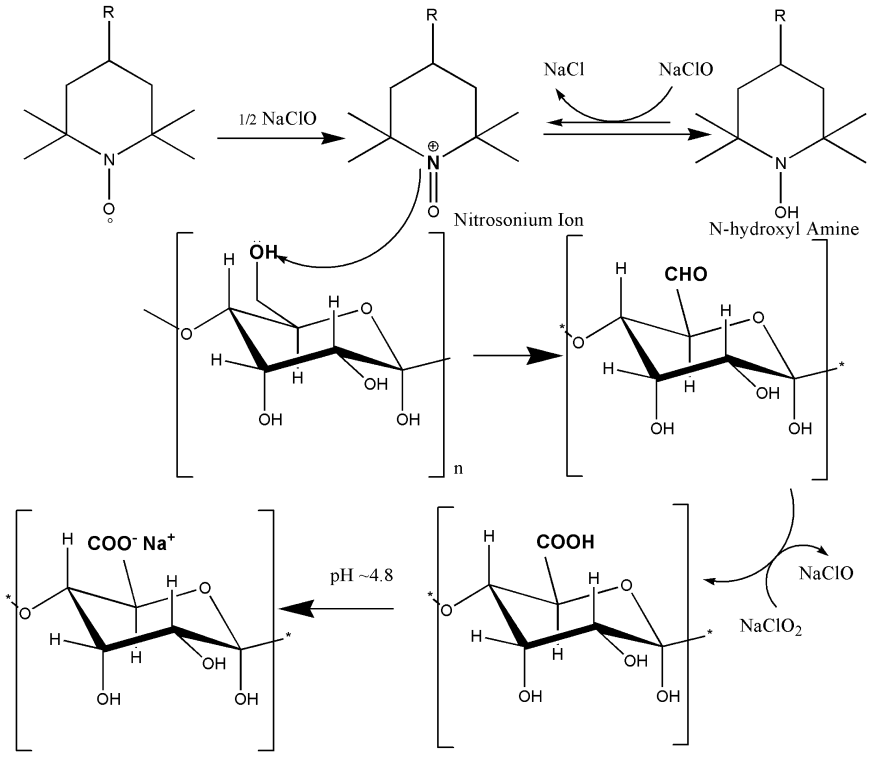


*Figure 5. Reaction mechanism of CNC production with TEMPO.*

Ammonium persulfate (APS), an inexpensive oxidizing agent with high water solubility and low stability, has also been used for the fabrication of CNCs. APS based CNC fabrication is a sustainable process that is less hazardous than sulfuric acid based process and is easy to scale up. Moreover, it has the capability to remove lignin, hemicellulose, pectin and other plant contents *in situ*, making CNC production a single step process. However, the yield of this process is lower compared to acid based systems and it requires a high purity of initial cellulose for better CNC yield. Leung et al. (*54*) fabricated CNCs from APS by heating cellulose slurries at 60°C with varied reaction times (in the range of 3-16 hours) depending on the complexity of the cellulosic biomass. The fabricated CNCs had average width of 3-7 nm with an yield of 28-36% from the complex flax and hemp fibers compared to the 81% yield from pure cellulose source from Whatmann filter paper (*54*).

Sadeghifar et al. (*55*) used hydrobromic acid coupled with ultrasonication for fabrication of CNCs. The optimized condition for CNC production, found to be 3 h of reaction time at 80°C under 2.5 M HBr acid concentration, resulted in a yield of ~80% with whatman filter paper as cellulose source. HBr modified CNC can be a potential candidate for site-specific grafting reactions (*55*). As HBr is a stronger acid than HCl or $H_2SO_4$, it offers appreciable savings when large scale production of CNC is considered. Figure 6 shows the reaction pathways of cellulose hydrolysis with different acids in detail.

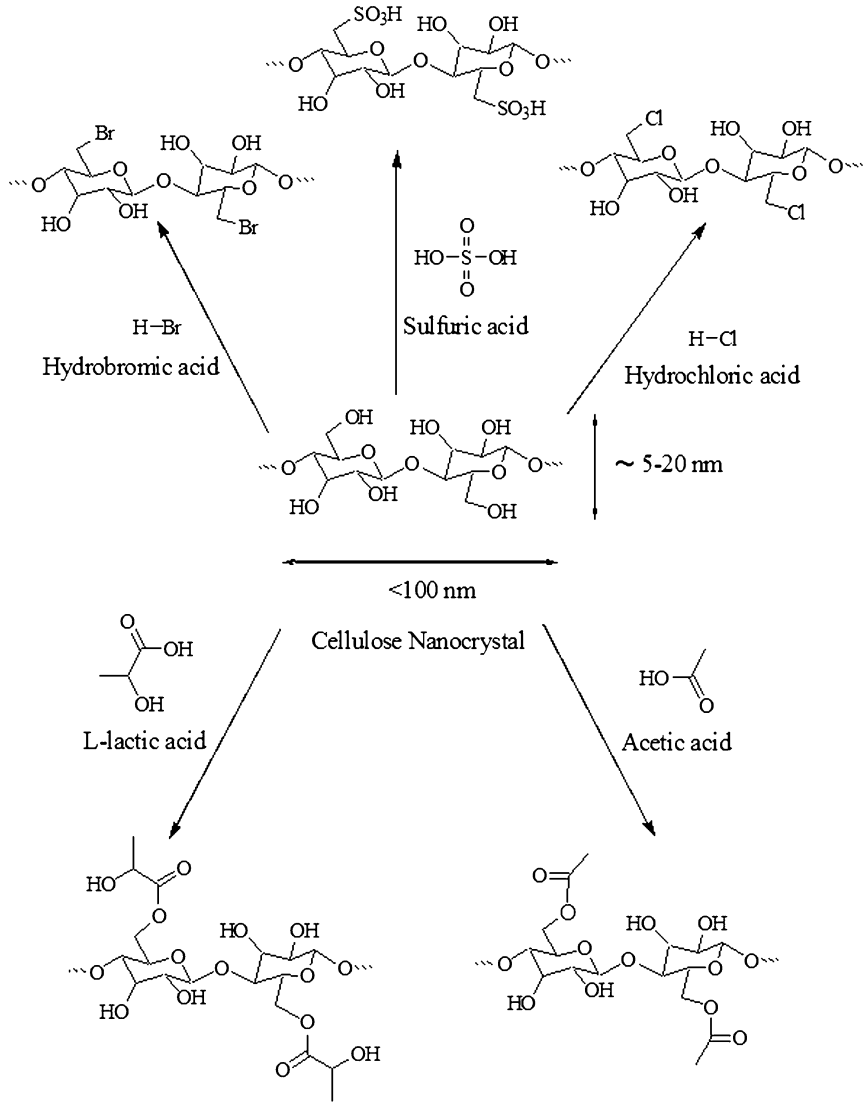


*Figure 6. Reaction pathways for acid hydrolysis with different acids.*

**Table 1. Comparison of Yield and Morphology of CNCs Extracted from Different Sources of Acid**

| *Acid* | *Optimized parameters* | *Morphology* | *Dimensions* | *Yield* | *References* |
|---|---|---|---|---|---|
| **Sulphuric Acid** | 64% sulphuric acid at room temperature | Circular and rod shape | Length: 50-60 nm<br>Width: 5-10 nm | <50% | (*45*) |
| **Hydrochloric Acid** | Hydro-thermal treatment | Rod Shape and Elliptical | Length: 250-300 nm<br>Width: ~15 nm | ~ 93% | (*58*) |
| **2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO)** | TEMPO coupled NABr/NaClO at pH~10 at room temperature | Rod Shape | Length: 100-200 nm<br>Width: 3-4 nm | ~80% | (*50*) |
| **Hydrobromic Acid** | 2.5 M Hydrobromic acid reaction temp 80-100 °C & reaction time 3 hours | Rod Shape | Length: 100-200 nm<br>Width: 7-8 nm | ~80% | (*55*) |
| **Phosphoric Acid** | 85% phosphoric acid reaction time 90 min at 100 °C | Rod Shape | Length: ~300 nm<br>Width: ~30 nm | ~80% | (*51*) |
| **Ammonium persulfate** | Reaction time 3-16 hours and reaction temperature 60 °C | Rod Shape | Length: ~300 nm<br>Width: ~3-7nm | ~28-36% (fibers)<br>~81% (filter paper) | (*54*) |
| **Acetic Acid** | Reaction temperature 70 °C and reaction time 5 hours coupled with ultrasonication | Rod Shape | Length:~200-300 nm<br>Width: ~10-100 nm | ~86% | (*57*) |
| **Cationic exchange resins** | Capacity 4.7 mmol/g [$H^+$] | Rod Shape | Length:~100-400 nm<br>Width:~10-40 nm | ~50% | (*56*) |

The use of harsh, strong acids for CNC production makes the overall process environmentally harmful, causing significant pollution. Moreover the cost of CNC production is increased due to the requirement of corrosion resistant equipment. Therefore, use of bio-based acids or other cation exchange resins have been explored for CNC hydrolysis to make the process less hazardous. Cation exchange resins are negatively charged solid catalysts which are easy and safe to handle, and can be readily recycled after reaction. In acidic medium the NKC-9 resin (styrene-divinyl benzene co-polymer) has an exchange capacity of 4.7 mmol/g [$H^+$] (*56*). CNCs fabricated using NKC-9 were 10-40 nm in diameter and 100-400 nm in length. Maximum yield for the process was ~50%. The main advantage of this process is that it does not require large amounts of water to stop the reaction; instead, direct removal of resin beads will stop the reaction (*56*).

Although bio-based acids have weak penetration power, they can impregnate and break the glycosidic linkages of cellulose chains when coupled with ultrasonication. Tang et al. (*57*) esterified cellulosic slurry with acetic acid in presence of sulfuric acid which converted the hydroxyl groups to acetic anhydride. The esterification was followed by high energy ultrasonication which led to fiber disintegration. The maximum yield of the CNCs thus obtained was ~86% for a reaction time of 5h at 70°C. Ultrasonication disintegrates the amorphous chains of cellulose thus facilitating the diffusion of weak acid molecules. The weak acid subsequently depolymerizes the cellulose to CNCs. The fabricated CNCs contain acetic anhydride functional groups which can be grafted with polymer chains to improve the dispersion of CNCs in polymer matrices. Table 1 compares yield and morphology of the CNCs extracted from different acid systems.

**Surface Modification of Cellulose Nanocrystals**

Effective dispersion of CNCs into polymer matrix has been a challenging problem because of the strong intermolecular hydrogen bonding between cellulosic chains. Therefore surface modification of CNCs is usually carried out to increase the compatibilization of polymer matrix and nanocrystals. Surface modification is accomplished by altering the highly accessible hydroxyl groups present on cellulose chains. Some of the important surface modification reactions include sulfonation, silylation, grafting with isocynate, acid anhydride, acid chloride and surfactants. These functional groups act as nucleating sites for grafting of polymers on CNC surface. Polymer grafting is usually carried out by two approaches namely “grafting from” and “grafting onto” methods. Figure 7 shows the different surface modification reactions carried out using different chemical entities.

Goussé et al. (*59*) performed surface silanization of CNCs with various alkyldimethylchlorosilanes (alkyl moieties ranging from 3 to 12 carbons) through partial silylation process with a degree of substitution of 0.6. Raquez et al. (*60*) functionalized CNCs with trialkoxysilanes ($R'Si(OR)_3$) allowing them to tune the dispersion of CNCs in various polymers by changing the organic functionality (which can be alkyl, amine, vinyl, methacrylic or long hydrocarbon chains). Mabrouk et al. (*61*) carried out one-step surface modification of CNCs with methacryloxypropyl triethoxysilane leading to a stabilized dispersion of

CNCs in poly(styrene-co-2-ethyl hexylacrylate) copolymer during miniemulsion polymerization. Similarly, surface modified CNCs, when melt blended with PLA using twin screw micro compounder, showed better dispersion and compatibility with the polymer matrix than the non-functionalized CNCs (*62*). Improved dispersion of CNCs leads to enhancement of the barrier and thermo-mechanical properties of PLA, since CNC acts as a nano-reinforcing agent (*60*, *63*).

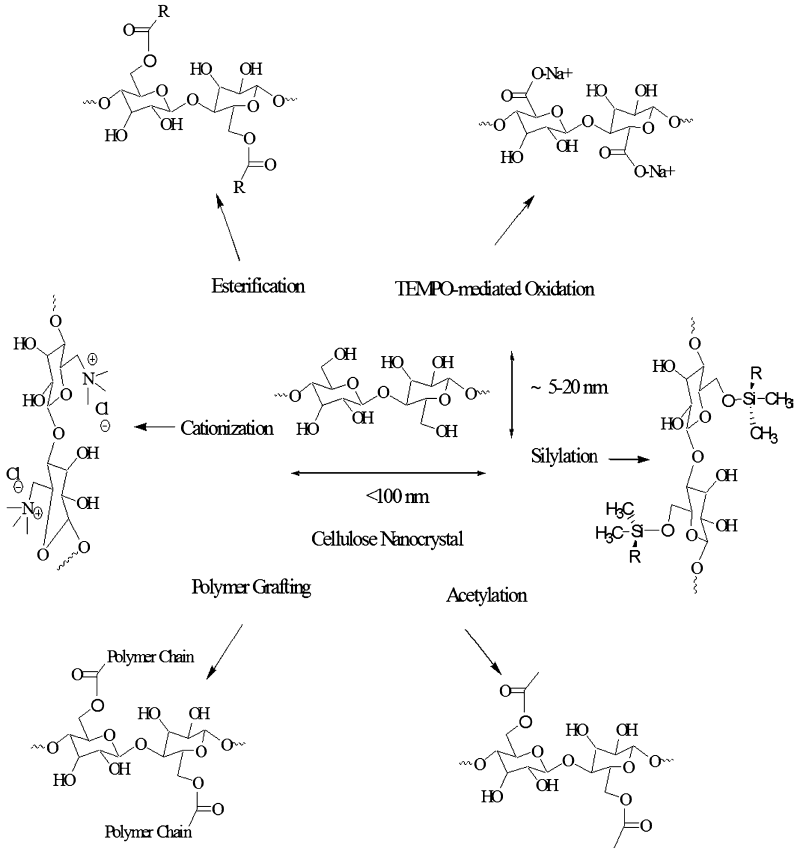


*Figure 7. Surface modification of CNC.*

Acetylation of CNC surface improves the dispersion of CNCs in organic solvents and increases adhesion of CNC with synthetic polymer matrixes. Acetylation can be carried out with alkenyl succinic anhydride (ASA), which is widely used as sizing agent in paper making. Nair et al. (*64*) studied the acylation of hydroxyl functional groups in chitin using ASA and other reagents. CNC

acylation using ASA was studied by Yuan et al. (*65*). In the study, a slurry of cellulose nanowhiskers is mixed with ASA, freeze dried and further heated to 105 °C, which introduced a degree of substitution of ~0.022. The acylated CNCs exhibited good dispersion in low polarity solvents such as 1, 4-dioxane, and also in polymers such as polystyrene (*65*). Acylation has also been carried out through Fischer esterification reaction in which a mixture of organic acids (such as acetic acid and butyric acid) is used for hydrolysis. In this process, a combination of 17.5 M acetic and 10.9 M butyric acid, along with a trace amount of hydrochloric acid, was optimized for CNC production resulting in nanoparticles of 10-20 nm diameter and 200 nm length (*62*). The butyrated and acetylated CNCs show better dispersibility in non-polar toluene compared to chlorinated and sulphonated CNCs. Surface modification of CNCs (Figure 7) with esterification process leads to improved dispersion in organic solvents and prevents the aggregation of CNC possibly due to hydrogen bonding. Introduction of these functionalities can lead to the synthesis of more hydrophobic CNCs by incorporating multiple complex chemical reactions (*65*).

**Polymer-Grafted Cellulose Nanocrystals**

Several polymers have been grafted on to the CNC surface using the "grafting onto" approach resulting in better dispersion and compatibility with the polymer matrix. Habibi et al. (*66*) grafted CNC surface with poly (ε-caprolactone) (PCL) using an isocyanate-mediated coupling agent. Nanocomposite films fabricated using PCL-grafted nanoparticles displayed high modulus and good ductility reflecting the reinforcement of polymer matrix by CNCs. Cao et al. (*67*) used a similar approach to graft waterborne polyurethane chains on CNC surface. Mangalam et al. (*68*) used TEMPO oxidized CNCs with -COOH functional groups to graft single stranded DNAs in the presence of carbodimide derivatives. Ljungberg et al. (*69*) grafted maleate functionalized atactic polypropylene of low molecular weight on CNCs extracted from tunicates. Films cast using grafted CNCs showed good compatibility with the polymer matrix leading to higher transparency and tensile modulus. Amine functionalized polyethylene glycol (PEG) was grafted on TEMPO oxidized CNC through carbodimide chemistry by Araki et al. (*70*) to obtain CNC suspensions of high stability. Surface modified CNCs have remarkable properties such as high colloidal stability, better dispersion in polar and organic solvents and thermo-reversible aggregation (*71*).

In the "grafting from" approach, either the monomer molecules are grafted on the CNC surface (and further polymerized in the presence of a catalyst) or *in situ* surface initiated atom transfer radical polymerization is carried out. This process leads to nanocomposites having high molecular weight polymers along with well dispersed nanoparticles in the polymer matrix. Habibi et al. (*72*) grafted PCL through ring opening polymerization (ROP) in the presence of a catalyst and grafting agent stannous octoate. PCL grafted CNCs showed higher stability in toluene and improved thermo-mechanical properties such as Young's and storage modulus, because of better compatibility with the polymer matrix. Similar studies when carried out in the presence of microwave irradiation (*73*) lead to higher grafting density, which in turn shield the hydrophilic surface of

CNCs thus enhancing its water resistance properties (*74*). Pranger et al. (*75*) carried out *in situ* polymerization of furfuryl alcohol in the presence of CNCs using sulfonic acid as catalyst at high temperatures. Furfuryl alcohol served both as a dispersing agent and the matrix precursor for *in situ* polymerization. The resulting nanocomposite showed higher onset degradation temperature of ~80°C for 0.75 wt. % CNC. Figure 8 shows different possible functionalization routes for CNCs.

## Biopolymer-Based CNC Composites

### PLA-Based CNC Composites

PLA, a completely biodegradable thermoplastic polyester, when reinforced with CNCs leads to the formation "green bio-composites" having a percolated nanofiber network in polymer matrix which leads to improvement in properties. Several technical problems have been reported with the dispersion of hydrophilic CNCs into hydrophobic PLA matrix.

CNCs should be handled in partially hydrated state during processing with polymers as complete freeze drying of the material creates the problem of redispersion (*76*) due to the strong hydrogen bonding between cellulosic chains and tendency of CNCs to self-assemble. However, hydration leads to incompatibility with and poor dispersion in hydrophobic polymers. Production of PLA based nanocomposites through industrial based processing techniques, such as melt compounding or extrusion, makes the situation even more complicated. In the melt state, polymer-nanoparticle interactions are extremely weak which leads to agglomeration of the filler nanoparticles.

Surface modifications of CNCs, carried out using complex chemical routes, have promoted dispersion to some extent. However, the surfactants used for dispersing nanoparticles have been observed to adversely affect PLA degradation (*77*). Further, surface modifications of CNCs may create complications related to deterioration of biodegradation characteristics, potential increase in toxicity and migration of additives in the host polymer, and other health related problems in food packaging applications.

Solution casting of CNC into PLA matrix at relatively low weight fraction has shown good dispersion, with improved mechanical properties. However solution casting has several drawbacks related to solvent entrapment and industrial scale up. Several studies on effectively dispersing CNCs in polymer matrices have been carried out to date. Bondeson and Oksman (*77*) investigated the effect of adding polyvinyl alcohol (PVA) to improve the dispersion of CNCs in PLA. It was observed that immiscibility of PLA and PVA led to phase separation. The CNCs was primarily localized in the PVA phase due to more favorable interactions, with negligibly small amount present in the PLA phase.

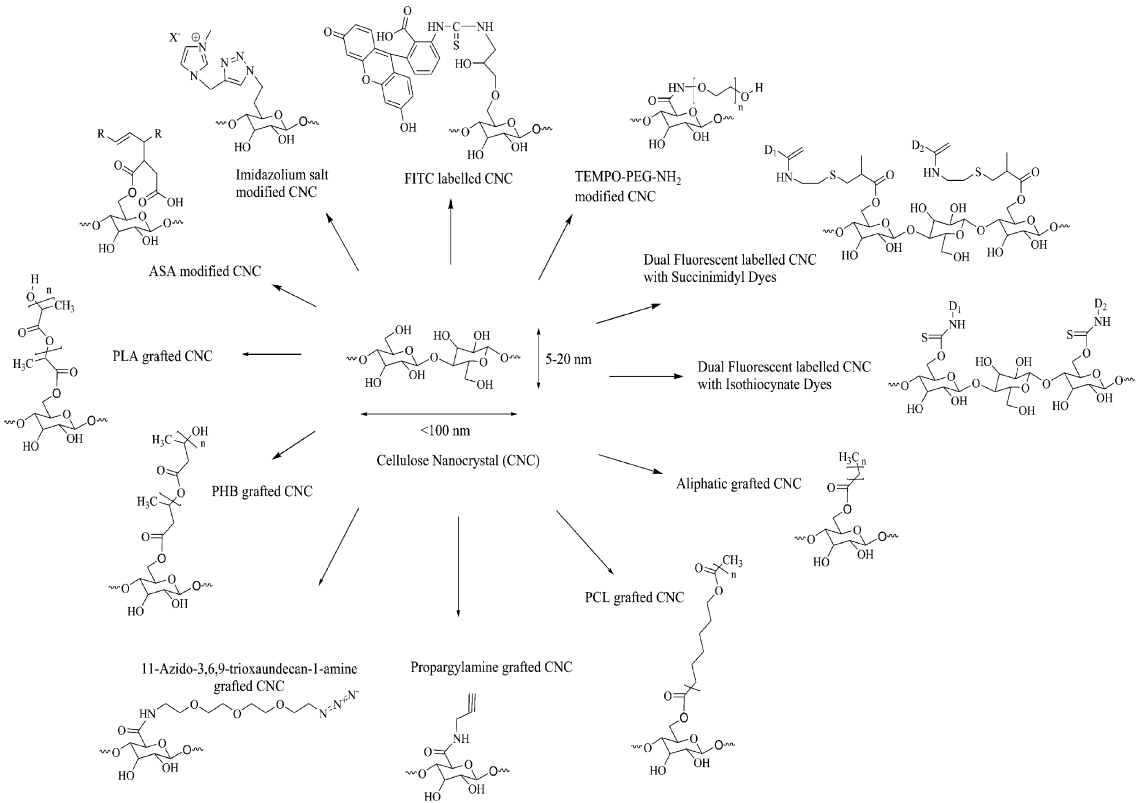


*Figure 8. Different methods for functionalization of cellulose nanocrystals.*

Due to phase separation and inadequate dispersion, there was no significant improvement in thermal and mechanical properties of PLA/CNC nanocomposite. Surface modification of cellulose fibers through silylation reaction with 3-aminopropyltriethoxysilane resulted in better dispersion into PLA matrix through melt compounding (*78*). The improvement in thermo-mechanical properties of the composites could be attributed to better interfacial adhesion between cellulose and the polymer matrix. CNCs exhibit good dispersion in ethylene-vinyl alcohol copolymer (EVOH) when preincorporation methods (such as electrospinning) are used before melt compounding, leading to improvement in mechanical properties over neat EVOH (*79*). Moreover, study on incorporation of bacterial CNCs into PLA by electrospinning and solution precipitation with polar EVOH has revealed significant improvement in water and oxygen barrier properties over neat PLA (*80*).

Braun et al. (*81*) successfully grafted PLA on the surface hydroxyl groups of CNC through *in situ* polymerization of lactide. The hydroxyl groups initiated lactide ring opening polymerization, which subsequently led to the grafting of PLA chains onto the CNC surface. Acetylation of a fraction of surface hydroxyl groups of CNCs using Fischer esterification process resulted in grafting of high molecular weight PLA chains on the CNC surface. The nanocomposite thus obtained displayed significant improvement in properties, such as heat distortion temperature, speed of crystallization and shear modulus, over neat PLA. In fact, the shear modulus obtained for the nanocomposite was two orders of magnitude larger than that obtained by Capadona et al. (*82*) for PLA nanocomposites prepared using unmodified CNC. The property enhancement is explained by the reinforcement effect of the percolating network formed by the CNC filler particles. Melt pressed films of the PLA-grafted CNC nanocomposite material retained transparency indicating good dispersion of the filler in the polymer matrix.

**Polyhydroxyalkanoate-Based CNC Composites**

It is well known that the incorporation of cellulose in polyhydroxyalkanoate (PHA) matrix leads to improvement in the physical and mechanical properties. Medium chain length PHA-cellulose composites show improvement in the mechanical properties of PHA without any significant effect on the degradability of PHA (*83*). Nanocomposites of cellulose and poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) have also been observed to possess improved mechanical properties. PHBV/CNC nanocomposites fabricated by Jiang et al. (*84*) using solution casting showed improved tensile strength and modulus and elevated glass transition temperature compared to neat PHBV. However, melt processed PHBV/CNC nanocomposites showed a deterioration of mechanical properties due to agglomeration and consequent poor dispersion of CNC particles. Srithep et al. (*85*) reported improvement in the mechanical properties (such as tensile and storage modulus) and crystallization kinetics of PHBV/CNC nanocomposites. However, the thermal degradation behavior was adversely affected as addition of CNC led to reduction of onset degradation

temperature. This behavior is explained by the presence of water bound to CNCs (through hydrogen bonding) which facilitates degradation through hydrolysis.

Hydrophobic PHB obtained from microbial source and hydrophilic CNC derived from plant source can be combined to make completely "green" PHB/CNC nanocomposites with potential application in food packaging industry. Further studies and technical developments are required to improve the compatibility and interfacial interactions between the biopolymer (PHB) and bio-nanoparticle (CNC) before PHB/CNC nanocomposites can be used for high end applications.

### Chitosan-based CNC Composites

Chitosan is a biodegradable and biocompatible natural biopolymer which possesses antimicrobial properties making it a suitable material for food packaging applications. However, the high water sensitivity of chitosan films coupled with poor mechanical properties have restricted their widespread use in moist environments. Chitosan has amine functional groups, which makes it an ideal candidate for grafting hydroxyl and carboxyl groups of polymers. Chitosan/CNC composites are easy to fabricate because both components are hydrophilic and highly compatible. In the presence of acidic medium, the amine groups of chitosan are protonated and can associate with the negatively charged sulphonyl groups on CNCs through electrostatic attraction. Grafting of both biopolymers can easily be carried out by amide linkages between the carboxyl functional groups of CNC with terminal amide groups of chitosan. De Mesquita et al. (*86*, *87*) fabricated chitosan/CNC nanocomposites by first functionalizing the CNCs with methyl adipoyl chloride (MAC) and reacting the surface functional groups of CNCs with amino groups of chitosan. The nanocomposite showed considerable improvement in mechanical properties and a remarkable reduction in hydrophilicity as suggested by water uptake results. Such property improvements make these completely bio-based nanocomposites promising materials for food packaging applications.

## Cellulose Nanocrystal Films: Properties

### Mechanical Properties

CNC has high aspect ratio and is one of the strongest biopolymers, attributes which makes it ideal for dispersion into polymeric matrices, especially biopolymers, to improve their properties. Tashiro et al. (*88*) theoretically calculated the Young's modulus of crystalline cellulose (polymorph I) along the chain axis to be 167.5 GPa. AFM study, performed by Lahiji et al. (*89*), revealed the transverse elastic modulus of an isolated CNC to be between 18 and 50 GPa. Using a combination of four-point bending test and Raman spectroscopy, Šturcová et al. (*90*) determined the elastic modulus of crystalline tunicate cellulose to be 143 GPa.

Good dispersion of CNCs in polymer matrices generally leads to significant improvement in mechanical properties over the neat polymer due to reinforcement effect of the CNC filler. Ljungberg et al. (*69*) observed that polypropylene/CNC

nanocomposites showed drastic improvement in mechanical properties over the neat polymer due to filler-filler interactions. They also investigated the effect of filler dispersion quality on the mechanical properties of the resulting nanocomposite. Nanocomposites in which the CNCs were well dispersed in the polymer matrix showed lower brittleness and higher elongation at break compared to nanocomposites containing aggregated CNCs. Preincorporation of CNCs into PLA fibers using electrospinning and subsequent melt-mixing with PLA has been shown to improve the dispersion of CNCs in the polymer matrix (*80*). The resulting nanocomposite possesses higher tensile strength and elastic modulus than neat PLA.

Extensive intermolecular hydrogen bonding in CNCs allows for the formation of a percolating network within the polymeric matrix (*91*). This is governed by the size, orientation, aspect ratio and interparticle interactions of CNCs, as well as by the polymer-CNC interactions (*92*). The classical mean-field micromechanical model for composites, developed by Halpin and Kardos (*93*), fails to predict the mechanical properties of polymer-CNC nanocomposites as it does not account for strong interparticle interactions and percolation effect (*91*). To account for the effect of percolation network on the mechanical properties of nanocomposites, Ouali et al. (*94*) applied the percolation concept to the classical series-parallel model of Takayanagi et al. (*95*). Elastic tensile modulus ($G'$) of the nanocomposite is then given by:

$$G' = \frac{(1 - 2\psi + \psi X_r)G_r'G_s' + (1 - X_r)\psi G_r'^2}{(1 - X_r)G_r' + (X_r - \psi)G_s'}$$

where t and s refer to the rigid (CNC) and soft (polymer) phase respectively, $\psi$ is an adjustable parameter that corresponds to the volume fraction of the percolating rigid phase and $b$ represents the critical percolation exponent. $\psi$ is given by

$$\psi = 0 \quad for\ X_r < X_c$$

$$\psi = X_r \left( \frac{X_r - X_c}{1 - X_c} \right)^b \quad for\ X_r > X_c$$

where $X_r$ is the volume fraction of CNCs, $X_c$ is the critical percolation volume fraction and $b = 0.4$ for a three-dimensional network (*96*).

When CNC is dispersed in hydrophobic polymers, no remarkable enhancement of mechanical properties is usually observed. Incompatibility between the CNC and the polymer matrix leads to a lack of reinforcement effect in such systems. Further, agglomeration of CNCs inside the polymer matrix can lead to a decrease in tensile and elastic modulus. Surface grafting and other methods have been applied to increase interfacial adhesion between polymers and CNCs (*97*). Mechanical properties of these composites, where no percolation network is formed, can be predicted from the Halpin−Tsai (*98*) equations

$$E = \frac{E_m(1 + \phi\eta\xi)}{1 - \phi\eta}$$

$$\eta = \frac{{E_r}/{E_m} - 1}{{E_r}/{E_m} + \xi}$$

where $E_m$ and $E_r$ refer to the Young's modulus of the polymer matrix and the reinforcing phase (CNCs) respectively, $\varphi$ is the volume fraction and $\xi$ is twice the aspect ratio. The threshold for percolation network, which strongly depends on filler aspect ratio, can be predicted from the following equation (*97*)

$$X_c = \frac{0.7}{{L}/{d}}$$

where L and d are the length and diameter of the nanoparticles.

Nanoindentation has been extensively applied to study the nano-mechanical properties of polymer/CNC nanocomposites and influence of polymer adsorbed on the CNC surface. Oliver and Pharr (*99*) equations can be used to extract Young's modulus ($E_r$) and hardness ($H$) from nanoindentation data as follows:

$$E_r = \frac{\sqrt{\pi}\left({dP}/{dh}\right)_{unloading}}{2\beta\sqrt{A_c}}$$

$$H = \frac{P_{max}}{A_c} = \frac{P_{max}}{24.5h_c^2}$$

where $P$ is the indentation load, $h$ and $h_c$ are the penetration and contact depths respectively, $\beta$ is a constant that depends on the geometry of the indenter and $A_c$ is the projected contact area which is a function of the contact depth.

Factors such as humidity and temperature can significantly affect the mechanical properties of CNC films (*100*). A study on the effect of humidity and temperature on the nano-mechanical properties of CNC films fabricated from sulfuric acid hydrolysis of switchgrass and cotton was carried out by Wu et al. (*100*). They observed that in comparison to cotton CNC, switchgrass CNC had a higher aspect ratio, and films cast from switchgrass CNCs possessed higher modulus ($E_r$) and hardness ($H$). The mechanical properties of CNC films improved with a decrease in humidity which disrupts the hydrogen bond network between CNC particles. Mechanical properties of polymer/cellulose nanocomposites strongly depend on the aspect ratio of the nanocellulose filler. This was demonstrated in a study by Xu et al. (*97*) where a systematic comparison of nanocomposites fabricated by dispersing CNCs and cellulose nanofibrils (CNFs) in polyethylene oxide (PEO) matrix was performed. PEO/CNF nanocomposites

displayed better strength and modulus than PEO/CNC nanocomposites at identical filler loadings. This was due to larger aspect ratio, greater fiber entanglement and stronger reinforcement effects of percolation network formed in the case of CNFs. However, fiber entanglement in CNFs resulted in lower strain-at-failure due to an increase in the tendency of the fibers to agglomerate.

Atomic force microscopy (AFM) has been successfully used by several researchers to measure the mechanical properties of CNCs and polymer/CNC nanocomposites. By applying the three-point bending test, Iwamoto et al. (*101*) measured the elastic modulus of tunicate cellulose microfibril (obtained by TEMPO oxidation) to be 145 GPa. Pakzad et al. (*102*) studied the mechanical characteristics of the interphase region (between CNC and the polymer matrix) using AFM. In the (PVA)–poly (acrylic acid) (PAA)–CNC nanocomposites studied by them, the elastic modulus increased from 9.9 GPa in the PVA-PAA matrix to 12.8 GPa at the surface of CNC. The thickness of the interphase region increased with increase in CNC diameter.

## Optical Properties

CNC based films are usually transparent which makes them ideal for packaging applications. Transparent packaging is preferred for food packaging because the quality and condition of food can be easily detected by visual inspection. CNCs fabricated using hydrochloric acid have lower stability and higher opacity compared to those fabricated using other acids (such as sulfuric acid). Films containing multiple layers of CNCs do not differ much in transparency from the native polymer films. Aulin et al. (*103*) observed that twenty bilayers of polyethylenimine/nanofibrillated cellulose reduced the transparency of the PLA substrate by less than 1.5%.

## Contact Angle/Wettability

As cellulose is hydrophilic in nature, CNC films exhibit a small contact angle for water. Contact angle measurements aid in understanding the degree of adhesion of liquid droplets on film surfaces. Contact angle depends on solid-liquid, liquid-gas and solid-gas interfacial free energies. Wettability of a film is influenced by several surface characteristics such as roughness, topography and chemical composition. Films fabricated from TEMPO oxidized cellulose nanofibers are hydrophilic due to the abundance of surface carboxylate groups. In several packaging application, affinity to moisture can be an undesirable attribute and hydrophobization of the film surface may be required. Fukuzumi et al. (*5*) measured the contact angle of water on such films to be 47° which decreased with time due to penetration of water into the film. Treatment of the films with alkylketene dimer (AKD), a common hydrophobizing agent, led to significant increase in water contact angle (94°). Adsorption of the cationic AKD at the anionic carboxylate sites on the cellulose surface reduced the hydrophilicity of the films.

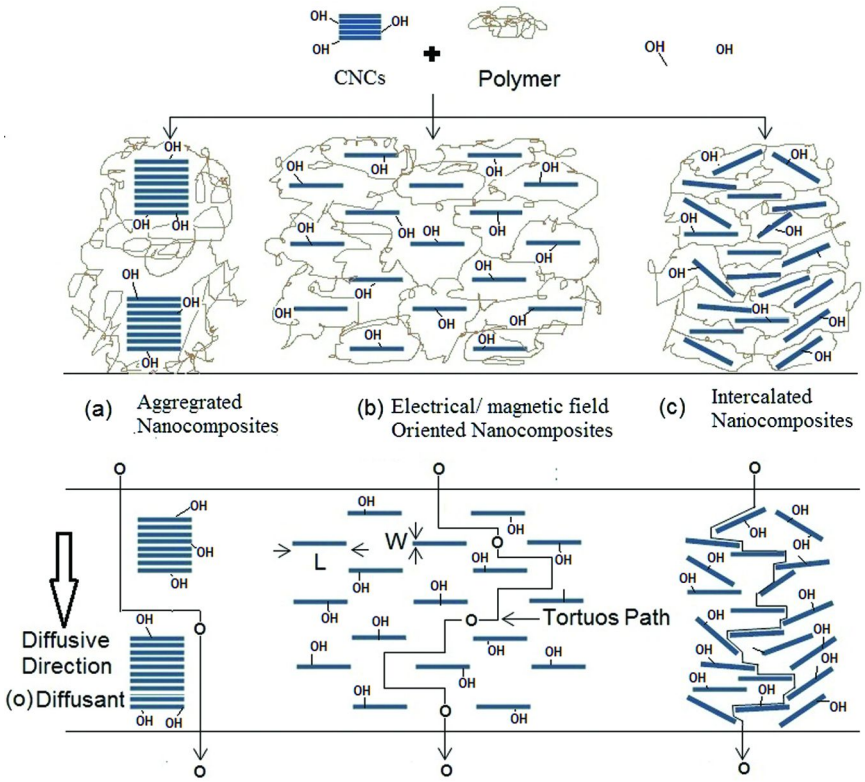


*Figure 9. The mechanism of gas diffusion through different types of CNC-polymer composites. (a) Aggregated CNC-polymer composites (b) Electrical/magnetic field-oriented CNC-polymer composites and (c) Intercalated CNC-polymer composites.*

**Barrier Properties**

Dispersion of CNCs in polymer matrix has been known to drastically reduce the permeability of gases and water vapor (*104*), making CNC a promising material for packaging applications. The permeability of atmospheric gases through the polymeric material depends upon the solubility of the gas in the polymer matrix and its diffusivity through the matrix. The diffusion rate depends on the fraction of free volume cavities dynamically created in the polymer matrix due to thermal motion of the polymer chains. Diffusivity also depends on the temperature and the size of the penetrant gas molecule. Incorporation of CNCs into polymer matrix creates a more tortuous path for the diffusing gas molecule than that encountered in neat polymers as shown in Figure 9. The diffusion pathway followed by the penetrant is greater in case of intercalated polymer/CNC nanocomposites than that

in aggregated composites. Hence, nanocomposites with well dispersed CNCs are expected to possess better barrier properties towards gases/vapors such as oxygen and water vapor. Figure 9 shows the effect of aggregated, oriented and intercalated CNCs on the diffusion pathway of gas through a polymer matrix. Nielsen (*105*) proposed a simple theoretical model for gas diffusion in polymer nanocomposites, in which the filler particles are modeled as rectangular platelets of finite width (*L*) and thickness (*W*), evenly distributed throughout the polymer matrix and oriented perpendicular to the direction of diffusion. Mathematical form of Nielsen model is given by

$$\frac{K_{composite}}{K_{matrix}} = \frac{(1-\phi)}{\left(1+\frac{\phi\alpha}{2}\right)}$$

where K values represent permeability of the composite material and of the polymer matrix in the absence of filler respectively, φ is the volume fraction of the filler and α is the aspect ratio (L/W) of the filler particle. φ is related to τ (tortuosity) through the following relation:

$$\tau = 1 + \left(\frac{L}{2W}\right)\phi$$

Several more sophisticated models have been proposed (*106*) to describe gas transport through polymer nanocomposites. Some such models are listed in Table 2.

Experimental measurement of gas permeation through polymer films/ membranes is done in a chamber/shell consisting of pressure transducers which regulate the pressure of both upstream and downstream chambers. Transport of a gas through the membrane is expressed in terms of permeability coefficient, *P,* and diffusion coefficient, *D*. Under steady state conditions, *P* can be expressed as (*107*):

$$P = \left(\frac{273 * VL}{76 * ATp}\right)\left(\frac{dp(T)}{dt}\right)$$

where *P* is in $cm^3$(STP).cm/$cm^2$.s.cm-Hg, *V* (in $cm^3$) is the volume of downstream chamber, *A* (in $cm^2$) is the effective area of film, *L* (in cm) is the thickness of the film, *p* (in cm-Hg) is the pressure of the penetrant gas in the upstream chamber, *T* (in K) is the absolute temperature and *dp(T)/dt* (in cm-Hg/s) is the rate of pressure increase measured by the pressure sensor in the low pressure chamber. The permeability coefficient is commonly expressed in barrers (1 barrer = $10^{-10}$

$cm^3$(STP).cm/$cm^2$.s.cm-Hg). The diffusion coefficient, *D*, is obtained from the time lag (intercept of the plot of downstream pressure versus time on the time axis) using the relation

$$D = \frac{L^2}{6\theta}$$

where $\theta$ is the time lag. Solubility coefficient (*S*) can then be calculated using the relation S = *P/D*. Transport of gases through polymeric membranes is an activated process that follows the Arrhenius equation. The temperature dependence of *P* and *D* can be written as follows:

$$P = P_0 \exp\left(\frac{-E_P}{RT}\right),$$

$$D = D_0 \exp\left(\frac{-E_D}{RT}\right)$$

where $P_0$ and $D_0$ are pre-exponential factors, and $E_P$ and $E_D$ are the activation energies associated with permeation and diffusion respectively. The temperature dependence of the solubility coefficient is given by

$$S = S_o \exp\left(\frac{-\Delta H_s}{RT}\right)$$

where $S_o$ is the pre-exponential factor and $\Delta H_s$ is the heat of sorption. Heat of sorption is given by the difference in permeation and diffusion activation energies, $\Delta H_s = E_P - E_D$.

Surface coating of PLA films with TEMPO-oxidized cellulose nanofibers has been observed to drastically reduce the oxygen permeability of the films by several orders of magnitude. Oxygen permeability of the coated PLA films is comparable to synthetic polymers such as polyvinylidene chloride which have excellent oxygen barrier properties. Deposition of multiple, alternating layers of nanofibrillated cellulose and polyethylenimine (PEI) on a PLA substrate by Aulin et al. (*103*) showed large improvement in oxygen barrier property and moderate improvement in water vapor barrier property over neat PLA. In fact, the oxygen permeability of a fifty bilayer assembly was comparable to that of PVA. This reduction in oxygen permeability can be attributed to the tortuous diffusion pathway created by the presence of high aspect ratio, impermeable nanofibrillated cellulose. Martínez-Sanz et al. (*76*) incorporated CNC/EVOH blends into PLA matrix and observed significant improvement in water and oxygen barrier properties over neat PLA. Thus, CNC based polymer nanocomposites can be potentially used as an environment-friendly alternative to synthetic polymers in packaging applications where excellent barrier properties are required.

**Table 2. Models for Gas Permeation in Polymer Nanocomposites**

| *Model* | *Filler Type* | *Orientation* | *Equation* |
|---|---|---|---|
| Nielsen (*105*) | Ribbon | Regular array, oriented | $R_P = \frac{(1-\phi)}{\left(1+\frac{\phi\alpha}{2}\right)}$ |
| Nielsen (*108*) | Ribbon | Non-uniform orientation | $R_P = \frac{(1-\phi)}{\left(1+\frac{\phi\alpha}{3}(S+0.5)\right)}$ where, $s = 0.5 * (3\cos^2\theta - 1)$ |
| Cussler – regular array (*109*) | Ribbon | Regular array, oriented | $R_P = \frac{(1-\phi)}{\left(1+\left(\frac{\phi\alpha}{2}\right)^2\right)}$ |
| Cussler – random array (*109*) | Ribbon | Random array, oriented | $R_P = \frac{(1-\phi)}{\left(1+\left(\frac{\phi\alpha}{2}\right)^3\right)}$ |
| Gusev and Lusti (*110*) | Disk | Random array, oriented | $R_P = \frac{(1-\phi)}{e^{\left(\frac{\alpha\phi}{3.47}\right)^{0.71}}}$ |
| Fredrickson-Bicerano | Disk | Random array oriented | $R_P = \frac{(1-\phi)}{\left[4\frac{(1+x+0.125x^2)}{(2+x)}\right]^2}$ where, $x = \frac{\phi\alpha\pi}{2ln\left(\frac{\alpha}{2}\right)}$ |
| Picard (*111*) | Flakes | Random parallel polydisperse thickness | $R_P = \frac{(1-\phi)}{\left[\frac{\left(1+0.33\phi\left(\Sigma n_i\left(\frac{w_i}{t_i}\right)^2\right)\right)}{\Sigma n_i\left(\frac{w_i}{t_i}\right)}\right]^2}$ |

$R_P$, Relative permeability; $\alpha$, aspect ratio; $\phi$, volume fraction of the nanoplatelets that are dispersed in the matrix; $\theta$, the angle between the diffusion direction and the unit vector normal to the surface of a platelet; $S$, an order parameter non-uniform orientation of the platelets; $n_i$ is the number of platelets having width $w_i$ and thickness $t_i$.

Water sorption isotherm and uptake studies are helpful in understanding the interaction of water molecules with the hydroxyl groups present in cellulose chains as shown in Figure 10. Several models are available in literature (*112*) which deal with sorption of water in polymers/biopolymers. Rogers (*113*) classified the models for sorption of gases and vapors in polymers into several categories: type I (Henry's law), type II (Langmuir isotherm), type III (Flory-Huggins isotherm) and type IV (sigmoid-shaped isotherm). Brunauer, Emmett and Teller (BET) model (*114*) and Guggenheim-Anderson-de Boer (GAB) model (*115*) describe multilayer adsorption (condensation) of gases and vapors. Semi-empirical models such as Peleg (*116*), Ferro-Fontan, Henderson (*117*), Smith (*118*), Oswin (*119*), and Halsey (*120*) models were used by Al-Muhtaseb et al. (*121*) to fit water sorption isotherms on starch. Water adsorption isotherm on cellulose materials can be described using BET, GAB or Park model (*122*, *123*). BET is a two parameter model while GAB model is a modification of the BET model and has three parameters. Mathematical equation for the GAB model is given by

$$C = C_m \frac{C_G K_{ads} a_w}{(1 - K_{ads})(1 - K_{ads} + K_{ads} C_G)}$$

where $C_m$ is the monolayer capacity, $K_{ads}$ is a measure of the difference in adsorption enthalpy of the second and higher layer from the heat of liquefaction, aw is the activity of water and $C_G$ is the Guggenheim constant ($C_G K_{ads}$ is analogous to the BET energy constant). The Park model contains three terms (five parameters) corresponding to contributions from three mechanisms: Langmuir adsorption, Henry's law sorption and water clustering. At low activity of water, adsorption on specific sites on the cellulose (which follows Langmuir isotherm) as well as non-specific sorption in the voids and amorphous polymer regions (which follows Henry's law) account for the net sorption of water. However, at high activity of water, aggregation and cluster formation will also contribute to the overall sorption. The mathematical form of the Park model is given by (*122*)

$$C = \frac{A_L b_L a_W}{1 + b_L a_W} + K_H a_w + K_a n a_w^n$$

where $A_L$ is the concentration of specific sites, $b_L$ the Langmuir affinity constant, $K_H$ the Henry's solubility coefficient, $K_a$ the equilibrium constant for the clustering reaction and $n$ the average number of water molecules per cluster.

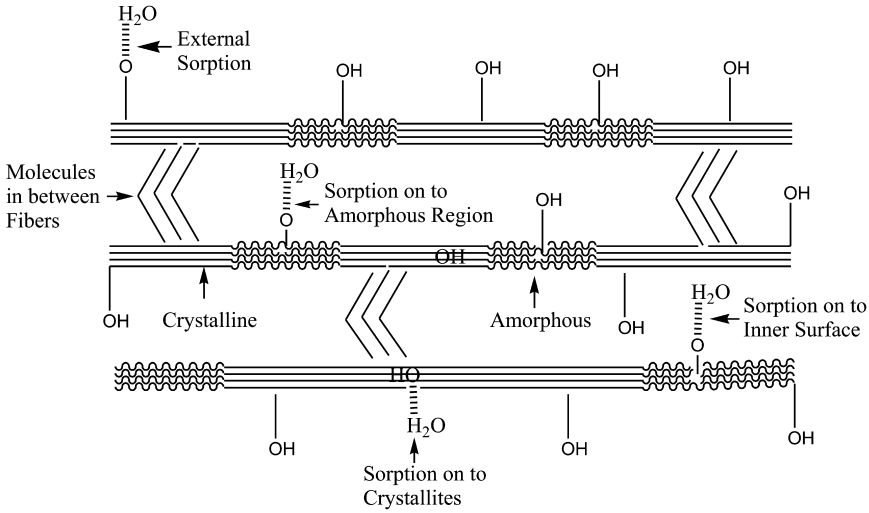


*Figure 10. Mechanism of water sorption on cellulosic fibers. (adapted from Okubayashi et al. (124))*

Figure 10 shows the typical sorption sites for water molecules on a cellulose fibril: hydroxyl groups on external surface, amorphous regions, inner surface and crystallites. Kinetics of water sorption in cellulosic materials can be effectively modeled by the parallel exponential kinetics (PEK) model which treats the

sorption phenomenon as two parallel independent first order rate processes. Direct sorption of water molecules on the external surface and amorphous regions of cellulosic fibers is a fast process while indirect adsorption onto the inner surface and crystallites is a slower process due to mass transfer (diffusion) limitations (*125*). PEK model equations for adsorption and desorption are given by

$$\text{Sorption:} \quad M_t = M_1\left(1 - e^{-\frac{t}{\tau_1}}\right) + M_2\left(1 - e^{-\frac{t}{\tau_2}}\right)$$

$$\text{Desorption:} \quad M_t = -M_1\left(1 - e^{-\frac{t}{\tau_1}}\right) - M_2\left(1 - e^{-\frac{t}{\tau_2}}\right)$$

where $M_t$ is the moisture content at any time $t$, $M_1$ and $M_2$ correspond to the moisture content at equilibrium states associated with the fast and slow processes respectively, and $\tau_1$ and $\tau_2$ are characteristic times for the fast and slow processes respectively.

Several studies have investigated the effect of adding CNCs to a polymer matrix on the water vapor barrier properties of the polymer. Membranes fabricated using CNCs dispersed in PVA matrix in the presence of a crosslinking agent (PAA) showed improved barrier resistance to water vapor transmission. Nanocomposites of positively charged triethyl ammonium-modified CNCs with various layered silicates showed enhanced water vapor barrier properties due to pronounced CNC-clay electrostatic interactions (*126*). Water vapor and gas barrier properties of films made of CNC and microfibrillated cellulose (MFC) extracted from sisal fibers were reported in a recent study by Belbekhouche et al. (*125*). Diffusion coefficients and water vapor permeabilities were found to be higher for CNCs than MFCs due to higher porosity of CNC films coupled with more entanglements in MFCs (leading to more tortuous diffusion pathways).

## Thermal Properties

Thermal properties are extremely important for evaluating the suitability of polymer nanocomposites for food packaging applications. Onset of thermal degradation of TEMPO oxidized CNCs occurs at around 200°C whereas native cellulose begins to degrade at 300°C. Thus, the formation of sodium carboxylate groups on the surface of cellulose nanofibers during TEMPO oxidation has an adverse effect on the thermal stability of cellulose (*29*). On the other hand, the coefficient of thermal expansion of TEMPO-oxidized CNCs is extremely low due to high crystallinity of the cellulose nanofiber.

Thermal stability of CNCs depends on the acid used for hydrolysis. CNCs fabricated from hydrochloric acid (HCl) show maximum thermal stability with an onset degradation temperature ($T_o$) of 220 °C (*58*). TEMPO oxidized CNCs show slightly lower $T_o$ (~200 °C), while CNCs fabricated using sulfuric acid have even lower thermal stability with $T_o$ ~150 °C (*29*). If phosphoric acid is used for hydrolysis, the resulting CNCs begin to degrade at approximately 200 °C (*51*). Thus, the thermal stability of fabricated CNCs follows the trend:

HCl-CNC > H3PO4-CNCs > TEMPO-CNCs > H2SO4-CNCs.

Films cast from CNCs show yellow discoloration when subjected to high temperatures due to degradation of cellulose. Further increase in temperature leads to depolymerization, dehydration and finally, degradation and char formation (*127*). Sulfuric acid-CNCs start showing yellow discoloration at 60 °C and turn nut-brown at 160 °C. In contrast, phosphoric acid-CNCs and HCl-CNCs start turning yellow at around 160 °C and do not turn dark brown even at 240 °C. Thus, unmodified sulfuric acid-CNCs are unsuitable fabricating nanocomposites by melt compounding or extrusion with the polymers which have melting points higher than 150 °C (e.g. PLA and PHB). Higher thermal stability of phosphoric acid and HCl based CNCs makes them ideal candidates for large scale nanocomposite film preparation by extrusion.

### Antibacterial Properties

CNCs have immense potential for application in biomedical engineering, especially in areas such as drug delivery and tissue engineering (*53*). However, due to the absence of any bactericidal/antimicrobial effect, microorganisms can easily attack CNC surface and degrade it which limits their application in field of drug delivery. Therefore, current research has focused on improving the antibacterial properties of CNCs through incorporation of nanoparticles or surface grafting of bactericidal molecules.

Cellulose films coated with Cu nanoparticles prepared from cellulose-cuprammonium solution through coagulation process showed enhanced antimicrobial effect against *Staphylococcus aureus* and *Escherichia coli* with bacteria being killed in 1 hour (*128*). Surface modification of CNCs by chemically grafting aminoalkyl groups, especially 3-aminopropyltri-methoxysilane, has shown enhancement in antimicrobial activity due to the presence of free amino groups on their surface (*78*). The surface-modified CNCs were found to be nontoxic to human adipose-derived mesenchymal stem cells. Such modifications can facilitate the use of CNCs in a wide array of fields, such as tissue implants, wound healing and drug delivery, without compromising their biodegradability and non-toxicity.

## Cellulose Nanocrystal Films: Fabrication Techniques and Advancements

CNC is an ideal material for food packaging applications because of its high structural strength 130-145 GPa (*129*), low density ~ 1.5 g/cc (hence strong but lightweight), ease of functionalization and fabrication as films. Although CNC based films have been fabricated using various processes such

as solvent casting, filtering etc., CNC film preparation suffers from two major problems. First, the presence of water necessitates long evaporation times for film preparation. Second, lower CNC concentration in aqueous solution limits its direct melt extrusion with a polymer. Therefore, selection of proper fabrication and processing techniques for uniform dispersion of CNCs into the polymer matrix is an important factor, which governs the percolation network structure and consequently the mechanical and barrier properties of the nanocomposite. CNC-based film preparation using different processing techniques, such as solution casting, electrospinning, extrusion and layer-by-layer approach, in different organic and polar solvents is discussed next.

**Solution Casting-Cum-Evaporation Technique**

Solution casting-cum-evaporation is a process in which nanoparticles are dispersed into the polymer solution and film is cast by subsequent solvent evaporation. It has been the most feasible technique of dispersing aqueous CNC solution into hydrophilic polymers due to favorable interactions between the nanoparticle and the polymer. Due to its hydrophilicity, CNCs can be dispersed either in a hydrated or a freeze dried state into the aqueous hydrophilic polymer solution. However, dispersing CNCs in synthetic hydrophobic polymers is more difficult. Recent studies have focused on first dispersing CNCs into polar or dipolar organic solvents to ensure good intercalation in hydrophobic polymer solutions for creation of better reinforced polymer nanocomposites. Freeze dried CNCs prepared from sulfuric acid can be dispersed in organic solvents, like DMSO and DMF, forming a stable solution (*77*). This is because of the negative charges imposed by the surface sulfate groups which increase intermolecular repulsion in CNCs. This in turn limits the formation of hydrogen bonds thereby preventing agglomeration and leading to a stable dispersion. However, a trace amount of water was found to be critical towards stability of the final solution, as aggregation would take place due to hydrogen bonding otherwise (*130*).

The type of acid used for CNC production greatly affects the stability as well. HCl based CNCs showed very poor dispersion in N,N-dimethyl formamide (DMF), N-methyl pyrrolidone (NMP) and dimethyl sulfoxide (DMSO), evident from the zeta potential values which fall within the unstable region (*131*). In the absence of surface charges on HCl based CNCs, the intermolecular hydrogen bonding causes agglomeration which leads to poor dispersion in solvents (*132*). Ten et al. (*133*) observed that dispersing CNC filler into the biopolymer PHB through melt compounding led to reduction in molecular weight. Van den Berg et al. (*134*) observed good dispersion of CNCs fabricated from sulfuric acid and hydrochloric acid in formic acid and m-cresol. They proposed that esterification of the surface hydroxyl groups was responsible for the good dispersion. Therefore, these polar aprotic solvents, which facilitate the dispersion of CNCs, have been extensively used for the fabrication of polymer nanocomposites.

Solution casting technique has been used for film preparation of CNC with several biopolymers especially PHB, PLA, chitosan etc. However, the maximum threshold limit of dispersion is 5 wt. % of CNC. Solvents for dispersing CNCs

(solvent-CNC) should be selected such that either the polymer is completely miscible in the solvent or the solvent-CNC dispersion is completely miscible with the polymer solution. As poly(methyl methacrylate) (PMMA) is completely miscible in DMF, Liu et al. (*135*) prepared PMMA/CNC nanocomposite using DMF as the solvent using solution casting by heating the solvent at 75°C for 10 hours. Similarly, as PHAs are completely soluble in solvents such as chloroform and DMF, CNCs are dispersed into PHAs through solvent exchange with these solvents. Grunert and Winter (*136*) solubilized cellulose acetate butyrate in acetone, and fabricated films by dispersion of CNC in acetone through solution casting method. However, the solution casting method has several problems related to solution entrapment within the films, which severely affects the mechanical properties. Moreover, uneven and uncontrolled solvent evaporation can lead to the formation of small pores which adversely affects the barrier properties. The solution casting method is not viable industrially as it cannot be scaled up to produce large sheets. Even though solution casting has several drawbacks it is extensively used to study the interfacial interaction between polymer and nanoparticles.

**Layer-by-Layer Assembly**

The layer-by-layer (LbL) technique is a cost effective, robust and efficient film fabrication technique, where oppositely charged polymers or nanoparticles are deposited through electrostatic interactions. By controlling the conditions of film preparation, it is possible to tune the morphology of the nanoparticle dispersion and its thickness on the substrate. Moreover, several other properties, such as optical and barrier, can be tuned by calculated multilayer deposition on the polymer substrate (*137*). Unlike solution casting, the probability of defect formation during drying in LbL approach is very low. In polyelectrolyte LbL approach the cationic polymer gets impregnated into the anionic void sites of nanofibrils thereby decreasing the void space and consequently the oxygen permeability.

The LbL approach is generally used for deposition of multilayer CNC on bio-based PLA. Desired gas barrier properties and transparency can be obtained by tailoring the number of layers deposited on the polymer substrate. Aulin et al. (*103*) studied the fabrication of multilayer CNC films on PLA. First, the PLA surface was made hydrophilic by treating it consecutively with ethanol and water several times. LbL deposition of CNC on the treated PLA films was achieved by dip coating the PLA films with cationic polyethylenimine (PEI) and anionic carboxylate CNC to make 20-50 bilayers of CNCs. Electrostatic interactions between CNC and polymer, in combination with hydrogen bonding and van der Waals interactions, lower the polymer free volume resulting in improved barrier properties (*103*). CNCs prepared from sulfuric acid hydrolysis contain surface sulfate groups that are negatively charged. Thus, polymers with positive charge are favorable for LbL assembly of CNC. Podsiadlo et al. (*138*) used poly(diallyldimethylammonium chloride), a polymer containing a high density of positive charge per unit length, to prepare CNC nanocomposites using the LbL

approach. With a 10 min interval of adsorption time, LbL assembly led to the formation of 11 nm thick bilayers, which were densely packed and uniformly covered with CNCs. In a recent study (*139*) to understand the complex hydrolysis process of lignocellulosic biomass, different multilayered model films of CNCs and xyloglucan were prepared by spin-assisted LbL approach. Fabrication of poly(allylamine hydrochloride)/CNC multilayered thin films has been studied by varying different process parameters such as the drying step between each layer of adsorption, ionic strength of polymer solution, dipping time and concentration of CNC (*140*, *141*). Drying process and ionic strength were found to be important parameters for successful film fabrication by the LbL approach; film thickness was primarily influenced by the dipping time and CNC concentration (*140*). The drying step is critical for film construction and architecture, because ejection of water molecules from the film leads to the formation of dense polymer layers with high cohesion. When cationic polymers are used with anionic CNCs in the LbL approach, the polymer gets adsorbed onto the CNC surface resulting in molecularly dense structured films thereby reducing the free volume fraction.

Chitosan/CNC "green" composite films were fabricated by LbL approach using alternating layer deposition (*86*). The growth of these multilayered films was driven by hydrogen bonding as well as electrostatic interactions between the positively charged amine groups of chitosan and negatively charged CNCs. TEMPO oxidized anionic CNCs deposited on chitin nanofibrils through LbL approach showed uniform layer deposition (*142*). The cast films showed antireflective properties making them potentially useful as thin nanolayer coatings on glass and window panes of vehicles. Renewable multi-layered alkyd resins were deposited over CNC-coated paperboards by a continuous roll-to-roll process (*143*). The water vapor permeability decreased from 1707 to 5.2 g mm/$m^2$ day.atm with 6 g/$m^2$ of CNC coating. The improvement was attributed to two reasons: decrease in pore size (CNC acts as a sealant) and increase in surface smoothness. Therefore, films fabricated using the LbL approach have potential for use in food packaging application.

**Extrusion Process**

Extrusion is a cost-effective, scalable, industrial process for the fabrication of polymer nanocomposites, with little or no solvent requirement. In melt extrusion, the polymer is forced to flow under shear along a helical screw direction. The extruder is divided into a number of zones such as the feed section, mixing and melting section, and compression section. In melt intercalation, biofillers such as CNCs can be directly mixed mechanically with the polymer melt to form a homogeneous mixture. This method is widely used for thermoplastic nanocomposites and can be applied to nanobiocomposite. Moreover, absence of solvent makes the technique industrially economical as well as environment friendly (*144*). However, proper dispersion of CNCs into polymer matrix by extrusion can be challenging as the agglomeration of CNCs must be prevented. The stage at which nanoparticle is introduced during polymer processing through extrusion is an important factor for the formation of the percolation network.

**Table 3. Comparison of Scale-up Strategies with Different Acid System (+ Sign Represents Stable Dispersion of CNCs and - Sign Represents Agglomeration of CNCs)**

| Acid | Sulphuric Acid | Hydrochloric Acid | TEMPO | High Pressure | Phosphoric Acid | Hydrobromic Acid | Acetic Acid | Ammonium persulfate |
|---|---|---|---|---|---|---|---|---|
| Methodology | Stringent hydrolysis at room temperature | Stringent hydrolysis at room temperature | TEMPO-mediated oxidation at room temperature | Repeated high pressure homogenization | Stringent hydrolysis at 100 °C for 90 min | Stringent hydrolysis at 80-100 °C for 3 hours | Ultrasonication assisted reaction time 5 hours | Directly using biomass reaction time 3-16 hours |
| Energy Consumption | <7 MJ/kg | ~7-10 MJ/kg | <7 MJ/kg | 700-1400 MJ/kg | ~10-30 MJ/kg | ~10-20 MJ/kg | 700-1000MJ/kg | ---- |
| Application | Polymer nanocomposites | High Thermal stable CNC films | High gas barrier films, health care materials | Filter aid nano fiber | Better dispersed CNC films with good stability | Surface modification through click chemistry | Bio-material, Drug delivery | CNC from mixed biomass as starting material |
| Stability (in water) | + | - | + | - | + | - | + | + |
| Advantage | Stable and uniformly dispersed in wide range of solvents | Thermally stable CNCs at higher temperature. | No swelling and change in morphology of fibers, carboxyl end groups | Process requires no acid treatment less health hazardous | Highly thermal stable and uniformly dispersed in solvents. | Surface grafting & chemical modifications | Bio-based acid, favors fabricated CNCs for biomedical application | Requires no Biomass pretreatment. |
| Disadvantage | Presence of sulphonyl groups degrades polymer nanocomposites | Difficult to disperse in solvents | Partial dispersion of biomass difficult CNC synthesis | High electricity consumption, non-uniform CNC size distribution | Slow CNC fabrication process, requires concentrated acid and high temperature | Low stability | Requires long reaction time. | Extremely low yield and very high reaction time |
| Scale Up Problems & benefits | Health hazardous, Environmentally unsafe process, requires acid resistant process equipments which increases cost. High end applications of fabricated CNCs | Limited application restricts large scale production of CNCs through these process. | CNC production from inexpensive wood pulps. Radical based reaction so difficult to scale-up. | Costly production due to high electricity consumption, efficiency of homogenization decreases at large scale. | Fabricated CNC suitable for polymer nanocomposites, Time consuming compared to other process. Costly process. | HBr highly corrosive in nature. Health hazardous process. | Ultrasonication coupled process difficult to scale-up, energy intensification process. | Suitable for CNC fabrication from mixed biomass resources, low yield and long reaction time come as hurdle for scale-up. |

Oksman et al. (*145*) fabricated PLA/CNC nanocomposites by incorporation of CNC suspension into polymer melt during extrusion process. However, the use of N,N-dimethylacetamide (DMAc) as solvent for dispersing CNCs through extrusion led to degradation of PLA at high temperatures. Dispersion of CNC filler into PHBV through melt compounding has also been observed to cause reduction in molecular weight (*85*, *133*) which in turn can adversely affect the mechanical and barrier properties of the polymer nanocomposites. Surface modified CNCs dispersed into PVA and PP through extrusion resulted in homogenous dispersion of nanoparticles in the polymer matrix. The biggest drawback of polymer/CNC nanocomposite fabrication using extrusion is polymer degradation as the sulfonyl end groups on CNCs act as initiation sites for polymer decomposition. Although CNCs fabricated using hydrochloric acid do not cause polymer degradation during processing, problems associated with proper dispersion in the polymer matrix are still present. Proper functionalization of CNCs and fabrication of CNCs with different acids should be optimized, which could simultaneously lead to better dispersion stability in polymeric matrix and improved resistance to thermal degradation.

## Scale-up and Industrial Scale Production of CNC: Strategies and Troubleshooting Information

CNC has found potential application in a wide variety of fields ranging from drug delivery in pharmaceutical industries to packaging industries. Hence large scale production of CNCs will become a necessity in the near future to address the required demand. While the large scale processing of CNCs is still in design stage, carried out at FPInnovation (http://fpinnovation.ca) in USA, the currently prevalent laboratory scale method of CNC fabrication using sulfuric acid hydrolysis is extremely difficult to scale up. Moreover, the sulfuric acid based process would require acid resistant reactors and pipelines which will add extra cost to the process. The huge amount of water required to stop the reaction during dilution process, and proper disposal of acid would make the fabrication process environmentally unfriendly. Therefore, alternative technologies for CNC production process should be developed to make the process “green” and eco-friendly. The type of biomass and pretreatment procedure followed for cellulose extraction significantly affects the CNC fabrication process. Thus, biomass selection and pretreatment procedures need to be optimized for maximum yield.

Recently, several advances have been made in the field of CNC synthesis using different acids. A comparison of scale-up strategies with different acid system is presented in Table 3. Ammonium persulfate (APS), a very cheap and weak oxidant, can successfully disintegrate the raw cellulose fibers from biomass into nanocrystals. However the yield is extremely low and stability of the CNCs has not been studied in detail, which makes the process unsuitable for commercialization. CNCs have also been manufactured with TEMPO, hydrochloric acid, hydrobromic acid and phosphoric acid. TEMPO based oxidation of CNCs has several advantages with regards to scale up and industrial

based production. The size of the original wood cellulose fibers, before and after TEMPO oxidation, is in the micron range and shows no welling except functionalization with sodium carboxylate groups. The fibers are thereafter washed thoroughly to remove the solvent, and finally disintegrated into nanofibers through onsite mechanical disintegration. The advantages of this procedure is that inexpensive wood cellulose, especially paper pulp, beached wood which contain 85-95% cellulose and 5-15% of hindered hemicellulose, could be used for production of TEMPO oxidized CNCs (*29*). Alternatives routes, such as high pressure homogenization, make the process energy intensive whereas use of other corrosive acids such as HBr and HCl has problems related to dispersion and stability. New technologies developed at the laboratory scale, using enzymatic and microbial route, suffer from low yield and high cost and hence are not suitable for scale-up. Thus, there is a strong need to develop commercially viable technologies for large scale, high yield production of CNCs to fulfill the demands of application industries.

## Summary

CNC or nanocellulose is a biodegradable, non-toxic, environmentally friendly nanoparticle with immense potential for application in fields such as biomedical engineering, food packaging, sensors, electronic devices etc. It is derived from renewable resources present in abundance in the form of biomass. This chapter discussed recent developments in the fabrication of CNCs from cheap biomass and industrial waste resources using different acids, and highlighted the issues related to scale-up. Also discussed were the use of surface modification and grafting to properly disperse CNCs into polymeric matrix which significantly improves the mechanical and barrier properties. Such property enhancements make CNC-based polymer nanocomposites attractive for food packaging applications. Proper selection and optimization of process parameters must be done to ensure good stability and dispersion of CNCs in the polymer matrix. Large scale production of CNCs will be required in the near future and intensive research must be carried out to overcome the technical problems associated with scale-up of current fabrication techniques.

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## Chapter 18

# Advances in Cellulose Hydrophobicity Improvement

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Cellulose is the most abundant natural occurring source of raw materials for the fabrication of environmental-friendly products. However, its water-proof property is poor, thus the hydrophobicity improvement for cellulose is critical for use in research and potential applications. The cellulose hydrophobicity improvement includes chemical modification and physical treatments. The fabrication process, mechanism and potential applications of the hydrophobic cellulose products as well as the assessments are described in detail in the present chapter. Moreover, the novel functional materials based on cellulose hydrophobic modification with novel methods are emphasized, hoping to broaden the applications of cellulose for replacing the fossil-based products partially.

## Introduction

Recently, the chemistry of the world became oriented to the exploiting of bio resources, considering the themes of "Chemistry for a sustainable world" "Chemistry of Natural Resources" and "Chemistry of energy and food" corresponding to 239th, 241st and 245th ACS meetings, respectively. The fabrication of environmental-friendly materials from the renewable bioresources via a "green" pathway has attracted much attention as a worldwide topic. Entering the 21st century, cellulose as the most abundant and reproducible biopolymer is considered to be one of the most promising resources to replace fossil fuel. However, as one of the most intransigent biomacromolecules, cellulose is difficult to dissolve due to the strong inter- and intra-hydrogen bonding interactions (*1*).

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It is noted that in our laboratory, novel green solvent systems, such as NaOH/urea aqueous solution with cooling, have been developed to dissolve cellulose in about 2 minutes (*2*). Compared with other organic solvents dissolving cellulose at high temperature such as *N*-methylmorpholine -*N*-oxide (NMMO) (*3*), LiCl/*N,N*-dimethylacetamide (DMAc) (*4*) and ionic liquids (*5*), the rapid dissolution at low temperature is attractive. These new solvents break the limitation of the traditional heat dissolving methods of cellulose in organic solvents. From these novel solvents, cellulose raw materials such as cotton linter pulps can be converted to environmental-friendly regenerated materials such as fibers, films, microspheres and hydrogels (shown in Figure 1), and these materials are safe, biocompatible and biodegradable, so they are environmental-friendly materials (*6–16*).

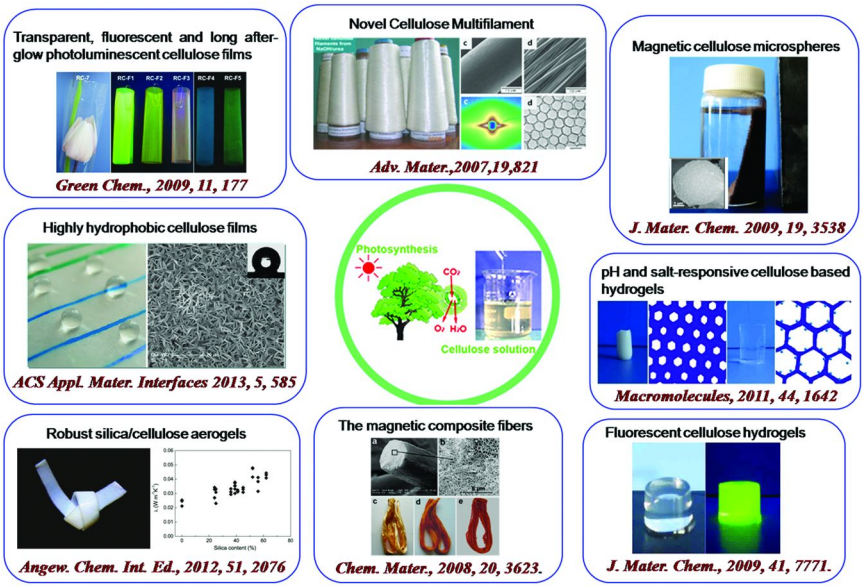



*Figure 1. Novel functional materials fabricated from cellulose in NaOH/urea aqueous solutions. Reproduced from references (6–12) and (15). Copyrights 2009 Royal Society of Chemistry; 2007 and 2012 John Wiley & Sons; 2008, 2011 and 2013 American Chemical Society.*

However, cellulose exhibits poor water resistance because of the abundant hydrophilic hydroxyl bonds, which restricts its applications, especially in the packaging field. Therefore, improving the cellulose hydrophobicity for better utilization has become a hot topic. There are two ways including chemical modification and physical treatments to construct the hydrophobic cellulose materials. Chemical modification contains size operation in paper making, cellulose esterification and atom transfer radical polymerization (ATRP) approach (*17*), and physical treatments such as the surface treatment with cold-plasma, coating and surface crystal growth have also been introduced in this chapter.

Moreover, the hydrophobic modified cellulose can also be used as microfluid platform (*18*), in oil-water separation (*19*, *20*) and medical dressing applications (*21*). The present chapter is mainly focused on the enhancement of the cellulose hydrophobicity via chemical or physical methods. The preparation process, characterization, and application of the hydrophobic cellulose materials from different methods are summarized in detail, including the discussion of their advantages and disadvantages.

## Chemical Modification

### Grafting Methods

The chemical modifications mainly consist of the derivative and grafting methods for improving hydrophobicity, which were performed by grafting hydrophobic groups such as long chain alkyl groups onto cellulose chains. Cunha et al have studied the effects of chemical modification by using different methods on the cellulose fibers hydrophobicity, and they have published a review on the cellulose hydrophobicity partially based on their systematic work (*17*). Notably, they have prepared highly hydrophobic/lipophobic cellulose fibers by hydrophobization and lipophobization of cellulose fibers through the reaction with gaseous trichloromethylsilane (TCMS) (*22*). $N_2$ was used as carrier gas for TCMS, and the reaction was conducted by streaming the $N_2$/TCMS mixture (0.1 L/min $N_2$ flow) through the filter paper for a given period of time. A three-dimensional Si-O-Si bridge network has been constructed, which was partly bound to the polysaccharide macromolecules to generate inorganic coatings around the fibers. The filter paper has been used as raw material, and the sample reacted for 30 min with 600 μL TCMS is denoted as FP2. Figure 2 shows the mapping and scanning electron microscopy (SEM) image of FP2. Obviously, silicon distributed evenly throughout the surface and cross section of the cellulose fiber, indicating that the reaction of TCMS was not limited on the surface. Moreover, there were many micro- and nano-asperities distributed along the fiber surface, resulting in high hydrophobicity. Therefore, they provided a simple and straightforward method for highly hydrophobic fiber fabrication.

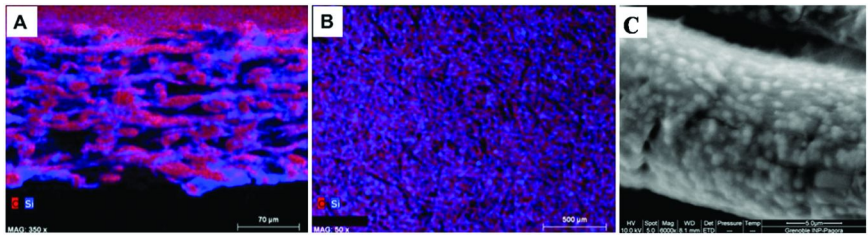
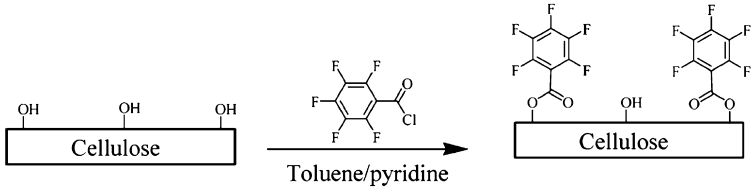



*Figure 2. C (red) and Si (blue) mapping of the FP2's cross section and surface (B), and scanning electron micrograph of FP2 at 6000×magnification (C). Reproduced from reference (22). Copyright 2010 Elsevier.*

In previous work, the authors prepared highly hydrophobic/lipophobic biopolymers by a simple esterification procedure using pentafluorobenzoyl chloride through controlled heterogeneous pentafluorobenzoylation of cellulose substrates (*23*). Scheme 1 shows the schematic view of pentafluorobenzoylation of cellulose fibers. The surface modification of plant and bacterial cellulose fibers was performed with pentafluorobenzoyl chloride in a nonswelling medium such as toluene. The fluorinated moiety on the surface endows cellulose fibers with remarkable hydrophobic/lipophobic properties. The pentafluorobenzoylated cellulose exhibits better hydrolysis resistance compared with trifluoroacetate cellulose. In view of the potential applications, it was claimed that the resistance to hydrolysis of these modified cellulosic substrates was a key property in major technologies such as papermaking, packaging, and biomedical commodities, where moisture-proof is very important. In related work, Rodionova et al fabricated hydrophobic cellulose fibrils through heterogeneous acetylating of microfibrillated cellulose (MFC) for physical properties modification and the cellulose fibrils morphology preservation (*24*). Before the acetylation reaction, successive solvent exchange using water, acetone and toluene was adopted, and the evaporation of the reaction mixture was prevented by using a water condenser system




*Scheme 1. Schematic view of pentafluorobenzoylation of cellulose fibers. Reprinted from reference (23). Copyright 2007 American Chemical Society.*

The superiority of the method was that the modification process did not affect the mechanical strength and morphologies. Moreover, the acetylating of MFC resulted in suitable barrier properties for sustainable packaging, broadening the application of cellulosic material in the packaging field. Cellulose fibers with hydrophobic surfaces have been fabricated after being grafted with norbornene utilizing surface-initiated ring-opening metathesis polymerization (SI-ROMP) method (*25*). The reaction scheme for SI-ROMP from cellulose fibers is shown in Figure 3A. Dichloromethane, 2-(dimethyl) aminopyridine and triethylamine are abbreviated to DCM, DMAP and TEA in the scheme, respectively. Field emission scanning electron microscopy (FESEM) images of unmodified cellulose and a filter paper grafted at room temperature for 1 minute are also shown in Figure 3. Obviously, the grafted filter paper was completely covered by polynorbornene and the original fibrillar structure almost disappeared (B, C), meanwhile the grafted filter paper changed from hydrophilic to hydrophobic (D). The authors provided a versatile and rapid method to modify cellulose fiber and improve the hydrophobicity.

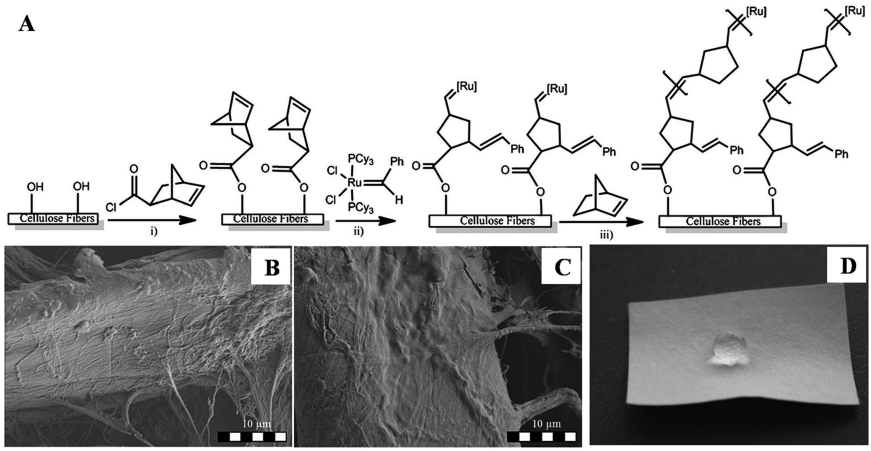



*Figure 3. Reaction scheme for the SI-ROMP from cellulose fibers (A): (i) TEA/DMAP/DCM, room temperature, (ii) heptanes, room temperature and (iii) DCM, room temperature or 0°C or -18 °C; FESEM images of an unmodified cellulose (B) and a filter paper grafted at room temperature for 1 minute (C); A water droplet on a filter paper grafted with polynorbornene for 1 min (D). Reproduced from reference (25). Copyright 2012 Royal Society of Chemistry.*

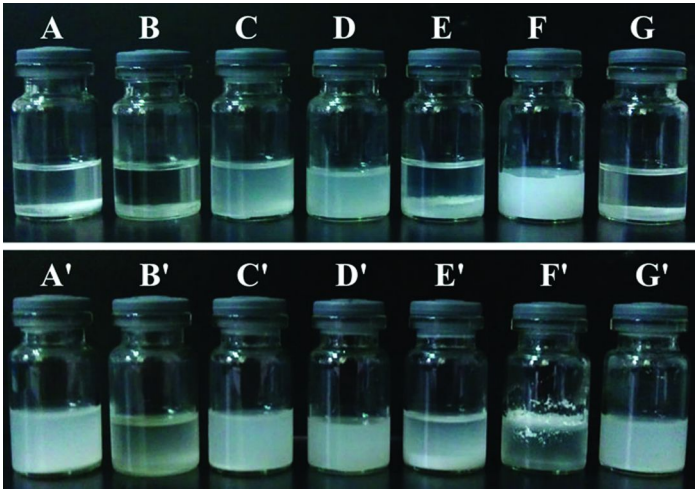
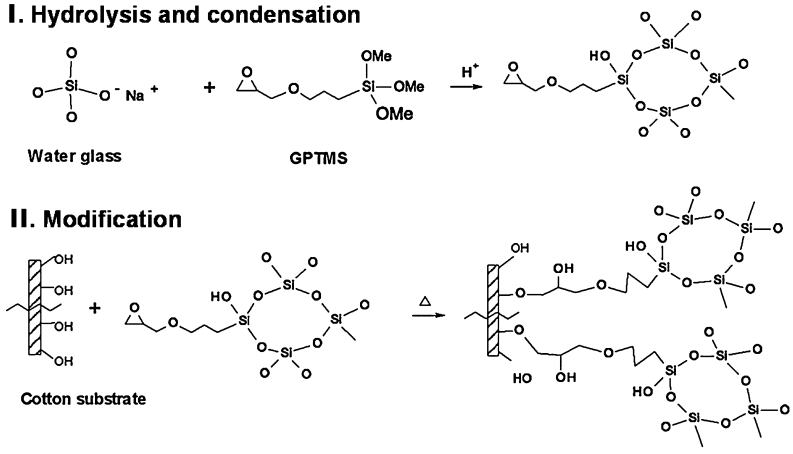



*Figure 4. Dispersion visualization of the CN (upper) and CO-g-CN (down) in ethyl ether (A and A'), toluene (B and B'), acetone (C and C'), N,N-dimethylformamide (D and D'), ethanol (E and E'), water (F and F') and tetrahydrofuran (G and G'). Reprinted from reference (26). Copyright 2013 Springer.*

Shang et al have prepared hydrophobic cellulose nanocrystals (CNs) by grafting isocyanate-terminated castor oil onto surface of CNs (*26*). Phenyl isocyanate (PI) was used to produce PI-terminated castor oil (PI-CO), and the molar ratio of NCO versus OH was 2.1:3 in order to guarantee that the CO molecules with the reactivity towards toluene diisocyanate (TDI). Figure 4 shows the dispersion visualization of the CN and castor oil-grafted cellulose nanocrystals (CO-g-CN) in different solvents. The CN covered with CO displayed a relatively good dispersibility in some low-polar solvents, such as ethyl ether and toluene. The novel CO-g-CN can be used as valuable alternatives to formulate bionanocomposites with non-polar polymers for optimized performances. They provide a pathway to improve the CN hydrophobicity for its application in some low-polar solvents.




*Scheme 2. The hydrophobic modification process by water glass and GPTMS. Reprinted from reference (29). Copyright 2010 Elsevier.*

## Sol-Gel Method

The sol–gel method is based on the hydrolysis and condensation reactions of suitable metal/non-metal alkoxides, such as using alkoxysilane to produce silica materials, and silica sol–gel films are prepared by dip-coating, spin-coating or electrodepositing the silica sol onto different substrates (*27*, *28*). Shang et al have fabricated durable superhydrophobic cellulose fabric from water glass and n-octadecyltriethoxysilane (ODTES) with 3-glycidyloxypropyltrimethoxy silane (GPTMS) as crosslinker by the sol–gel method (*29*). Native cotton fabrics were immersed in the silica sol, padded with a wet pickup, dried at 80 °C, and then rinsed with water. The treated fabrics were immersed in ethanol solution of hydrolyzed n-octadecyltriethoxysilane , dried at room temperature, and cured at 120 °C for 1 h in an oven. Scheme 2 shows the hydrophobic modification process, the hydrolyzed siloxane part in GPTMS molecule reacted with Si-OH groups in silica sol, and the epoxy ring of GPTMS further reacted with hydroxyl groups on cellulose fiber to

complete surface modification. The interfacial adhesion between cellulose fiber and hydrophobic silica layer was enhanced by modification, resulting in a durable superhydrophobic surface. The treated cotton still remained hydrophobic after 50 washing cycles, which can meet customers' demands. The authors provided a new bridge-crosslinking method to improve the durability of a superhydrophobic surface, which is very important for daily applications.

Cappelletto et al have fabricated hydrophobic siloxane paper by using siloxane coating on pure cellulose paper through sol–gel dipping in sols (*30*). $SiO_2$ sols were prepared by dispersing the appropriate amount of precursor (tetraethoxysilane, methyl triethoxysilane, dimethyl diethoxysilane, trimethyl monoethoxysilane) in absolute ethanol with stirring, and then HCl or $NH_4OH$ was added dropwisely to complete hydrolysis. Whatman filter papers were dipped in the sols, conditioned at room temperature for 1 h, and the coated samples were conditioned in the oven at 60 °C for 30 min for the second dipping. The hydrophobicity of the paper surface increases with the methyl number of siloxane precursor. Moreover, the mechanical and thermal properties can also be improved by increasing the coating thickness. The authors systematically studied the effects of different siloxane coatings, which provided an effective and direct method for cellulose hydrophobic modification with siloxane coatings.

The surface properties of the cellulose fibrous mats were modified by Ding et al from superhydrophilic to superhydrophobic with a simple sol-gel coating of decyltrimethoxysilane (DTMS) and tetraethyl orthosilicate (TEOS) (*31*). Cellulose acetate nanofibrous mats are fabricated by electrospinning cellulose acetate solutions dissolved in acetone/DMAc, the resultant cellulose acetate samples were fixed on the slide glass, immersed in sol–gel solutions and air dried for 1 h subsequently. Then, the sol–gel film-coated samples were heated. Figure 5 shows a schematic diagram of a sol–gel (I) film on a cellulose acetate surface and several water droplets were placed on the 10 wt% cellulose acetate fibrous mat coated with the sol–gel (I) film. The superhydrophobicity of fibrous mats was attributed to the combined effects of the high surface roughness of the electrospun nanofibrous mats and the hydrophobic DTMS sol–gel coating. Additionally, hydrophobic sol-gel nanofilms were found to be transparent according to UV–visible spectra. Therefore, the authors provided an effective pathway to fabricate superhydrophobic cellulose film with TEOS and DTMS coating.

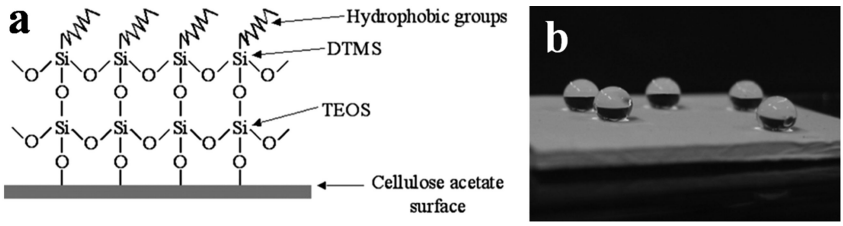



*Figure 5. A proposed schematic diagram of sol–gel (I) films on cellulose acetate surface and the photograph of superhydrophobic 10 wt% cellulose acetate fibrous mat coated with the sol–gel (I) film (b). Reproduced from reference (31). Copyright 2006 Iopscience.*

Vasiljević et al have created superhydrophobic and oleophobic cotton fabric by surface modification of cellulose fibers. The pretreatment plasma was prepared with the use of low-pressure water vapor plasma and the following sol-gel coating was accomplished with the fluoroalkyl-functional siloxane (FAS) (*32*). The FAS used in this work was a water- and oil-repellent organic-inorganic hybrid precursor. The cellulose fabric treated by plasma pre-treatment (PT) and sol-gel coating with the precursor FAS was denoted as CO (PT)-FAS sample. Figure 6 shows the SEM image and representative atomic force microscopy (AFM) image of the CO (PT)-FAS sample. The plasma pre-treatment introduced the nanoscopic topography (a,b) and led to the formation of a micro- and nano-structured binary composite fiber surface, resulting in superhydrophobicity. Obviously, the CO (PT)-FAS sample exhibited self-cleaning property (lotus effect), as shown in Figure 6c. The effective concentration of the FAS network on the fabric was enhanced by the plasma pre-treatment, resulting in improved repellency before and after repetitive washing compared with FAS network without plasma treatment. The authors provided an effective method to create superhydrophobic/oleophobic cellulose fibers with self-cleaning properties.

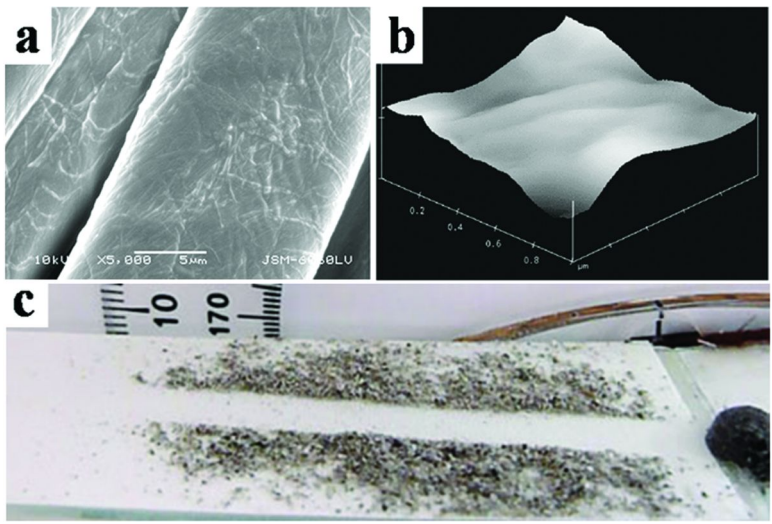
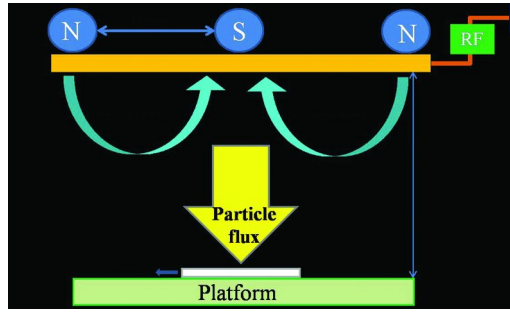



*Figure 6. SEM image (a) and Representative AFM image of CO (PT)-FAS sample (b), and demonstration of the self-cleaning effect on the surface of CO(PT)-FAS sample covered with solid particles of dirt(c). Reproduced from reference (32). Copyright 2013 Springer.*

**Polymerization on the Surface**

Hydrophobic cellulose films can be obtained by surface modification via plasma polymerization, using RF (Radio Frequency) magnetron plasma method, where acetylene and nitrogen are precursor gases (*33*). Scheme 3 shows the schematic diagram of the RF magnetron plasma source. In the discharge region near the cathode, dense plasmas were produced under a strong RF electric field to obtain high density particle fluxes. Particle flux density of ions, radical, and excited molecules and atoms decreased with the distance (h) from the cathode. Small pieces 4×4 $cm^2$ of cellulose membranes were placed on the glass stage at distance h from the cathode for plasma deposition. Thus, high hydrophobic cellulose membranes with a water contact angle (WCA) of 130° could be prepared by the appropriate conditions of deposition time (t), distance between cathode and the membrane etc. Moreover, the hydrophobic hydrocarbon films showed good stability for normal usage.




*Scheme 3. Schematic diagram of the RF magnetron plasma source.*

A robust and durable superhydrophobic cotton fabric for oil/water separation has been fabricated by Zhou et al via in situ deposition of polyaniline and fluorinated alkyl silane to the cotton fabric via a facile vapor phase process (*20*). The clean cotton fabric was steeped in the $FeCl_3/C_{14}H_{19}F_{13}O_3Si$ solution for about 2 min, and dried at room temperature. The vapor phase deposition of aniline was performed by placing the treated fabric into a small chamber filled with aniline vapor. Then the fabric was washed and dried to remove the solvent. Figure 7 shows the fabrication process (a). Obviously, the resultant cotton fabric was superhydrophobic with WCA of 156° (b, left). Hexadecane could spread quickly on the coated fabric and permeate thoroughly within 10 s, indicating superoleophilicity (b, right). Moreover, the coated fabric exhibited high oil-water separation efficiency (up to 97.8%), which can withstand the severe environmental conditions to be reused for at least 30 times with stable superhydrophobicity and constant separation efficiency. Therefore, the authors provided a simple and versatile method to prepare superhydrophobic cotton fabric for oil-water separation, broadening the application of cellulose.

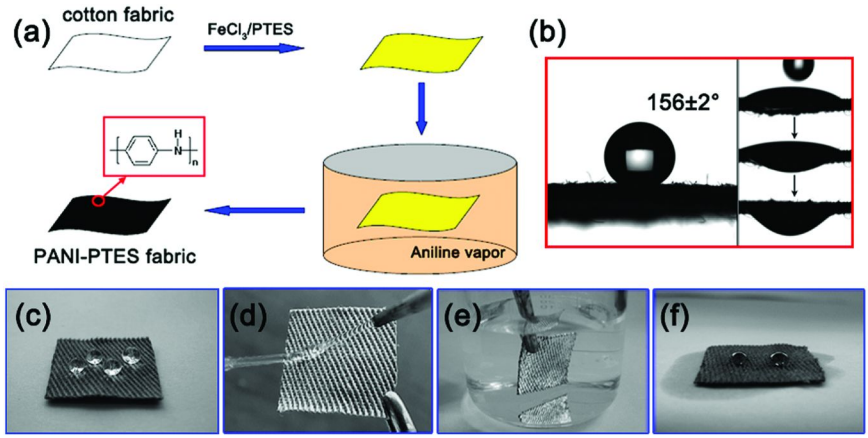



*Figure 7. (a) Schematic of the fabrication of superhydrophobic cotton fabrics through vapor phase deposition process. (b) Water droplet sitting on the superhydrophobic cotton fabric and hexadecane droplet spreading on and permeating through the fabric. (c) Water droplets on the coated textile, (d) a jet of water bouncing off the surface, (e) the textile immersed in water by an external force, (f) water droplets on the oil contaminated textile. Reproduced from reference (20). Copyright 2013 American Chemical Society.*

A superhydrophobic surface on a cellulose-based material such as cotton fabric or paper has been fabricated by Li et al through a solution-immersion method with the condensation of an industrial waterproof reagent potassium methyl siliconate (PMS) (*34*). Figure 8 shows the coating process (a), the images of water droplets on different surfaces (b,c), and FESEM image of the modified cotton fabric at low-magnification (d). The method involved a hydrogen bond assembly and a polycondensation process. The silanol solution was prepared by reacting the PMS aqueous solution with $CO_2$ at room temperature, cotton fabric (or filter paper) was soaked in deionized water followed by the addition of PMS aqueous solution with stirring. The silanol was formed by a reaction of PMS aqueous solution with $CO_2$ and assembled on the cellulose surface via hydrogen bond interactions of the hydroxyl between cellulose and silanol to prepare the PMS coatings. Obviously, the surface of the modified cotton fabric and filter paper was superhydrophobic, and a thin layer of nanoscaled spherical protuberances was observed on the modified fiber. The superhydrophobic coatings were satisfactory due to both chemical and mechanical durability. The facile and versatile method in this work is expected to fabricate superhydrophobic cellulose materials as compared to organic silicon halides.

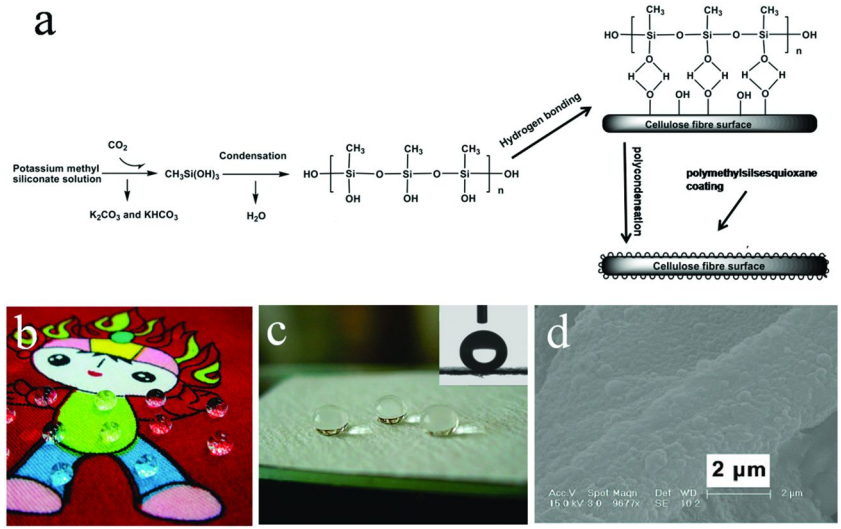



*Figure 8. The formation process of a superhydrophobic polymethylsilsesquioxane coating on a cellulose fiber surface (a), images of water droplets on the surface of the modified colorized cotton fabric (b) and the modified filter paper (c), and FESEM image of the modified cotton fabric at low-magnification (d). Inset shows the image of water droplet. Reproduced from reference (34). Copyright 2008 American Chemical Society.*

**Polymerization and LBL Coating**

Layer by layer (LBL) assembly can be used to generate nanocoatings by selective deposition of polymers on various substrates, which can be used to modify the surface architecture with different components and roughness. Guntari et al have rendered different substrates such as cotton and filter paper superhydrophobicity by using the versatile continuous assembly of polymers (CAP) process mediated with ring-opening metathesis polymerization (ROMP) ($CAP_{ROMP}$ ) approach to tune the surface composition and properties of nanocoatings (*35*). Figure 9 shows different macrocrosslinkers, surface-bound catalyst and fabrication of multilayered amphiphilic films via $CAP_{ROMP}$. All planar substrate manipulations were conducted in individual oven-dried vials under argon, and the functionalized substrates with catalysts were placed in vials followed by the addition of CAP-active macro-crosslinker. Polymer-coated substrates were removed, washed with DCM and then exposed to ethyl vinyl ether (EVE). In their findings, the authors demonstrated that hydrophilic substrates in nature can be rendered with superhydrophobicity by rational selection of the macromolecules crosslinkers.

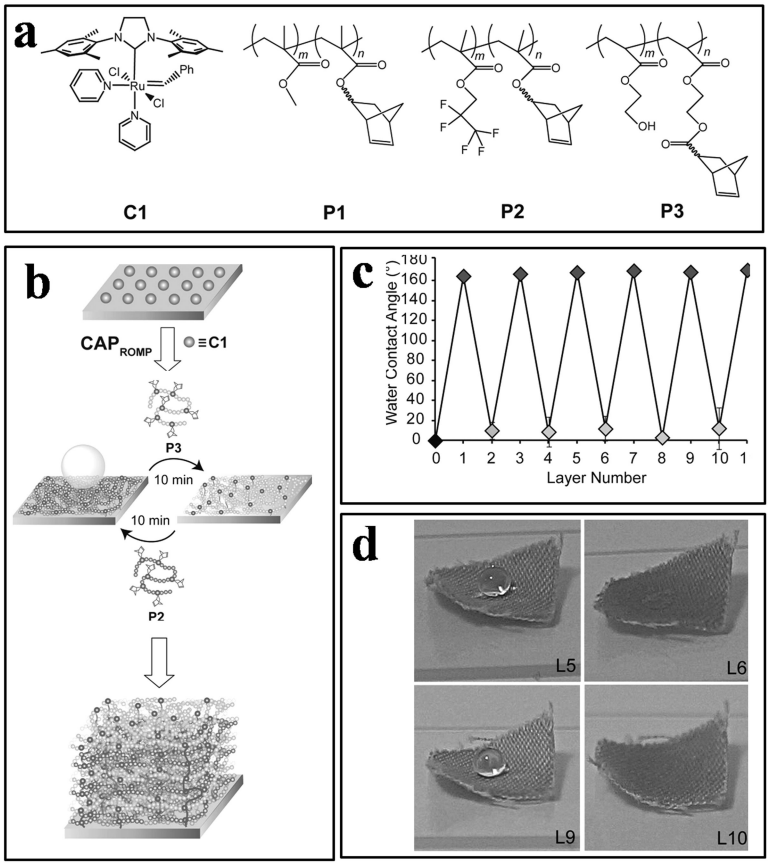



*Figure 9. Formulas of different macrocrosslinkers (P1, P2 and P3) and surface-bound catalyst C1 (a) and fabrication process of multilayered amphiphilic films via CAPROMP : general scheme of iterative $CAP_{ROMP}$ reactions on cotton using macrocrosslinkers P2 and P3 (b); contact angle measurements with respect to number of layers (odd and even layer numbers correspond to outermost coatings of P2 and P3, respectively) (c); and superhydrophobic and superhydrophilic behavior of the cotton after alternate layering with P2 and P3. L5, L6, L9, and L10 correspond to 5, 6, 9, and 10 layers, respectively (d). Reproduced from reference (35). Copyright 2013 John Wiley & Sons.*

As is known, native cotton exhibits superhydrophilic behavior with contact angle of 0°. As shown in Figure 9c, native cotton is converted to superhydrophobic (168° ± 4°) by rapid exposure to P2. The superhydrophilicity of cotton was retrieved by exposure to P3, and the reversible switching lasted for 11 times, highlighting the surface confined ROMP catalyst robustness and the $CAP_{ROMP}$ approach versatility (Figure 9c,d). Moreover, the hydrophobic behaviors of the

filter paper can be also controllable. Therefore, the authors further provided a versatile and effective method to control wetting properties, showing wide and promising applications such as micro fluidic devices and self-cleaning surfaces.

**Surface-Initiated Atom Transfer Radical Polymerization (SI-ATRP) Method**

Recently, the SI-ATRP technique has attracted much attention because it can be used for the surface grafting high-density polymer with well-defined structure. Moreover, the corresponding molecular weight and its distribution are tunable in this process. Up to now, the SI-ATRP technique has been successfully employed to graft polymers to various surfaces (*36–40*). Xiao et al have created controllable hydrophobic chains on cellulose microfibrils (CMF) by SI-ATRP of butylacrylate (BA) on CMF (*41*). They selected BA for SI-ATRP because the resultant PBA on CMF surfaces would improve the compatibility between CMF and PBA polymer matrix, showing great potential to act as elastomeric interfacial layer for the reinforcement of biocomposites. Therefore, the modified CMF with hydrophobicity broadens the application of CMF as a promising reinforcement of biocomposites for automobile applications.

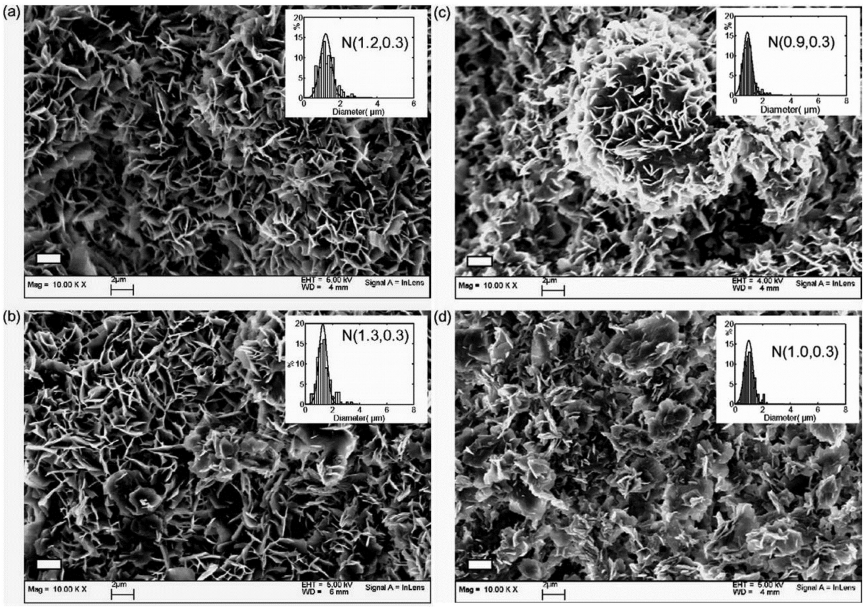



*Figure 10. SEM micrographs of AKD particles collected at different pre-expansion pressure and temperature: (a) 300 bar, 40 °C; (b) 200 bar, 40 °C; (c) 300 bar, 60 °C; (d) 200 bar, 60 °C. The white scale bar corresponds to 2 μm. Reproduced from reference (43). Copyright 2009 Elsevier.*

**Introduction of Alkyl Ketene Dimer (AKD)**

AKD is a typical crystallizing wax and hydrophobizing chemical, which is used as a common sizing agent in the papermaking industry (*42*). Quan et al have produced superhydrophobic AKD layers on top of untreated cellulose paper surfaces by a rapid expansion of supercritical $CO_2$ solution (RESS) process (*43*). Firstly, a certain amount of AKD granules were loaded into the pre-expansion vessel of stainless steel. Liquid $CO_2$ was transferred into the vessel and compressed to the pre-expansion pressure, and the AKD granules were then dissolved in supercritical carbon dioxide (SC-$CO_2$) during agitation. The AKD-$CO_2$ solution was sprayed through a sapphire nozzle into an expansion chamber at atmospheric pressure after 1 h. Figure 10 shows the SEM images of AKD particles collected at different pre-expansion pressure and temperature. The average AKD particle size decreases with the increase of pre-expansion pressure or pre-expansion temperature and decrease of spraying distance. The porous structure of AKD flakes and aggregates of AKD flakes with inherent low surface energy of AKD led to the superhydrophobic properties to the target surface. The new method was fast and more environmentally sustainable than most other current techniques for producing superhydrophobic surfaces, avoiding the utilization of fluorine compounds and organic solvents.

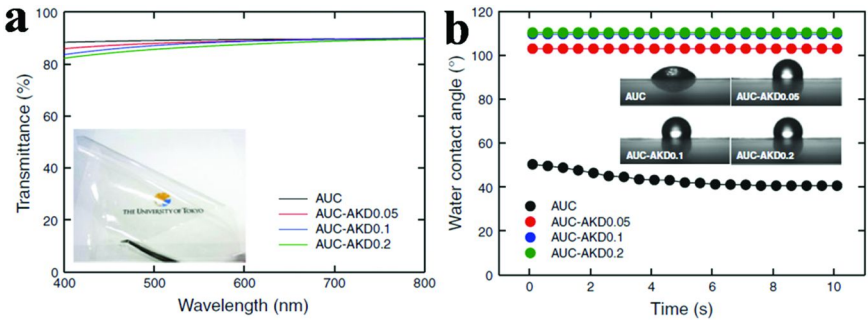



*Figure 11. Visible light transmittance of AUC and AKD-treated AUC films with 30 μm thickness (a); Changes in water contact angle on different films over time and photographs of water droplets on the films, taken 0.1 s after landing (b). The inset photograph for a shows AUC–AKD0.1 film. Reproduced from reference (44). Copyright 2012 Springer.*

More recently, Yang et al have fabricated the transparent and water repellent cellulose films by using surface modification of alkali/urea regenerated cellulose (AUC) films through soaking in cationic AKD dispersion and drying process (*44*). Cellulose solution was prepared by dissolving cotton linters pulp in LiOH/urea/$H_2O$ solution with cooling, and sheet-like cellulose hydrogels were prepared by casting method to prepare AUC films. The once-dried AUC films were soaked in

AKD dispersion and then rinsed thoroughly with water. The wet films were dried in the same way and then heated at 100 °C for 10 min. The AKD-treated AUC films obtained from AKD concentration of 0.05, 0.1, and 0.2 wt% are denoted as AUC–AKD0.05, AUC–AKD0.1, and AUC–AKD0.2 respectively. Figure 11 shows the transmittance of different films, the photograph and WCA change of water droplets on different films. The AKD-treated AUC films exhibited high light transparency (88 % at 600 nm) and high hydrophobicty with water contact angle of 110°. The WCA of AUC–AKD0.1 was similar to AUC–AKD0.2 and the curve for AUC–AKD0.1 was almost covered by AUC–AKD0.2, indicating that the required AKD concentration was about 0.1 wt%. Moreover, the AKD-treated AUC films had high tensile strength, low water uptake, and gas barrier properties. Therefore, the authors provided a simple and effective method to prepare transparent and highly hydrophobic cellulose films.

### Formation of Nanosilica and Polymer Composites

Gashti et al have modified cotton fibers by embedding silica nano-particles on the surface to improve the hydrophobicity and thermal stability using 1,2,3,4-butanetetracarboxylic acid (BTCA) as a crosslinking agent and sodium hypophosphite as a catalyst (*45*). Embedding particles on cotton included four steps:

1 colloidal dispersions preparation;
2 addition of polycarboxylic acid crosslinking agent (BTCA) with sodium hypophosphite (60% of BTCA);
3 padded (85% wet pick up) in prepared solutions; and
4 heat-treated by ironing at 200 °C for 10 s.

Figure 12 shows the SEM images of the modified cotton fiber cross-linked with BTCA and 40 g/L nano-silica at different magnifications. Obviously, BTCA-silica nanocomposite was well dispersed on the surface of fiber with a thin layer of the silica coating, resulting in hydrophobicity and water repellent properties. The authors provided a pathway to fabricate hydrophobic cellulose fiber. However, the drawback of this method, as the authors mentioned, was a great loss in abrasion resistance for the crosslinked structures of the cotton fibers due to the irreversible acid catalyzed depolymerization or the hydrolytic action of BTCA on the cellulose chains.

## Physical Treatments

### Treatments on Electrospun Fibers

Micro- to nano-scale polymer fibers such as cellulose acetate fibers can be fabricated by electrospinning, which is a unique and effective technology to produce nonwoven-type mats with a hierarchical micro/nanostructure for a broad range of applications (*46*). Yoon et al have prepared nano/micro-fibrous cellulose triacetate (CTA) mats by electrospinning CTA dissolved in DCM/ethanol

solvent (*46*), which were endowed with superhydrophobicity by further carbon tetrafluoride ($CF_4$) treatment. In the solvent composing of 80/20 (v/v) for DCM/ethanol, the untreated CTA mat of 5 wt.% CTA concentration exhibited high surface roughness with corresponding WCA of 142° and good electrospinning process ability, showing high hydrophobicity. Figure 13 shows AFM images of CTA fibrous mats with various plasma treatment times. The non-treated electrospun CTA mat displayed a rough surface, resembling a lotus-leaf structure. The surface roughness of the CTA mat was maintained up to 60 s (WCA of 153° and a minimum water tilting angle (WTA) of 4°) by plasma treatment, because the excessive plasma treatment would reduce surface roughness due to the etching (collapse) of the protrusion peaks in the surface. They provided useful message and experience for hydrophobic modifying cellulose fibers.

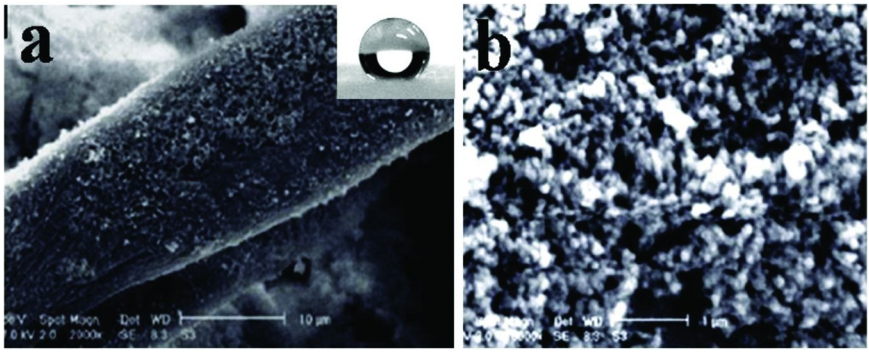



*Figure 12. SEM image of the cotton fiber cross-linked with BTCA and 40 g/L nano-silica of 2000× and 15,000× magnification. Inset shows the water droplet on its surface. Reproduced from reference (45). Copyright 2012 Elsevier.*

Anitha et al have fabricated water-repellent and antibacterial cellulose acetate (CA) fibrous membrane embedded with ZnO nanoparticles through electrospinning method (*47*). Zinc acetate dihydrate was dissolved in a mixture of DMF/acetone with the ratio of 4:1, and then the cellulose acetate was added into the sol solution and stirred to achieve the homogeneous solution for electrospinning. Figure 14 shows SEM image of 14 wt% CA composite fibrous membrane, TEM image of the composite fiber and bactericidal effect of CA composite fibrous membrane against *Staphylococcus aureus* (c). The ZnO nanoparticles were distributed evenly in the CA fiber. Furthermore, the CA composite fibrous membrane exhibited high hydrophobicity and strong bacteria repellency. They provided a good synthesis method for the fibrous composite membrane by electrospinning to prevent agglomeration of nanoparticles and increase the contact area between the surface and the microorganisms.

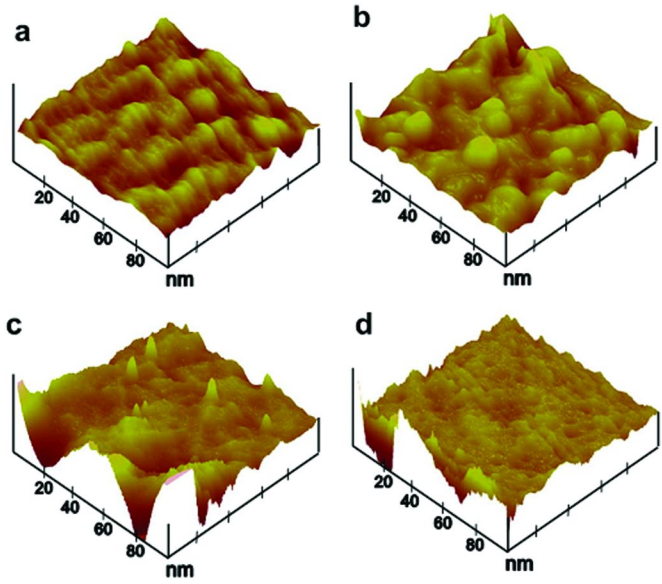



*Figure 13. AFM images of CTA fibrous mats with various plasma treatment times; (a) 0s, (b) 60s, (c) 180s, and (d) 300s. Reprinted from reference (46). Copyright 2009 Elsevier.*

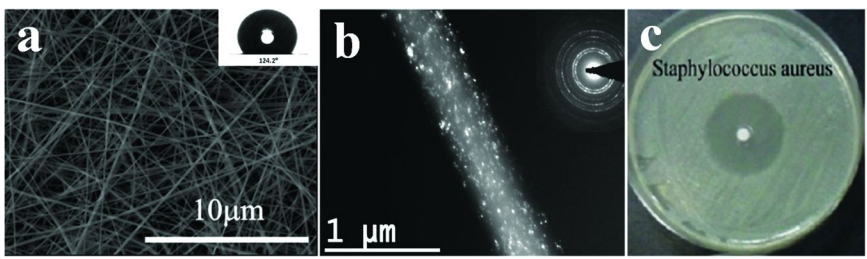



*Figure 14. SEM of 14 wt% CA membrane embedded with ZnO and inset photograph of water droplet ZnO embedded CA fibrous membrane (a); TEM image of the composite fiber and the inset SAED patterns (b); Image of bactericidal effect of ZnO embedded CA fibrous membrane against Staphylococcus aureus (c). Reproduced from reference (47). Copyright 2012 Elsevier.*

Thorvaldsson et al have coated a textile cellulose microfiber with electrospun cellulose nanofibers, and subsequently treated it with fluorine plasma (F-plasma) to create superhydrophobic microfiber (*48*). Figure 15 shows the SEM image of a Lyocell™ filament microfiber decorated with cellulose acetate (CA) nanofibers after F-plasma treatment (A) and corresponding electron spectroscopy for chemical analysis (ESCA) spectra (B). Obviously, the nanofibers of 450–600 nm in the dimension were smooth and randomly collected on the microfiber, resulting in a rough surface of fiber. The F-plasma treatment deposited fluorine groups onto the fibers, leading to the superhydrophobicity. They provided an environmental friendly method to create non-wetting cellulose fibers with superhydrophobicity.

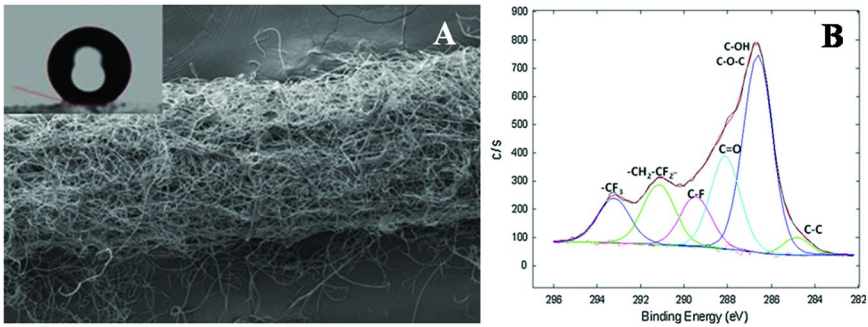



*Figure 15. A cellulose microfiber coated with cellulose nanofibers after plasma treatment (A) and corresponding ESCA spectra (B). The insert of A shows a drop of water on the fiber surface. Reproduced from reference (48). Copyright 2012 Springer.*

**LBL Technique and Adsorption of a Colloidal Wax**

LBL technique is a versatile method for surface modification, which can be used to treat polyelectrolytes for a multilayer structure formation with tailored properties. Gustafsson et al have improved the water resistant of the cellulose paper by combining the LBL technique with the adsorption of a colloidal wax onto the multilayer structure (*49*). Two polyelectrolytes of poly(allylamine hydrochloride) (PAH) and poly(acrylic acid) (PAA) were added separately to the fiber suspension, and allowed to adsorb for 20 min. After each polyelectrolyte adsorption at suitable PH step, the fibers were thoroughly rinsed with deionized water. Figure 16 shows the scheme of cellulose fiber after PAH/PAA LBL assembly and adsorption of wax, and the corresponding WCA. After the adsorption of five layers of PAH and PAA and the following adsorption of 8 mg paraffin wax per gram fiber, the contact angle (measured 60 s after a drop of water was applied to the sheet) was about 138°. Furthermore, the contact angle increased to be 150° when the sheets were cured for 30 min at 160 °C after sheet making.

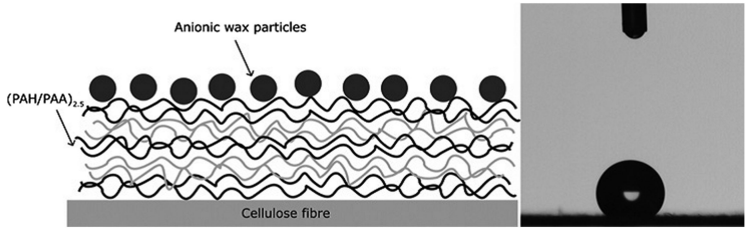
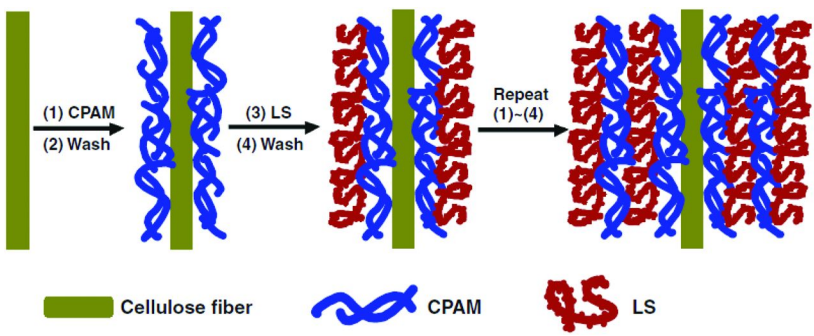



*Figure 16. Scheme of cellulose fiber after poly(allylamine hydrochloride) (PAH)/poly(acrylic acid) (PAA) LBL assembly and adsorption of wax, and the corresponding WCA. Reproduced from reference (49). Copyright 2012 Elsevier.*

Li et al have modified cellulose fiber into controllable hydrophobicity using LBL self-assembly technique to construct multilayer structure on cellulose fiber with cationic polyacrylamide (CPAM) and lignosulfonate (LS) (*50*). Scheme 4 shows the illustration of the LBL self-assembly process of CPAM and LS on cellulose fiber. The fiber surface roughness and hydrophobicity enhances as self-assembly proceeds by gradually covering with LS granules and CPAM/LS multilayers construction respectively. Moreover, the print density of handsheet increased with an increase of the number of bilayers, indicating that CPAM/LS multilayers construction on cellulose fiber surface improves printability of the handsheet. This pathway exhibited a positive impact and potential application value in cellulose fiber-based hydrophobicity modification and printing process control.




*Scheme 4. Illustration of the LBL self-assembly process of CPAM and LS on cellulose fiber. Reprinted from reference (50). Copyright 2012 Springer.*

**Coating with Inorganic Nanoparticles**

Nanocellulose hydrogels can be converted to highly porous nanocellulose aerogels by using vacuum-drying, freeze-drying or supercritical $CO_2$ drying. $TiO_2$ is inexpensive and nontoxic with photocatalytic properties, and nano $TiO_2$ coating creates low surface energy surface on the nanocellulose aerogels and renders cellulose aerogels with photoswitchable superabsorbency (*51*). Korhonen et al have coated the native cellulose nanofibrils aerogels with $TiO_2$ by using atomic layer deposition for their functionalization (*19*). Titanium isopropoxide was used as the titanium precursor and water as the oxygen source. $TiO_2$ was coated onto the nanocellulose aerogels by using atomic layer deposition, where a flow-through geometry along with long pulse and purge times was used for homogeneous coating inside the cellulose aerogel. The resultant aerogel was highly hydrophobic and oleophilic, which can float on water, and can be used as oil-absorbing material. Figure 17 shows the photos of the process of oil spill removal from water by cellulose aerogel coated with $TiO_2$ It is noted that such environmental-friendly cellulose aerogels can be reused after washing, recycled, or incinerated with the absorbed oil. Therefore, this work sheds light on nanocellulose based oil absorbents for environmental applications such as cleaning oil spills.

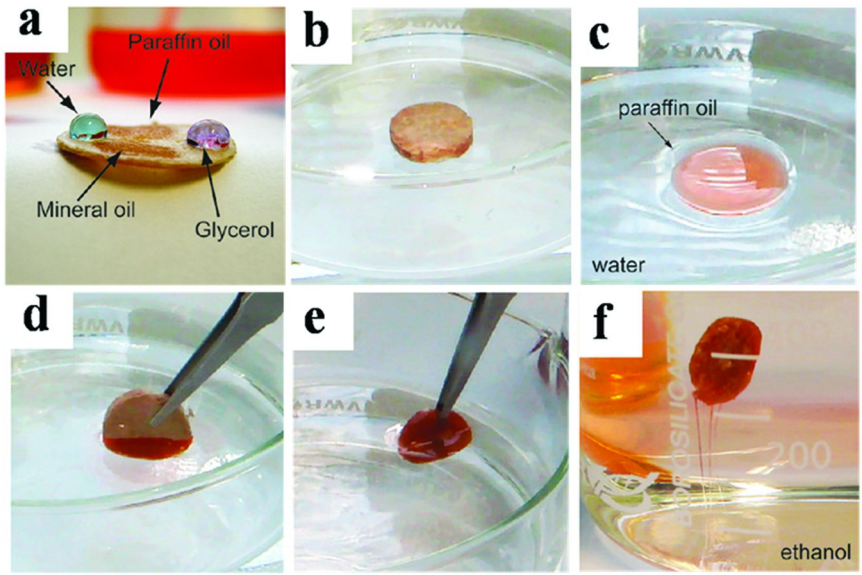



*Figure 17. Photos of $TiO_2$ coated aerogels and oil spill removal process from water. With different droplets, whereas paraffin oil and mineral oil are readily absorbed. (a), floating on water (b), paraffin oil floating on water (c), the oil being absorbed into the aerogel (d), all of the floating oil has been absorbed (e) and the oil-filled aerogel can be washed (f). Reproduced from reference (19). Copyright 2011 American Chemical Society.*

Ogihara et al have fabricated superhydrophobic and transparent cellulose paper surfaces by spraying nano $SiO_2$/alcohol suspensions at room temperature and under atmospheric pressure to deposit the coatings (*52*). The $SiO_2$/alcohol (ethanol, 1-propanol, or 1-butanol) suspensions were manually sprayed over standard printer-grade paper with a glass vaporizer. The resultant samples were placed horizontally and dried overnight at room temperature. Figure 18 shows SEM images of $SiO_2$ nanoparticle coatings prepared using ethanol (a-b) and photograph of paper spray-coated with $SiO_2$ nanoparticles dispersed in ethanol. The $SiO_2$ coating prepared from ethanol suspension exhibits micro- and nano- roughness consisting of nano $SiO_2$, resulting in micro-nano hierarchical structure to give superhydrophobicity. In their findings, superhydrophobicity was depended on the aggregation states of $SiO_2$ nanoparticles and the particle size, which were basically determined by the $SiO_2$ types of particles and alcohol for the suspensions, respectively. They provided a simple method to fabricate superhydrophobic and transparent paper surface, avoiding the use of costly instrumentation, extreme reaction conditions, and specialized nanomaterials.

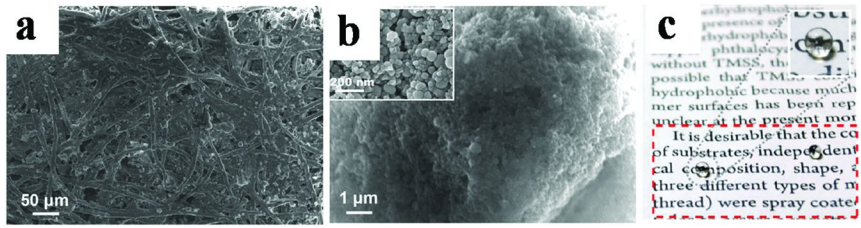



*Figure 18. SEM images of $SiO_2$ nanoparticle coatings prepared using ethanol (a-b) and photograph of paper (c) (red dashed square of which was spray-coated with $SiO_2$ nanoparticles (25 nm) dispersed in ethanol). Reproduced from reference (52). Copyright 2012 American Chemical Society.*

**Painting with Nanoporous Polymer Chalk**

A superhydrophobic paper has been fabricated by Tucker et al using a nanoporous polymer chalk, which is synthesized by a simple solvothermal polymerization using divinylbenzene (DVB) with azobisisobutyronitrile (AIBN) as an initiator (*53*). Nanoporous PDVB powder was directly wiped on the paper surface to prepare superhydrophobic paper, which adhered well to the paper due to the abundant lacunes of the paper surface and the electrostatic interaction. Figure 19 shows the photograph of water droplets on nanoporous polymer-coated paper and the SEM image of the corresponding surface. The paper changed remarkably from hydrophilicity to superhydrophobicity because the nanoporous coating layer increased significantly surface roughness. The red lines of the paper can be identified clearly due to the very thin polymer coating layer, which was further estimated to be about 10 μm. They provide a valid, facile and efficient approach to construct the superhydrophobic surfaces without complexity or technical difficulty, broadening the applications in fundamental science and industry.

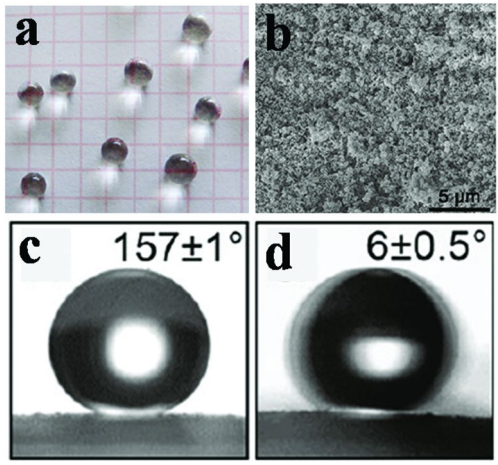



*Figure 19. Photograph of water droplets on nanoporous polymer-coated paper (a), SEM image of nanoporous polymer-coated paper surface (b), CA of a water droplet on nanoporous polymer-coated paper is 157° (c) and water droplet could roll down with a sliding angle of ~6°(d). Reproduced from reference (53). Copyright 2011 American Chemical Society.*

**Modified with Polyacrylate Latex**

Pan et al have improved the hydrophobicity of cellulose fiber network or handsheets to be hydrophobic by using cationic nanosized latex with a core-shell structure (*54*). A two-step "seeded" semi-batch emulsion polymerization was employed for constructing core–shell latex. The nanosized latex was prepared via an emulsion copolymerization of butylacrylate-costyrene/2-ethylhexylacrylate-co-methyl methacrylate (BA-co-St/EHA -co-MMA) using cetyltrimethyl ammonium bromide (CTAB) as a surfactant. To absorb the CPBA-co-MMA/EHA-co-St latex on the fibers, latex were added to the pulp suspension with 1 % consistency with stirring. The hydrophobicity of the modified handsheet was improved significantly as a result of the high retention of cationic latex within fiber networks. Meanwhile, the moisture barrier and the mechanical properties of the paper were also improved to some extent. This approach sheds light on improving the hydrophobic properties of handsheet without negative impact on fiber bonding strength.

**Cold Plasma Treatment**

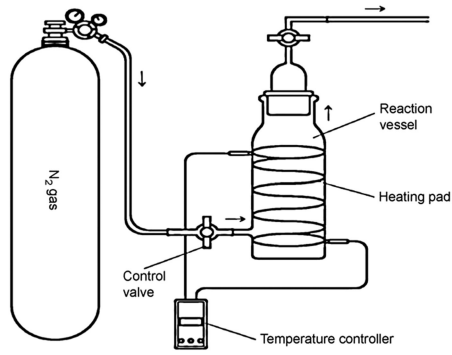
Cold plasma is an ionized mixture of gases with entire temperature approach the room temperature, which can be used to create hydrophobic surfaces (*55*). Shi et al have fabricated cellulose aerogels with good heat insulation performance

from cellulose solution, which has been dissolved in a NaOH/thiourea/$H_2O$ solvent system (*56*). In their work, the cellulose aerogels were converted from hydrophilic to hydrophobic by $CCl_4$ cold plasma modification technology. The contact angle of modified cellulose aerogel reached as high as 102°, and hydrophobic modification did not affect the heat insulation performance of aerogels. They avoid complicated and uncontrollable chemical crosslinking process, and provided an effective method to prepare cellulose aerogels for the applications in the insulating material field for 24h.

**Chemical Vapor Deposition (CVD)**

Chemical vapor deposition is a simple and effective method to form coatings on various substrates, which can be used to prepare superhydrophobic and photocatalytic surfaces (*57*). Oh et al have prepared hydrophobic filter papers with water contact angle over 120° under sufficient reaction time and silane concentration by self-assembled monolayers on paper surface with silanes through CVD method (*58*). Such monolayers can be prepared by both solution-immersion method and CVD method, and dimethyl-dichlorosilane (DMDCS) was chosen for vapor deposition method to form the monolayer due to relative low boiling point of 68–70 °C. Scheme 5 shows the schematic diagram of the apparatus used for CVD method. The reaction chamber was flushed with a stream of high purity nitrogen gas, and calcium chloride was used as a desiccant agent to keep a minimum of residual water in the reaction system. The temperature outside the reaction chamber was kept at 80 °C by a heating pad and temperature controller to evaporate the dimethyl-dichlorosilane (DMDCS). They provided a simple and effective process for the preparation of the high hydrophobic papers. Superhydrophobic cellulose paper has been fabricated by Balu et al through domain selective etching of amorphous cellulose in an oxygen plasma and subsequently coating the etched surface with a thin fluorocarbon film. The fluorocarbon film was deposited by plasma-enhanced CVD using pentafluoroethane (PFE) as a precursor (*59*). The full process of etching and deposition was denoted as superhydrophobic (SH)-treatment, control experiments of deposition process only was designated SH-control-treatment, and the treatment of samples exposed to fluorocarbon deposition only was termed PFE-treatment. Figure 20 shows a comparison of the WCA and WCA hysteresis of copy paper, handsheet, and silicon wafer subjected to three tests.

Obviously, the SH-treatment results in a water CA>150° and CA hysteresis<10° for both copy paper and handsheet. Moreover, both control experiments (SH-control and PFE-treatment) yield the same result for all three substrates, suggesting that the processing conditions of the plasma etching were not the main cause of the observations. It is claimed that the nanometer scale roughness, obtained by delineating the internal roughness of each fiber and the micrometer scale roughness, are robust compared to roughened structures from other approaches such as traditional polymer grafting, because it relies on uncovering roughness present inherently on cellulose fibers.




*Scheme 5. Schematic diagram of the apparatus used for chemical vapor deposition method. Reproduced from reference (58). Copyright 2011 Elsevier.*

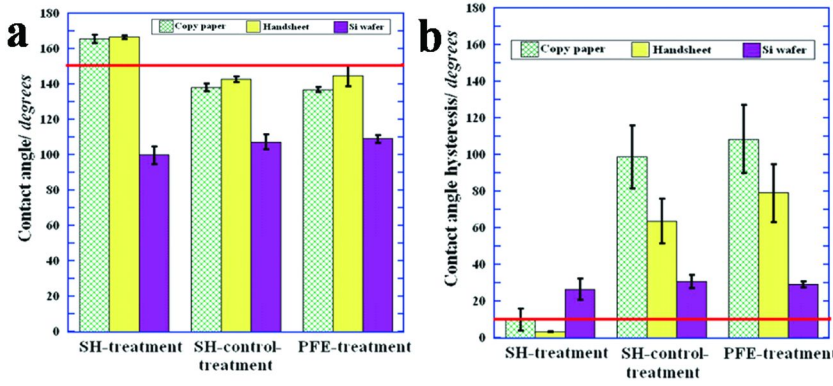



*Figure 20. Plot of WCA (a) and WCA hysteresis (b) measurements for the copy paper, handsheets, and Si wafers for the three plasma treatments. Red lines in (a) and (b) indicate the cutoff value for "roll-off" superhydrophobicity. Error bars represent 95% confidence intervals. Reproduced from reference (59). Copyright 2008 American Chemical Society.*

## Introduction of Compound Coatings

Stanssens et al have synthesized poly(styrene-maleic anhydride) copolymers by imidization under pure conditions or in presence of palm oil to fabricate organic nanoparticles, which were coated on cellulose substrates to obtain the water-repellent and superhydrophobic surface (*60*). The nanoparticles were applied as a coating on standard grade paper or tissue papers with a K303 Multi-coater (RP-Print Coat Instruments, Ltd), and the resultant coatings were

dried for 2 min at 100 °C and stabilized for at least 1 day. Figure 21 shows Atomic force microscopy of papers coated with (a) pure SMI nanoparticles, (b) SMI/oil nanoparticles. The nanoparticles agglomerated into a micro domain structured coating after drying, resulting in micro to nano structured morphology. Thus, the surface hydrophobicity increased and the printing characteristics were also improved in this process. In their findings, the barrier properties can be further improved by forming a more continuous surface coating through filling oil. Furthermore, surface treatment of tissues with the organic nanoparticles provided superhydrophobic surfaces (contact angle 148°). They provided a simple and attractive alternative for papers and textiles surface treatments to improve hydrophobicity, avoiding the use of environmental unfriendly fluoroderivates.

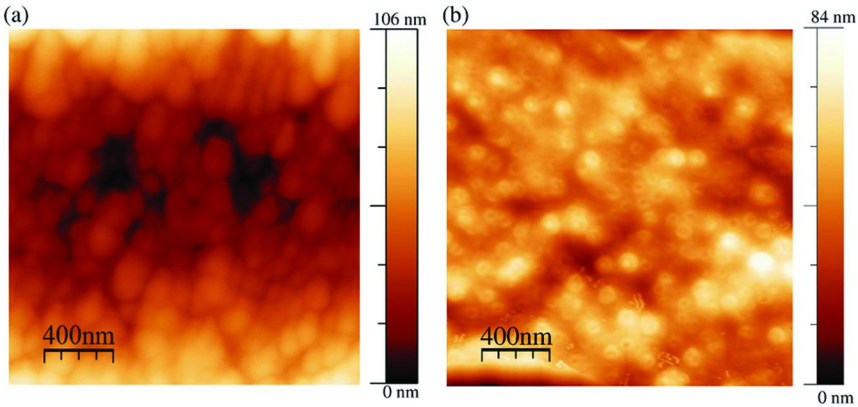
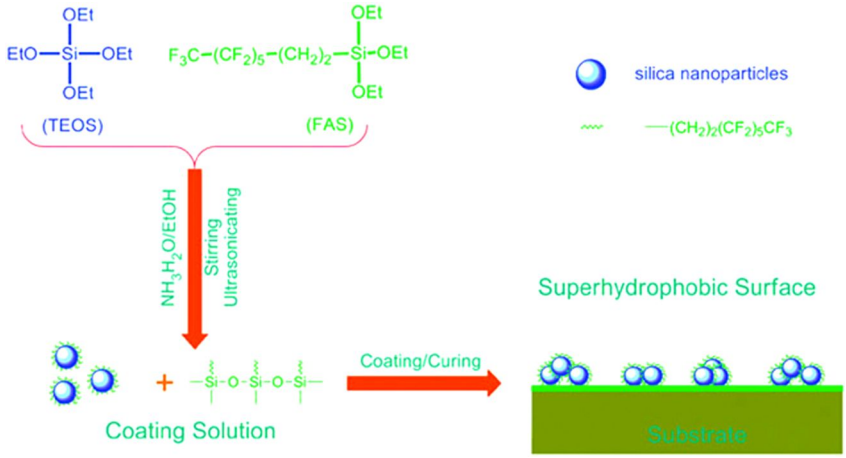



*Figure 21. Atomic force microscopy (height images) of papers coated with (a) pure SMI nanoparticles, (b) SMI/oil nanoparticles, both as deposited (no curing). Reproduced from reference (60). Copyright 2011 Elsevier.*

Wang et al have produced a superhydrophobic coating by simply coating a particulate silica sol solution of tetraethyl orthosilicate (TEOS)/ tridecafluorooctyl triethoxysilane (FAS) on various substrates such as filter paper (*61*). The sol solution containing silica nanoparticles was prepared by co-hydrolysis and condensation of TEOS and FAS in $NH_3 \cdot H_2O$/ethanol solution, which can be easily used for coating simply by dipping, spraying, or spin coating. Scheme 6 shows the reaction route of silica sol preparation and coating procedure. Superhydrophobic filter paper with contact angle of 172.4° and sliding angle of 6.3° has been fabricated after the coating process. They provided a simple and universal method to create superhydrophobic surface on various substrates. Furthermore, other functionalities such as antibacterial property can also be produced via surface treatment with nanoparticle materials for different applications.




*Scheme 6. Reaction route of silica sol preparation and coating procedure. Reprinted from reference (61). Copyright 2008 Royal Society of Chemistry.*

Guo et al have prepared superhydrophobic coatings on various substrates by using different water-repellent coatings (PE, PVC, PMMA) with the addition of modified silica nanoparticles by a one-step technique (*62*). The surface modified $SiO_2$ was added into the polymer solution such as PE in xylene, and the mixture solutions after stirring were dropped and dried to fabricate superhydrophobic coatings. Figure 22 shows SEM images of PVC coatings with addition of $SiO_2$ nanoparticles (a-b) and photo of water droplets on the surface a filter paper with PVC nanocomposite coating (c). The surface-modified $SiO_2$ nanoparticles exhibited a rough surface with clear porous structure, resulting in superhydrophobicty. Obviously, superhydrophobic filter paper was fabricated after PVC nanocomposite coating. It is noted that the superhydrophobicity was well maintained in acidic and basic solutions. This method is simple and inexpensive as well as utilizing non-fluorine-containing compounds, which may bring great advantages in industrial and civil applications.

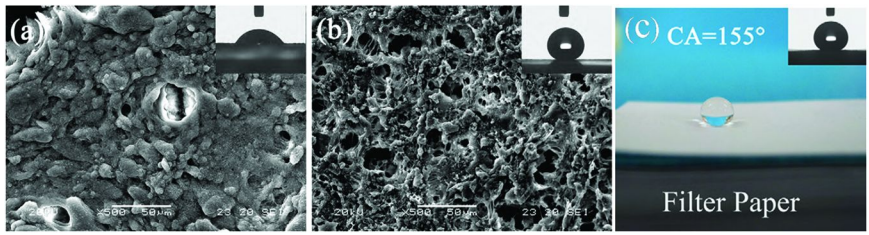



*Figure 22. SEM images of PVC coatings with addition of: unmodified $SiO_2$ nanoparticles (a) and surface-modified $SiO_2$ nanoparticles (b), and optical images of water droplets on the surface of filter paper with PVC nanocomposite coating (c). Reproduced from reference (62). Copyright 2010 Elsevier.*

## Growing Nano ZnO on the Surface

Growing inorganic nanocrystals such as ZnO on the surface of different substrates usually forms a rough surface, and a superhydrophobic surface can be created by further modification. Athauda et al have fabricated superhydrophobic cotton fibers by anisotropic growing single nano crystalline ZnO with 1-dodecanethiol modification (*63*). Figure 23a shows the scheme of the two-step hydrothermal process for growing ZnO nanowires on cotton surfaces. The growth process involved seed treatment of a cotton substrate with ZnO nanocrystals that would serve as the nucleation sites for subsequent anisotropic ZnO nanowires growth. There are two kinds of ZnO nanocrystalline, namely ZnO nanorods and nanoneedles (Figure 23b,c), grown on cotton surfaces. Variation of concentrations ratio for the seed-to-growth solutions ([S]/[G]) during the synthesis process led to a morphological transformation from nanorods to needle-like structures (Figure d), which was in conjunction with a drastic change in the physical and optical properties of the ZnO modified cotton surfaces. They provided a facile and green method to improve cellulose hydrophobicity. Moreover, the coated controllable ZnO nanoneedles hold great promise for the development of wearable and/or flexible photovoltaic, transparent conductors, and protective clothing with self-cleaning properties.

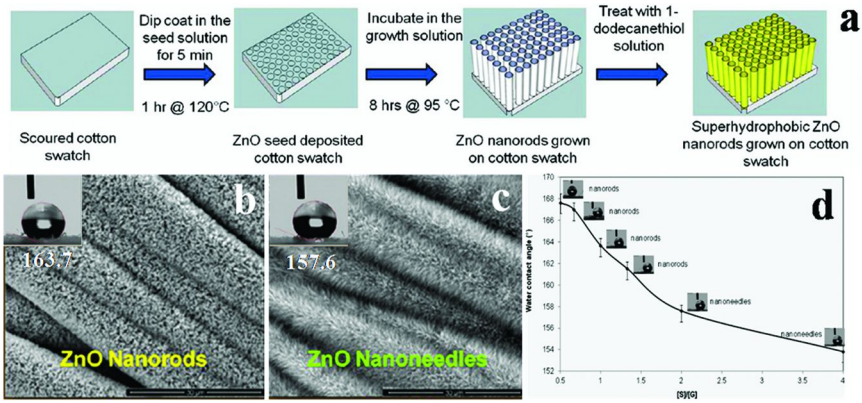



*Figure 23. Scheme of the two-step hydrothermal process for growing ZnO nanowires on cotton surfaces (a); SEM images of the resultant ZnO nanorods (b) and nanoneedles (c) grown on cotton surfaces; and variations in the water contact angles on cotton surfaces treated with 1-dodecanethiol as a function of [S]/[G] (d). Insets show images of water droplets on different surfaces. Reproduced from reference (63). Copyright 2013 American Chemical Society.*

## Incorporation with Stearic Acid

Stearic acid is a saturated crystalline fatty acid derived from animal and vegetable fats and oils, which can be used in the field of moisture barrier due to its low surface energy, inertness and low toxicity. Nisin and stearic acid have been

incorporated into a hydroxypropyl methylcellulose (HPMC) based film by Sebti et al to develop biodegradable packaging with moisture barrier and antimicrobial activity (*64*). Stearic acid was added to the HPMC film-forming solution with nisin before film formation, and the film-forming solution was heated to dissolve stearic acid, homogenized and then poured. The incorporation of 15% (w/w HPMC) of stearic acid can decrease Water Vapor Transmission Rate (WVTR) of60%, due to the apolar nature of stearic acid, which decreases the moisture affinity of films. Recently, a novel and highly hydrophobic cellulose composite film (RCS) with biodegradability has been fabricated in our lab via solvent-vaporized controllable crystallization of stearic acid in the porous structure of cellulose films (RC) (*15*). The stearic acid crystallization in micropores of the regenerated cellulose film surface can lead to the formation of the rough surface structure, resulting in high hydrophobicity. Figure 24 shows the preparation process of the cellulose/stearic acid films (RCS). The cellulose gel sheets were soaked in stearic acid/ethanol solutions with stirring to fabricate the cellulose/stearic acid composite gels, and then the high hydrophobic cellulose films embedded with stearic acid were obtained by hot-pressing the composite gels. Interestingly, the cellulose/stearic acid film can be drawn on just like ordinary paper (Figure 24).

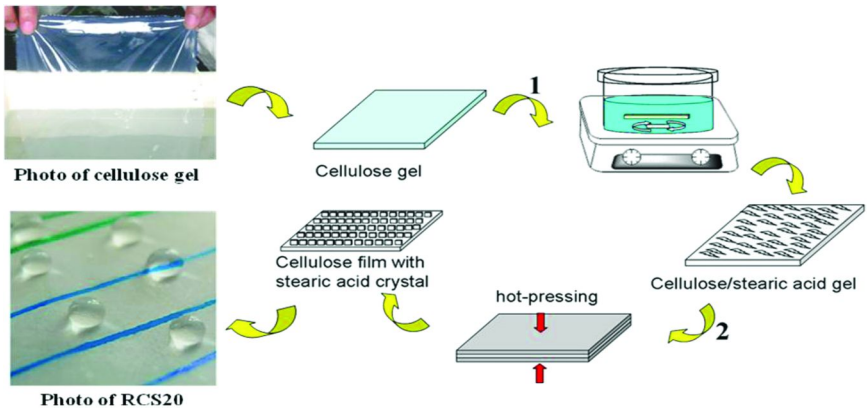



*Figure 24. The preparation process of cellulose/stearic acid films (RCS).1: soaking in the stearic acid/ethanol solution at 60 °C with stirring; 2: hot-pressing at 90 and 100 °C. Reproduced from reference (15). Copyright 2013 American Chemical Society.*

As shown in Figure 25, there are rough surface with a hierarchical structure containing micro- and nano-space on the RCS film surface which can trap abundant air, leading to the high hydrophobicity. Interestingly, a green superhydrophobic filter paper incorporated with stearic acid, similar to the lotus leaf (Figure 26a,c), has also been fabricated under similar conditions, demonstrating that stearic acid played an important role in hydrophobicity improvement. Moreover, the RCS films are flexible, biodegradable, and low-cost, showing potential applications in biodegradable water-proof packaging. This “green” method provides a facile way to increase cellulose hydrophobicity and broaden the cellulose applications.

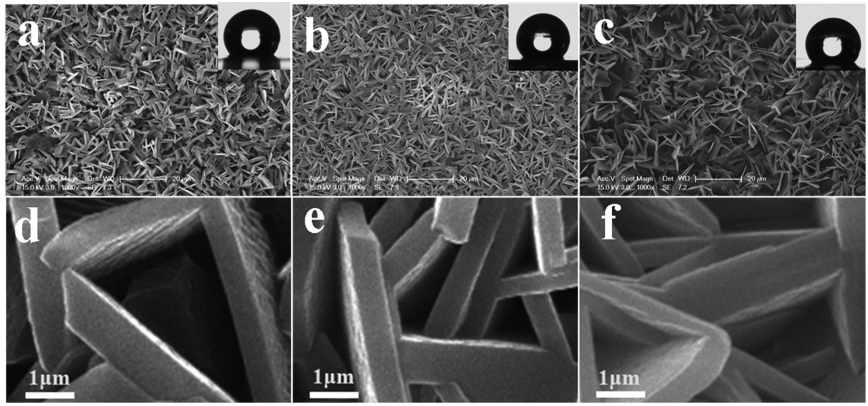



*Figure 25. The FESEM images of surface for RCS5 (a), RCS20 (b), RCS40 (c) films and magnification of RCS5 (d), RCS20 (e) and RCS40(f) prepared by hot-pressing dried process. Inset is water CA of each surface. Reproduced from reference (15). Copyright 2013 American Chemical Society.*

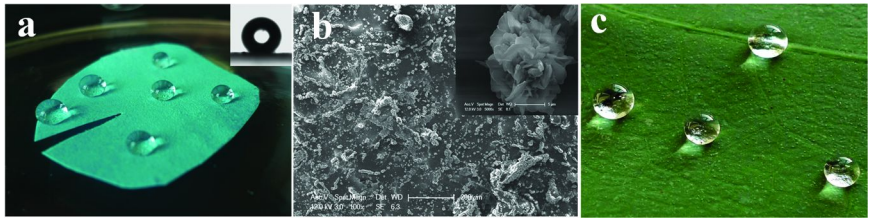



*Figure 26. A photograph of a superhydrophobic filter paper floating on water (a), the corresponding SEM image of its surface (b). and a photograph of a lotus leaf (c). Insets show the photograph of a water droplet for a and the enlargement of b, respectively. Reproduced from reference (15). Copyright 2013 American Chemical Society.*

**Electrospraying**

Sarkar et al have constructed cotton fabric or paper with differential superhydrophobi city and hydrophilicity on each side of the surfaces by coating with polyvinylidene fluoride (PVDF) and fluorinated silane molecules via electro-spraying (*21*). PVDF and $[CF_3(CF_2)_7CH_2CH_2Si(OCH_3)_3-]$ (FSM) are used to prepare different coatings. The electro-spinning machine was used to spray solutions on a plain woven cotton fabric surfaces. Seven samples were made by one-step process, spraying the seven solutions directly onto the surfaces of

seven different fabrics. Two more fabric samples were produced using a two-step process based on the outcome of one-step process. Figure 27 shows the SEM images of cotton fabric surfaces with or without coatings and the photos of water droplets on different surfaces. Obviously, the hydrophobicity of the cotton fabric was improved largely, reaching superhydrophobic. The pure cotton fabric was totally hydrophilic with smooth fiber and circular beads formed on the surface of the fabrics coated with a 10% PVDF solution, so the surface roughness increases. There were micro-scale bumps interspersed with honeycomb like microporous structure on the surface for fabric samples coated with 1% PVDF and 9% FSM, resulting in superhydrophobicity with a very high contact angle (WCA=158°). Moreover, the nano-structures of the two-step coated fabric surfaces for 1% PVDF and 9% FSM on PVDF coated fabric are spike-like, different from the sphere-like fabric surfaces of the one-step coated fabric. The spike-like architecture can help trap air during the solid–liquid contact to increase the hydrophobicity. Filter paper or cotton fabric with different superhydrophobicity and hydrophilicity on both side have been prepared via a two-step coating method. Such structure was advantageous for medical dressing and clothing next to the skin because the air permeability of the material was less affected by the coating. Furthermore, the hydrophilic side can effectively absorb the body sweat so as to keep the skin dry, while the superhydrophobic side exhibits anti-wetting and self-cleaning property. They provided an effective pathway using electro-spraying to improve cellulose hydrophobicity and broaden the applications of the cellulose fibers.

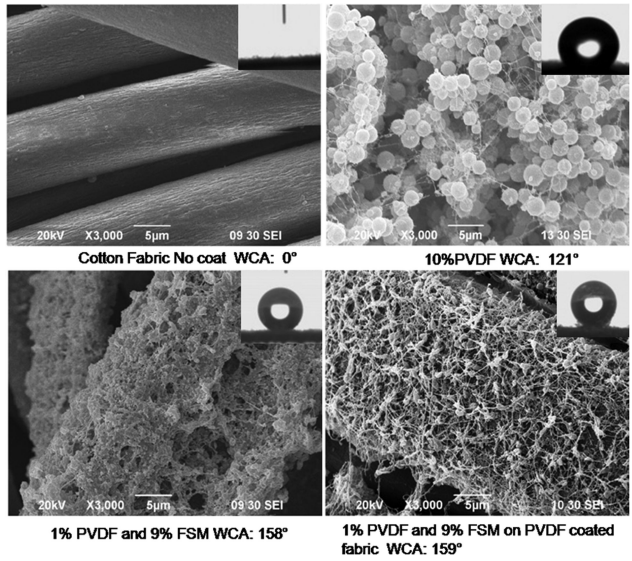
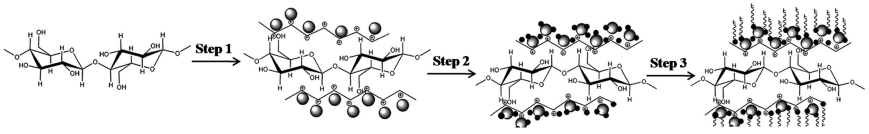



*Figure 27. SEM images of the surface of cotton fabric and modified cotton fabric from different coating process. Insets show the photos of water droplets on different surfaces. Reproduced from reference (21). Copyright 2010 Elsevier.*

**Multistep Nanoengineering**

Cellulose fiber with superhydrophobicity has been fabricated by Gonçalves et al through a multistep nanoengineering process (*65*), and the combination of different techniques made it possible to construct novel features at the ensuing surface. Scheme 7 shows schematic representation of the cellulose surface modification process. Poly(diallyldimethylammonium chloride) (PDDA) was used as a positive polyelectrolyte to promote the attachment of the silica spheres to the cellulose surface through electrostatic forces (step 1). Five PDDA/SS (sodium silicate) bilayers were added in the second step to their surface to increase the stability and roughness of the cellulose/silica composites (step 2). A silica-type network was formed by the sol–gel treatment and the post-cure combine the other surface entities. The perfluoro moieties of the siloxanes can reduce the surface energy (step 3), resulting in superhydrophobicity. Therefore, the cellulose-silica-silane composite materials opened the way to broaden the cellulose applications such as water repellence and self-cleaning.




*Scheme 7. Schematic representation of the cellulose surface modification in three steps: (1) $SiO_2$ particles, (2) 5 PDDA/SS bilayers, and (3) fluorosiloxane. Reproduced from reference (65). Copyright 2008 Elsevier.*

## Summary and Outlook

Facing the issues of the becoming-exhausted unsustainable resources and the white pollution of synthetic plastics, the exploiting and fabrication of renewable cellulose become urgent. Undoubtly, the original and novel methods for the improvement of the cellulose hydrophobicity are very important for the applications of cellulose such as water-proof packaging. Kinds of water-proof cellulose materials including papers, films and fabric have attracted more and more attentions all over the world. The relating achievements dealing with different aspects acting on different patterns of cellulose such as fiber, film and aerogel are reviewed in this chapter. These works would not only have a great impact on academical researches in improving cellulose hydrophobicity, but also shed light on industrial processing for exploiting cellulose in our daily life. The novel methods mentioned in this chapter are good to a large extent, but truly facile and green methods for robust hydrophobic cellulose fabrication are still needed urgently due to the exhausting fossil fuel and little utilization of the most abundant cellulose. It is inspiring that the energy issue have been listed as the top of the research all over the world as highlighted by the theme “Chemistry and materials

for energy" of the upcoming 247th ACS meeting, so more and more researchers will focus on renewable biopolymers especially for cellulose. Therefore, we believe that the issue of cellulose hydrophobicty improvement will be solved progressively on the basis of our prolific creativity and effective cooperation.

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## Chapter 19

# Polyhyroxyalkanoates (PHA)-Cellulose Based Nanobiocomposites for Food Packaging Applications

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Fabrication of biopolymers suitable for food packaging applications is currently an active area of research worldwide due to biodegradable and environmental-friendly nature of these polymers. Biodegradable polymers obtained from microbial fermentation, especially polyhydroxyalaonates (PHAs), utilizing cheap biomass sources, can be tuned to incorporate desired properties for different applications by altering the fraction of the hydroxyalkanoates. Nanocellulose can be employed as a unique filler material to enhance the properties of biopolymers, such as polyhydroxyalkanoates, while ensuring that the biodegradable nature is retained. Nanofillers such as cellulose nanocrystals (CNC) when dispersed into the polymer matrix of polyhydroxybutyrate (PHB) can improve the physical, barrier, thermal, mechanical and rheological properties of the biopolymer. However, CNC is hydrophilic whereas PHB, derived from microbial source, is hydrophobic in nature. This limits the dispersion of CNC in the polymer matrix which in turn can adversely affect the properties of the resulting nanocomposite. The optimum amount of CNC, ideal for improvement of biopolymer properties, has been observed to be ~2% as higher fractions lead to nanoparticle agglomeration or polymer degradation during processing. This chapter begins with a general introduction to polyhydroxyalkanoates (focusing on derivatives of PHB in particular) and CNCs. A detailed discussion on PHB-CNC nanocomposites. with particular

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emphasis on the potential of such materials for food packaging applications, is provided next. We compare various existing techniques used for the fabrication of PHB-CNC films, and report a cost effective technique for dispersion of CNCs into PHB through solvent exchange-cum-casting method.

## Introduction

The field of polymer-based food packaging has witnessed remarkable advancements in recent times, with packages now available that perform several functions, from protecting the food from dust, oxygen, light pathogenic micro-organisms, moisture, and other destructive/harmful substance to real time food quality monitoring using active sensors. Packaging is necessary to protect the packaged foods from the environment and other hazards, to curb the waste of foods, for quality maintenance, for ease of transportation (so that the foods reach distant destinations without any loss or harm) and most importantly, to increase the shelf life of foods. Due to the immense practical importance of packaging, research on the development of superior packaging material has attracted scientists, researchers, and engineers worldwide.

As per the World Packaging Organization (WPO), USA was the leader in the national packaging market during the period 2003–2009, followed by Japan and China respectively (*1*). Global packaging industry includes packaging markets for food, beverages, cosmetics and healthcare. Food packaging alone comprises around 50% of the packaging market. Modern polymeric packaging is capable of retaining freshness and healthy attributes of the packaged food and hence are widely used for packaging ready-to-eat food products. Hence, development in polymer packaging would also enhance the commercialization of processed food. Figure 1a illustrates the percentage world packaging consumption in the year 2009, while Figure 1b shows the growth of the global packaging market from 2000 to 2009 (*2*). Presently, most of the packaging market is dependent on petroleum-derived precursor polymers such as polyethylene, polyethylene terephthalate, polystyrene etc. Increase in global population will lead to increase in demand for packaging materials, and consequently, increased production requirement. As the source of most conventional packaging plastics are limited to rapidly depleting fossil-fuels, increased production may not be sustainable. Further, due to the non-degradable nature of most conventional packaging materials currently in use, proper disposal is a serious concern and increased use of such non-degradable materials may lead to environmental issues such as land pollution. Thus, proper waste management approaches are eseential to protect human health and the environment. This may be achieved by proper recycling, composting, combustion, landfilling and source reduction, or by switching over to the use of biodegradable polymers (*3*). Moreover, other than traditional plastic materials, metals, ceramics, and tinplated steel are used for packaging, which can contribute towards environmental pollution. Therefore, there is considerable interest at present in developing packaging materials derived from biopolymers.

Sustainable polymers can be broadly classified into three types based on the source and origin of monomer/polymer:

1. *Biopolymers* can be directly obtained from renewable resources. Examples include cellulose and chitosan (derived from plant and animal resources respectively) which can also be used as filler to improve polymer properties.
2. *Bio-derived Polymers* can be synthesized from renewable resource based precursor. These polymers are synthesized from monomers which are either derived from microbial or renewable resources. Common bio-derived polymers include poly (lactic acid) (PLA), thermoplastic starch (TPS), polyhydroxyalkanoates (PHA) etc. PHAs can be obtained from microbial fermentation of inexpensive biomass resources and have potential application in food packaging.
3. *Petroleum-based Bioplastics* are derived from petrochemical feedstock. Poly (butylene succinate) (PBS), poly (ε-caprolactone) (PCL), etc. can be produced from petrochemical feedstock and are biodegradable in nature.

In recent past, extensive research has been carried out and new technologies have been developed for the commercialization of PLA-based food packaging products by companies such as ThyssenKrupp (*4*), SKS Science (*5*), Alpha Packaging (*6*) etc. PLA is the most widely used bioplastic because the biomass resource available for production of lactic acid is cheap and abundant, and the properties of PLA satisfy the ASTM standards required for food packaging (*7*). The thermal and mechanical properties of PLA are comparable to those of poly(ethylene terephthalate) (PET). PLA possesses favorable melt flow index which makes it a readily processable material through extrusion for large scale production of PLA films (*8*). However, PLA has a low heat distortion temperature (HDT) (~ 60 °C), low melting temperature (~160 °C) and is non-biodegradable at ambient conditions which limits its application (*9*). Comparatively, polyhydroxybutyrate (PHB) has higher degree of crystallinity and melting temperature (~175 °C) than PLA. PHB has a high heat distortion temperature (HDT) (~115 °C) and is readily biodegradable at room temperature (*9*, *10*). But the low microbial production rate and high cost of extraction of PHB makes large scale production challenging. Further, its brittle nature, low melt viscosity and thermal instability limits its extrusion based scale up route (*11–13*). A major drawback of PHB is the degradation in molecular weight of PHB while processing due to the effect of moisture. We have tried to address this by fabrication of PHB nanocomposite films through solvent-cum-solution casting approach, discussed later in the chapter (*14*). Blending PLA and PHB has been observed to improve heat distortion temperature, mechanical and biodegradation rate. However, other properties such as oxygen and water transmission rates which are critical for packaging applications, need to be addressed (*9*).

To address the limitations of poor mechanical and gas barrier properties of sustainable polymers such as PLA and PHB, modern approaches have been introduced where nanoscale, organically modified clays, layered silicates, and nanocrystals dispersed into polymer matrices have been utilized to prepare

biodegradable nanocomposites with improved properties. Metal nanoparticles are known to migrate through the polymer packaging into food leading to its contamination, through diffusion (*15*). Cytotoxicity studies with silver and gold nanoparticles have shown antibacterial effects and under threshold concentration don't have adverse effects on eukaryotic cell; however, heavy metal nanoparticles are yet to be studied (*16*). The U.S. Food and Drug Administration (FDA) have recently drafted guidelines addressing concerns over regulated application of metal nanoparticles in different food packaging and cosmetic based products.

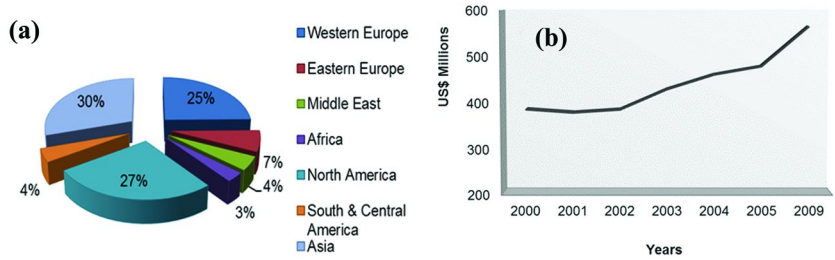


*Figure 1. (a) Percentage World Packaging Consumption in the year 2009.(b) The growth of the global packaging market from 2000 to 2009 (2).*

In this chapter, we discuss the classification, properties, and microbial synthesis of different types of PHAs. Next, we describe description of synthesis and fabrication of CNCs, and the fabrication of PHB based nanobiocomposites with CNCs. We also highlight the challenges in fabrication of PHB-CNC films due to the hydrophobic nature of microbial derived PHB and hydrophilic nature of plant-based CNCs, which limits the dispersion of CNC in PHB and subsequently its application. Finally, we evaluate the morphological, thermal, mechanical, optical, and rheological properties of fabricated PHB-CNC films for its potential application as a sustainable food packaging.

## Polyhyroxyalkanoates: Classification, Property Evaluation, and Microbial Synthesis

PHAs are biodegradable polyesters produced chemically or biosynthetically by microbes. PHAs and their nanocomposites have applications in a variety of fields such as biomedical (sutures, bone plates, drug delivery (*17*) etc.), tissue regeneration, packaging (*18*) etc. PHA monomers include 3-hydroxybutyrate, 3-hydroxyvalerate, 3-hydroxyhexanoate, 3-hydroxyoctanoate and 4-hydroxybutyrate (see Figure 2 for structures). PHAs are produced by various microorganisms (such as *Ralstonia eutrophus* or *Bacillus megaterium*), apparently in response to conditions of physiological stress. The type of PHA produced depends on the synthesis route, bacteria type and growth conditions. PHAs are produced by bacteria in the form of intracellular granules (*19*) for use

as energy reserves. PHAs may consist of $10^3$-$10^4$ monomer units and accumulate as inclusions having diameters ranging from 0.2 to 0.5 μm (*20–22*). PHAs have been synthesized in large scale by different industries worldwide for commercial purpose as listed in Table 1.

**Table 1. Worldwide Polyhyroxyalkaonates Commercial Producers and Their Applications (*23*, *24*)**

| *PHAs type* | *Company* | *Trade Name* | *Production Scale (tons/annum)* | *Applications* |
|---|---|---|---|---|
| P(3HB) | Mitsubishi Gas Chemical Company Inc | BioGreen® | 10000 tons | |
| P3HB4HB | Tianjin Green Bio-Science | Green Bio | 10000 | Raw materials & packaging |
| PHB | Jiang Su Nan Tian, China | | Pilot scale | Raw materials |
| PHB, PHBV | BASF, Germany | Enmat® | Pilot scale | Blending with Ecoflex |
| PHBH | P&G, USA | Nodax ™ | 20,000-50,000 | Packaging |
| Several PHA | Shantou Lianyi Biotech, China | | Pilot scale | Packaging and medical |
| PHBV and PHB | Biomer Inc. (Germany) | Biomer® | 50 | Packaging and drug delivery |
| PHBHHx | Jiangmen Biotech Ctr, China | | Unknown | Raw materials |

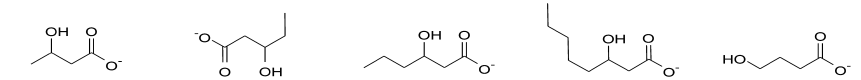


*Figure 2. Various monomer units for different PHAs.*

PHAs are primarily a product of carbon assimilation (from glucose or starch) under various ratios of carbon and nitrogen source and is employed by microorganisms as an energy storage molecule to be metabolized when other common energy sources are not available. Microbial biosynthesis of PHB starts with the condensation of two molecules of acetyl-CoA to give acetoacetyl-CoA which is subsequently reduced to hydroxybutyryl-CoA. This latter compound is then used as the monomer for synthesizing PHB with molecular weight in the range of 50,000-1,000,000 Da (*25*). PHAs can be classified in terms of the length of carbon chains in the respective PHA: short chain length PHAs are stiff, brittle and have a high degree of crystallinity, whereas medium chain length PHAs are flexible and have low crystallinity (*26*, *27*). Typical PHA yields achieved are ~2 g/L per hour using different bacteria such as *Alcaligenes eutrophus*, *Alcaligenes latus*, *Azotobacter vinelandii*, *methylotrophs*, *pseudomonas* and *recombinant Escherichia coli21* (*28*, *29*). Industrial scale production of PHAs is feasible which includes strain development, fermenter studies, and optimized purification process (*30*). Extracellular PHA depolymerases (carboxyesterases) are enzymes secreted by various microorganisms in soil that help in the biodegradation of PHAs by hydrolyzing the ester bonds of PHAs into monomers and oligomers which can solubilize in water are discussed in Table 2. Factors that significantly influence the biodegradability of PHAs include temperature and polymer crystallinity (higher crystallinity leads to lower biodegradation rates) (*33*).

**Polyhyroxyalkanoates: Classification and Property Comparison**

PHAs are thermoplastic in nature and can be processed by conventional processing equipment such as extruders depending on their composition, crystallinity and elastic behavior. PHAs can be classified on the basis of their chemical structures as shown in Figure 3. The thermal and mechanical properties of different PHAs are compared with those of conventional polymers namely polypropylene (PP), polystyrene (PS) and polyethylene terephthalate (PET) in Table 3.

*Poly(3-hydroxybutyrate)*

Poly(3-hydroxybutyrate) (PHB) is the simplest and most popular PHA synthesized from 3-hydroxybutyrate (3HB). It was first isolated and characterized by French microbiologist Maurice Lemoigne in the year 1925. It can be produced by various microorganisms such as *ralstonia eutropha* or *bacillus megaterium* (*37*) and accumulated as intracellular granules by at least 75 genera of bacteria (*38*). First commercialization of PHB has been accomplished by W.R. Grace & Company, US (*39*). Biocompatibility of PHB is well known which makes it a promising material in biomedical applications such as medical devices, tissue engineering and drug delivery.

**Table 2. Rate of Hydrolytic and Enzymatic Degradation of the PHAs at Different Temperatures and Enzyme Sources (*31*, *32*)**

| ***PHA type*** | ***Enzymatic Degradation at 37 °C (mg/h)*** | | | | ***Hydrolytic Degradation at 55 °C (day$^{-1}$)*** |
|---|---|---|---|---|---|
| | *A. faecalis (Sample: 14mg)* | *P. stutzeri (Sample: 14 mg)* | *C. acidovorans (Sample: 14 mg)* | *A. faecalis (Sample: 3-8 mg)* | |
| P(3HB) | 0.06 | 0.17 | 0.05 | 0.17-0.15 | - |
| P(4HB) | 0.15 | 0.07 | 0.18 | - | - |
| P(3HB-co-10% 4HB) | - | - | - | 0.80±0.05 | (8.3 ± 1.5) x $10^{-6}$ |
| P(3HB-co-17% 4HB) | - | - | - | 0.90 | (11 ± 2) x $10^{-6}$ |
| P(3HB-co-27% 4HB) | - | - | - | - | (17 ± 2) x $10^{-6}$ |
| P(3HB-co-45% 3HV | - | - | - | 0.03±0.01 | (4.5 ± 1.5) x $10^{-6}$ |
| P(3HB-co-71% 3HV) | - | - | - | 0.04±0.01 | (2.3 ± 0.5) x $10^{-6}$ |

It is a crystallizable polymer which crystallizes in the form of spherulites upon cooling from the polymer melt (*40*). Due to the brittle nature of PHB, its copolymers, which have lower crystallinity in comparison, are commercially more viable.

Neat PHB is thermally stable with a glass transition temperature ($T_g$) of 4 °C and melting temperature ($T_m$) of 175–180 °C (see Table 3). Theoretical enthalpy of 100% crystalline PHB is 146 J/g (*41*). Crystallization peak temperature ($T_c$) can be found during the cooling cycle and is found to depend on the cooling rate. An increase in cooling rate from 5 °C/min to 25 °C/min, leads to a decrease in the $T_c$ from 106.6 °C to 85.8 °C (*42*). Tensile strength of neat PHB is approximately 40 MPa (which is greater than that of PP but lower than PS and PET) while elongation at break is 6%. Oxygen permeability through PHB molded cups is 0.18 mL/(cup×24h×0.21 atm $O_2$) at 23 °C and 50% relative humidity (RH) whereas that for PLA and high density polyethylene (HDPE) cups of the same volume at the same conditions are 0.21 and 0.26 mL/(cup×24h×0.21 atm $O_2$) respectively (*43*, *44*). The low oxygen permeability of PHB coupled with its biodegradability makes it a promising material for food packaging applications. However, mechanical and barrier properties of PHB are less impressive, and must be improved with the addition of biocompatible nano-fillers.

**Table 3. Physical Properties of Polyhyroxyalkanoates in Comparison to Petroleum-Based Polymers (*20*, *21*, *24*, *26*, *27*, *34–36*)**

| *Sr. No.* | *Polymer Name* | *Glass Transition Temperature (°C)* | *Melting Temperature (°C)* | *Tensile Strength (MPa)* | *Elongation at Break (%)* |
|---|---|---|---|---|---|
| 1 | PHB | ~ 4 | ~175–180 | ~40 | ~6 |
| 2 | PHBV | ~ –7 | ~150 | ~25 | ~20 |
| 3 | PHBV | — | ~170 | ~38 | — |
| 4 | PHBV | — | ~137 | ~30 | — |
| 5 | P3HB4HB | — | ~166 | ~28 | ~45 |
| 6 | P3HB4HB | — | ~50 | ~65 | ~1080 |
| 7 | $PHBHH_x$ | ~ –4 | ~52 | ~20 | ~850 |
| 8 | $PHOHH_x$ | — | ~61 | ~10 | ~300 |
| 9 | PET | ~34 | ~262 | ~56 | ~7300 |
| 10 | PP | ~45 | ~170 | ~34.5 | ~400 |
| 11 | PS | ~21 | ~110 | ~50 | — |

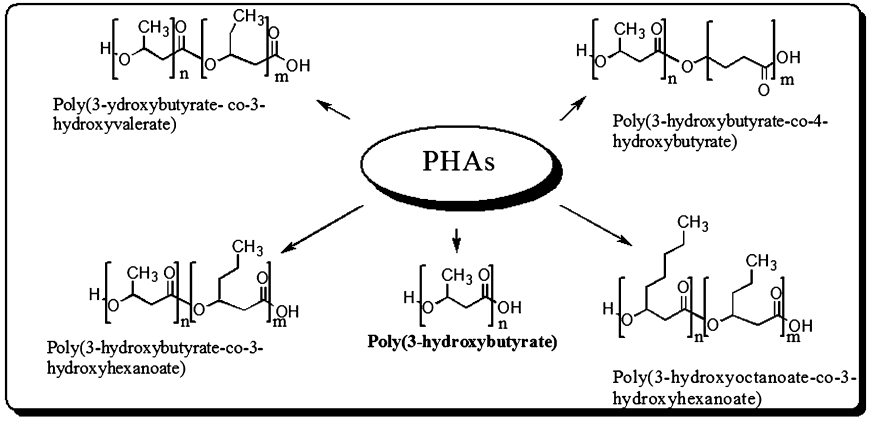


*Figure 3. Chemical structure of polyhyroxyalkanoates (PHAs).*

*Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)*

Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV), is a biodegradable thermoplastic copolymer made from monomers of 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV) (*45*). It may be obtained from *Alcaligenes eutrophus*

using carbon sources such as propionic acid, glucose, pentanoic acids and butyric acids having 0-95 mol% 3HV (*46*). In this composition range PHBV is highly crystalline with a crystallinity of more than 50%. Increase in 3HV content reduces the rate of crystallization in the PHBV (*47*). Degradability of PHBV has been observed to improve upon blending it with PHB and biodegradation increases with the percentage increase of PHB in the PHBV-PHB blend (*48*).

Composites of PHBV with agro-residue, fabricated using melt compounding technique, display improvement in mechanical properties over neat polymer. For example, using 30% agro-residue led to 256 % and 308% increase in tensile and storage modulus respectively (*49*). PHBV is used in tissue engineering, and helps in fabrication of orthopedic devices, medical implants, specialty packaging, bottles etc (*50*, *51*).

*Poly(3-hydroxybutyrate-co-4-hydroxybutyrate)*

Poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) (P3HB4HB) is a copolymer synthesized using monomers 3-hydroxybutyrate and 4-hydroxybutyrate. P3HB4HB is produced by the bacterium *Comamonas acidovorans* DS-17 using glucose, 1,4-butanediol or 4-hydroxybutyric acid (*34*). Increase in 4HB content has been observed to improve the tensile strength of the copolymer; however, it may decrease the erosion rate of the copolymer. On the other hand, hydrolytic and enzymatic degradation may be accelerated by the presence of 3HB units in a copolyester of PHAs (*52*). P3HB4HB has also been produced from 4-hydroxybutyric and butyric acids by fermentation using *Alcaligenes eutrophus* (*53*). This copolymer has potential for application in biomedical research.

*Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate)*

Poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) ($PHBHH_x$) is a random copolymer obtained from monomers of 3-hydroxybutyrate (3HB) and 3-hydroxyhexanoate (3HHx) (see Figure 3 for chemical structure) (*54*). Copolymers with 3HHx content in the range of 2.5–35 mol% are well known; the percentage component fraction can be evaluated using $^1H$ NMR spectroscopy. *Aeromonas hydrophila* is used to produce polymers having 2.5–9.0 mol% 3HHx while polymers synthesized by *Pseudomonas putida* have 9.0–35 mol% content of 3HHx, using fatty acids as the carbon source (*54*). Both genera have also been used to produce PHBHHx using glucose and gluconate as the carbon source (*55*). *Aeromonas caviae* is also found to produce a random copolymer of 3HB and 3HHx using sodium salts of alkanoic acids having $C_{2n}$ from $C_{12}$ to $C_{18}$ and olive oil as carbon source. This microbe can also produce $PHBHH_x$ using alkanoic acids having $C_{2n+1}$ from $C_{11}$ to $C_{17}$ as carbon source, resulting in weight average molecular weight in the range of $2\times10^5$ – $11\times10^5$. Recombinant *Ralstonia eutropha* also possesses the capability to produce $PHBHH_x$ from fructose (having 1.5 mol% 3HHx) (*56*).

$PHBHH_x$ has a lower melting point (150 °C) than that of PHB (175 °C), and presence of 1.5 mol% 3HHx decreases the crystallinity of PHB from 60±5 % to 48±5 %. Increase in 3HHx content of the copolymer decreases both the melting temperature as well as the glass transition temperature. For example, increase in 3HHx composition from 0 to 17% leads to decrease in melting temperature from 177 °C to 130 °C and change in glass transition temperature from 4 °C to –2 °C (*57*). In vivo degradation of $PHBHH_x$ is faster than neat PHB and hence, $PHBHH_x$ is a promising candidate for *in vivo* biomedical applications (*58*). Fractionation of $PHBHH_x$ has been well studied where polymers having 13.8, 18, 22 and 54 mol % of 3HHx composition have been fractionated using a solvent system of chloroform and n-heptane (*59*). PHBHHx production on the industrial scale with *Aeromonas hydrophila* in a 20 kL fermenter utilizing glucose as the sole carbon source resulted in a random copolymer with 11 mol% 3HHx (*60*). PHBHHx blended with poly(D,L-lactide) have shown to possess decreased crystallinity and higher elongation at break (*61*), and is of interest in biomedical applications such as nerve regeneration and tissue engineering (as scaffolds).

*Poly(3-hydroxyoctanoate-co-3-hydroxyhexanoate)*

Monomers 3-hydroxyoctanoate (3HO) and 3-hydroxyhexanoate (3HHx) polymerize to form the biocompatible polymer poly(3-hydroxyoctanoate-*co*-3-hydroxyhexanoate) ($PHOHH_x$). It is especially produced by the strain *Pseudomonas putida* GPo1 using octanoic acid as the carbon source (*62*).

## Microbial-Based Polymerization of Polyhyroxyalkanoates: Modelling Approach

PHB, the first member of PHA family, is a biocompatible, compostable and biodegradable aliphatic polyester. Its high crystallinity, hydrophobicity, and resistance to hydrolytic degradation make it an attractive candidate for environment-friendly packaging material. Undesirable properties such as high brittleness and low deformability may be controlled by the addition of fillers.

Several microbial strains have been known to produce PHAs of varying composition. PHAs are generally accumulated as energy source in microbial cells under stress induced conditions, during which certain nutritional factors such as nitrogen, phosphate, potassium, oxygen or magnesium are limited in the presence of excess carbon source (*63*). The culture media and microbes are tuned in such a way as to have the maximum yield of desired products. In literature, production of PHAs using several bacterial strains (both recombinant and naturally derived) has been widely studied along with rigorous process optimization listed in Table 4.

Life cycle assessment studies of PHA synthesis have showed that the cost of feed or carbon source significantly alters the overall production cost. Therefore recent research studies have focused on using industrial waste products, such as whey lactose, activated sludge etc., as the carbon source. Use of cheap carbon feed source not only makes the process economical but also leads to better

industrial waste resource management. In Table 4, several PHB production processes focusing on cheap carbon feed source have been listed. Recent studies by Simou and Pantazaki (*76*) show that the lactose present in whey byproduct could be utilized for production of high molecular weight PHB. Wang et al. (*77*) have reported the conversion of activated sludge waste into high molecular weight PHB for high end application. Moreover, single carbon $C_1$ feed source such as methanol, carbon dioxide and methane can also be used for the synthesis of PHB (*78*). Further investigations and rigorous process intensification studies need to be carried out to scale up the PHB production process.

The microbial production of PHBs follows a sequence of inter-related complex process steps (such as culture growth, intracellular polymer accumulation, cell harvesting, polymer separation etc.) that can affect the final polymer quality. Moreover, the PHB yield and polymer quality (e.g., molecular weight distribution) depend on a number of process parameters such as the carbon source type, the bacterial strain and its inherited metabolic pathway, the concentrations of the various feed nutrients, the cultivation strategy, etc. Therefore, proper optimization of process operating conditions should be carried out to maximize the biomass production rate and the PHB yield.

Molecular weight distribution of the accumulated PHB is influenced by the combined effect of metabolic, polymerization and macroscopic factors. Integration of the following three simplified strategies leads to development of models to analyze the system in discrete steps (*79*, *80*).

i. Metabolic model for production of monomer 3-hydroxybutyric CoA in cells.
ii. Polymerization kinetic model describing the dynamic evolution of molecular weight distribution of the intracellular accumulated PHB.
iii. Macroscopic model describing the dependence of PHB production rate on the ratio of carbon and nitrogen, and variation of other parameters.

*Metabolic Model*

The central aerobic carbon metabolism in the production of PHB is through Entner-Doudoroff pathway. Sucrose as feed source is initially converted to acetyl coenzyme A (AcCoA), which passes through PHB biosynthetic pathway that consists of three sequential enzymatic reactions (*81*):

(i) The condensation of AcCoA into acetoacetyl coenzyme A (AcAcCoA) by β-ketothiolase (phaA),
(ii) The reduction of AcAcCoA to 3-hydroxybutyric coenzyme A (3-HBCoA) by NADH-dependent acetoacetyl reductase (phaB), and
(iii) The polymerization of 3-HBCoA monomer into P(3HB) by synthase (phaC).

Presence of ammonium sulphate ((NH4)2SO4) favors residual biomass growth via metabolism of AcCoA (i.e., the Krebs cycle) (*81*).

*Polymerization Model*

The polymerization of 3-HBCoA is catalyzed by PHA-synthase (phaC) into PHB and depolymerization of PHB is catalyzed by PHA-depolymerase (phaZ) into 3HB by the following kinetic scheme.

Initiation:

$$E - SH + M \xrightarrow{Kml} E - SH - M \xrightarrow{Ki} P1 - ES + CoA - SH$$

Propagation:

$$Pn - ES + M^* \xrightarrow{kM2} Pn - ES - M^* \xrightarrow{Kp} P_{n+1} - ES + CoA - SH$$

Chain Transfer/ Termination:

$$Pn - ES + H_2O \xrightarrow{Kt} D_n + E - SH$$

Depolymerization:

$$Dn + E - OH \xrightarrow{Kd} D_{n-1} + D_1 + E - OH$$

where the symbols *E-SH*, *M**, *E-SH-M**, *CoA-SH* and *E-OH* denote the synthase dimer, the monomer-coenzyme A complex (M-SCoA), the monomer–synthase complex (MS#), coenzyme A and the depolymerase, respectively. Similarly, *Pn-ES* (*Pn*), *Dn* and *Pn-ES-M** (*Pn**) denote the respective "live" and "dead" polymer chains and the polymer–monomer–synthase complex ("intermediate"), with a degree of polymerization equal to *n* (*82*).

The polymerization kinetic model consists of a series of ordinary differential equations which are solved numerically. The key variable that connects the metabolic and polymerization model is intracellular monomer production rate, which depends on the metabolic pathway (*82*, *83*).

*Macroscopic Model*

Biomass growth is assumed to be an unstructured residual growth in a homogenous culture which is solved by simple dynamic mass balance equations. The most studied parameter for the production of PHB is the type of bacterial strain selected for production. The bacterial strain selected and microbial growth conditions play an important role in determining the final PHB yield obtained. The cell growth culturing method, for example continuous or fed-batch, affects the PHB biomass production and polymer yield. Table 4 provides a detailed list of the different types of natural and recombinant strains used for PHB production under various culture method. It has been observed that fed-batch process with

small monomer chains as food source lead to a higher yield of PHB (*69*, *84*, *85*). Recent development of recombinant strains produce very high yield of PHB at higher substrate and cell concentration. The production of PHAs can be classified on the basis of conditions prevailing during microbial growth, namely i. Aerobic, ii. Anaerobic, iii. Microaerophilic.

## Cellulose Nanocrystals: Synthesis and Fabrication Process

In recent years, intensive research attention has been directed towards development of functional materials from sustainable renewable resources (*86*). The cellulosic component of the lignocellulosic biomass, which is the most abundant renewable organic material available, has the capability to exist in crystalline form of nanoscale dimension; this form is called cellulose nanocrystal (CNC) (*87*). These CNCs have been extensively used as filler in biodegradable polymers such as PLA and PHB to fabricate nanobiocomposites.

Cellulose is a high molecular weight homopolymer of β(1→4) D-glucopyranose having cellobiose as the repeating unit. It can be extracted from lignocellulosic biomass by removing hemicellulose and lignin, the other two major constituents of lignocellulosic biomass. Relative amounts of hemicellulose, cellulose and lignin are different in different lignocellulosic biomass (*88*). For example, pine needles consist of 38–42% cellulose, 17–22% hemicellulose and 20–37% lignin, water hyacinth contains 18–31% cellulose, 22–43% hemicellulose and 4–26% lignin (*89*) and bamboo contains 57–62% cellulose and 26–30% lignin. Cellulose is a high strength material due to strong intramolecular and intermolecular hydrogen bonding, which occurs because of the presence of a hemiacetal hydroxyl, an acetal linkage and multiple hydroxyl functional groups (*90*, *91*) as shown in Figure 4. Strong intramolecular hydrogen bonding between the crystalline cellulosic chains makes them impermeable to most of the solvents. However, surface functionalization of hydroxyl end groups is easily carried out for industrial applications (*92*). In recent years, existence of cellulose in nano form has attracted numerous researchers to explore various cellulose-based nanobiocomposites. In this chapter, major emphasis will be on use of cellulose and CNCs as filler material for the fabrication of biocomposites.

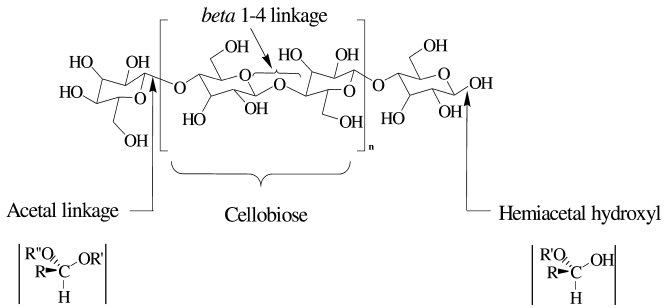


*Figure 4. Structure of cellulose.*

**Table 4. Composition and Type of PHAs Produced by Different Microbial Strains**

| *Micro-organism* | *Carbon source* | *PHA* | *PHA content (%w/v)* | *Culture method* | *Properties $M_w$ (Da)* | *References* |
|---|---|---|---|---|---|---|
| *Alcaligenes eutrophus* | Gluconate | PHB | 46-85 | Batch | | (*64*) |
| *C. vinosum* | Fructose | PHA | ~81 | Batch | 400000±2000 | (*65*) |
| *Escherichia coli (pJC4)* | Whey + lactose | PHB | 87 | Fed-batch | -- | (*27*) |
| *P. oleovorans* | Glucanoate | PHB | ~75 | Batch | -- | (*65*) |
| *Halomonas campisalis* | Maltose | PHA | 45–81 | Batch | $5–10 \times 10^5$ | (*66*) |
| *Methylobacteriumrhodesianum MB 1267* | Fructose/methanol | PHB | 30 | Batch | -- | (*67*) |
| *Pseudomonas aeruginosa* | Euphorbia and castor oil | PHA | 20-30 | Batch | -- | (*68*) |
| *C. necator B5786* | Gas mixtures containing CO | PHA | 70-75 | Batch | -- | (*69*) |
| *Methylocystis GB2* | Methane | PHB | 55 | | $2.5\times 10^6$ | (*70*) |
| *M. extorquens DSMZ13* | Methanol | PHB | 65 | Batch | -- | (*71*) |
| *Synechococcus sp.* | Carbon dioxide | PHB | 20-27 | Batch | -- | (*72*) |
| *Acidobacteria* | Acclimated activated sludge | PHB/PHV | 74 | Batch | -- | (*73*) |

| *Micro-organism* | *Carbon source* | *PHA* | *PHA content (%w/v)* | *Culture method* | *Properties $M_w$ (Da)* | *References* |
|---|---|---|---|---|---|---|
| *Ralstonia eutropha* | Whey & inverted sugar | P(3HB-co-3HV) | 38 | Fed-batch | -- | (*74*) |
| *Pseudomonas hydrogenovora* | Whey permeate | PHB | 21 | Batch | $353.5\times 10^3$ | (*75*) |
| *Thermus thermophilus HB8* | Lactose from whey | PHA | 35 | Batch | $3\times 10^6$ | (*76*) |

Cellulose disintegration through acid hydrolysis is shown in Figure 5. Hydrogen ions are liberated by acid when added to water forming more hydronium ions. The highly electronegative oxygen $O^{\delta-}$ of the cellobiose unit attracts the hydronium ion. The hydronium ion provides a proton to $O^{\delta-}$ giving rise to [OH]+ which has the tendency to attack either C4–O or C1–O bond giving rise to a carbonium ion. In the presence of water, carbonium ion accepts the lone electron pair of oxygen in water. Hydroxyl group from a water molecule is transferred to the carbonium ion while the remaining proton is accepted by electronegative oxygen atom of another water molecule simultaneously to form hydronium ion. Finally, the cellulose chain is broken into two strands of n′ and n-n′ cellobiose units. Progressive attack of an acid in a similar fashion leads to the formation of CNCs. Hydrolysis disintegrates the amorphous regions of cellulose while long exposure to acid may degrade the crystalline regions of cellulose as well (*93*).

Young's modulus of CNCs is ~100 GPa and its surface area is of the order of several hundred $m^2.g^{-1}$ making it an ideal filler for fabricating polymer nanocomposites. CNCs have good physical and mechanical properties such as nanoscale dimension, high aspect ratio, high specific strength and modulus, high surface area, unique optical properties etc. Different sources with different acid systems can be used to obtain CNCs of various shapes such as rod-like, spherical (*94*) and elliptical (*95*). The most commonly used acid for cellulose hydrolysis to synthesize CNCs is sulfuric acid where the cellulose fibrils are disintegrated into microfibrils to nanocrystals in stepwise reaction as shown in Figure 6. Hydrolysis in sulfuric acid leads to the introduction of charged sulfate ester groups on the CNC surface (*96*). A significant amount of time can be consumed in the removal of unreacted sulfate groups from the suspension by dialysis (*97*). Presence of sulfate groups on the surface tends to decrease the degradation temperature of CNCs (*98*).

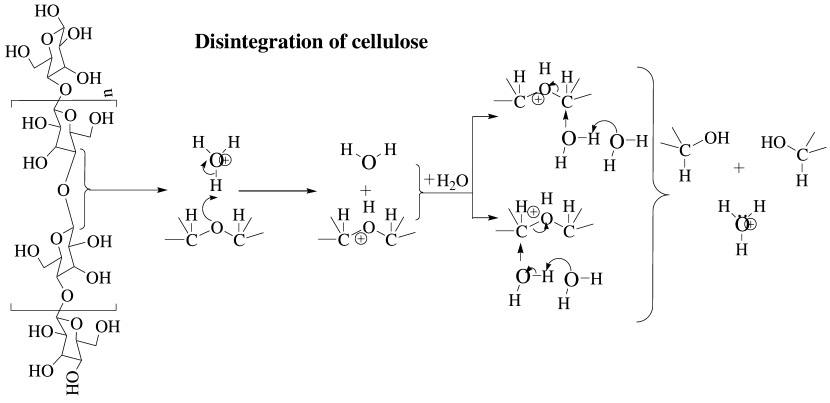


*Figure 5. Cellulose disintegration.*

Maximum degradation temperature of CNCs can be increased by the use of hydrochloric acid (instead of sulfuric acid) as a hydrolyzing agent for cellulose hydrolysis under hydrothermal conditions (*99*). However, use of hydrochloric acid requires thorough removal of acid as compared to sulfuric acid (*100*). Although no surface modification occurs on CNC surface using hydrochloric acid, the resulting CNCs have a strong tendency to aggregate due to strong intra-molecular and intermolecular hydrogen bonding (*101*). To avoid limitations associated with the use of sulfuric acid and hydrochloric acid, other acids such as phosphoric acid, acetic acid, formic acid etc. have been considered by researchers for cellulose hydrolysis. These acids modify the cellulose surface; for example, hydrolysis using phosphoric acid leads to attachment of phosphate groups to cellulose by ester linkage (*102*) while hydrolysis using acetic acid yields esterified CNCs (*103*).

## Polyhydroxybutyrate-Based Composites

An extensive amount of literature exists on PHB nanocomposites produced through several methods such as solution casting and various molding methods. In this section an overview of recent advancements in the area of PHB nanocomposites will be provided along with a brief discussion on the improved properties of the nanocomposites in the context of food packaging applications.

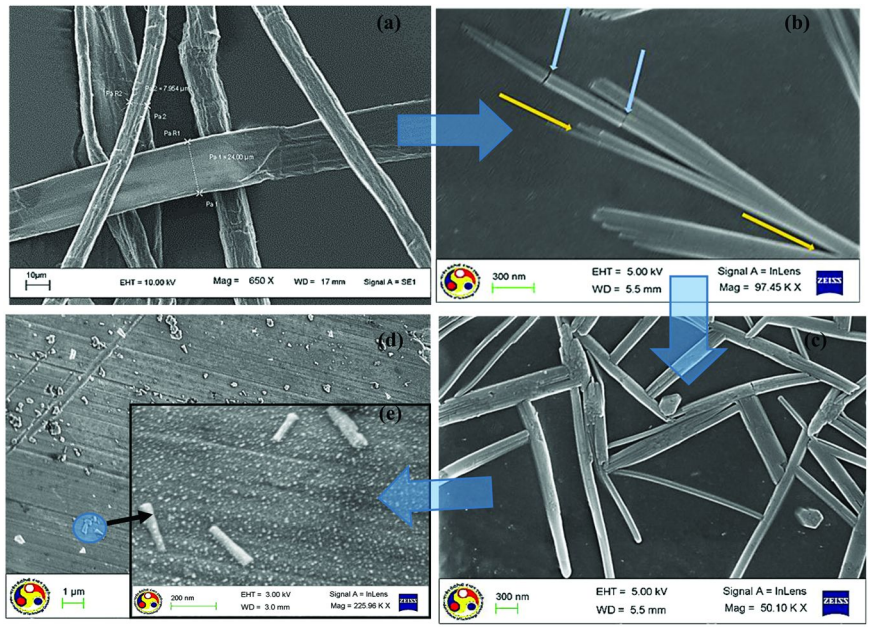


*Figure 6. Stepwise disintegration of cellulose fibers (a,b) during acid hydrolysis into microcrystalline cellulose (MCC) (c) to cellulose nanocrystals (CNC) (d,e).*

PHB compatibilized with modified montmorillonite clay has been observed to display significantly lower biodegradation rate and better mechanical strength than neat PHB (*104*, *105*). Botana et al. (*104*) fabricated PHB nanocomposites using two commercial montmorillonites namely Na-M and 30B-M by melt mixing at 165 °C. The organically modified montmorillonite 30B-M showed good dispersion in and intercalation with PHB due to better compatibility between the clay filler and polymer matrix. This resulted in enhancement of Young's modulus of the nanocomposite over neat PHB. Montmorillonite modified by neopentyl (diallyl) oxy tri(dioctyl) pyrophosphate titanate was used as reinforcement agent by Parulekar et al. (*105*), and the PHB-clay nanocomposite was prepared by extrusion followed by injection molding. Characterization studies using transmission electron microscopy (TEM), melt rheology and X-ray diffraction (XRD) suggested exfoliation of the organically modified clays.

PHB-silver sulphide nanocomposites were prepared by Yeo et al. using a two-step aqueous-to-organic phase transfer method, in which $Ag_2S$ particles were added to an organic solution of PHB in chloroform (*106*). An increase in $Ag_2S$ loading in PHB resulted in a decrease in the onset temperature of degradation; the thermal degradation rate constant was found to be linearly related to $Ag_2S$ loading. Further, these silver based PHB nanocomposites are expected to possess antimicrobial activity which would make them ideal for food packaging applications. Gardolinski et al. prepared PHB-kaolinite layered nanocomposites by melt intercalation at 180 °C (*107*). XRD studies showed ordered arrangement of the polymer-clay intercalates in flattened monolayers with an intercalation expansion of 0.453 nm. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) revealed that nanocomposites possessed enhanced thermal stability.

Lioa and Wu (*108*) prepared nanocomposites of PHB with multi-walled carbon nanotubes (MWNT) by melt blending. Dispersion of MWNT was improved by using PHB grafted with acrylic acid (PHB-g-AA) and multi-hydroxy functionalized MWNTs. An enhancement in the thermal and mechanical properties was observed due to the formation of ester carbonyl groups through reaction between the carboxyl groups of PHB-g-AA and hydroxyl group of functionalized MWNT. Silva et al. (*109*) prepared PHB-thermally reduced graphene (TRG) based films via a solution and coagulation method varying the TRG loading between 0.5-1.0 wt %. The TRG loading didn't alter the crystal structure of polymer but there was increase in nucleation density of spherulites compared to neat PHB. Further investigation is needed to explore the applicability of these PHB/graphene and PHB/carbon nanotube films as sensors for detection of toxic compounds in active food packaging.

Incorporation of cellulose in PHB has been observed to produce nanocomposites with enhanced properties. Patrício et al. incorporated cellulose nanowhiskers (CNWs) into PHB by dispersion in plasticizing agent polyethylene glycol (PEG) (*110*). For concentrations of CNWs up to 0.45 wt. % there was a remarkable increase in the elongation at break (as much as 50 times that of the neat PHB) without any significant loss in the tensile strength. This increase was attributed to the alignment of polymer chains in the direction of the applied load due to the presence of CNWs. Ten et al. dispersed PHB-CNW nanocomposites in

N-N Dimethyl Formamide (DMF) through ultrasonication and cast the solution into films on a glass substrate at 80 °C (*111*). The casting was performed under DC electric fields in an attempt to align the CNWs unidirectionally. Degree of alignment was found to be strongly influenced by the CNW concentration; decrease in alignment was observed with increasing CNW loading. The aligned nanocomposites exhibited considerable mechanical anisotropy with the storage modulus showing strong directional dependence. At CNW loadings above 4% (by weight), high suspension viscosity and consequent reduction in mobility of CNWs prohibited any significant alignment along the direction of the applied electric field. Even though the nanocomposite is effectively isotropic at CNW concentrations above 4%, samples prepared under the influence of electric field showed significantly higher storage modulus than those prepared in the absence of electric field. This suggests that the presence of electric field hinders CNW agglomeration and induces better dispersion. Zhijiang et al. dispersed bacterial cellulose (BC) in PHB to obtain biocompatible nanocomposites having a homogenous dispersion of nano-sized spherulites of PHB and nano-fibrils of BC (*112*). The dimensions of the PHB spherulites and BC were smaller than the wavelength of visible light and the nanocomposite films obtained were transparent. Morphological studies through TEM and SEM showed that the sperulitic PHB molecules filled the space between BC nano-fibrils, which led to significant increase in the mechanical strength and thermal stability of the nanocomposite over neat PHB. In particular, the tensile strength, elongation at break and Young's modulus of the PHB-BC nanocomposite films increased by 150%, 300% and 120% respectively. As both PHB and BC are biodegradable and transparent, these nanocomposites show promise for application in display devices, tissue engineering scaffolds, and food packaging.

## Polyhydroxy Butyrate/Cellulose Nanocrystal: Nanobiocomposites for Food Packaging Applications

### Fabrication of PHB-CNC Films

Extensive research is being carried out at present on fabrication of PHB-CNC based green nanocomposite films for food packaging and other applications, because of its bio-origin, complete biodegradability and non-toxicity. Different fabrication techniques ranging from commercially viable extrusion, melt compounding, compression molding and cost effective solvent exchange have been used todate for fabrication of PHB-CNC films. Ten et al. (*111*) reported on the fabrication of PHB-CNC films by solution casting followed by dispersion of CNCs into PHB solution (dissolved in DMF) through ultrasonication. However the ultrasonication process leads to a decrease in the molecular weight of PHB due to chain degradation. This in turn adversely affects the mechanical strength of fabricated films. Srithep et al. (*113*) reported the formation of PHB-CNC nanocomposite films through melt compounding, where they extruded the PHB-CNC freeze dried powder at 180 °C. However, even after keeping the

time of exposure and mixing time short, significant degradation of PHB (up to 21%) was observed. Degradation of the molecular weight during processing significantly reduces the thermal, barrier and mechanical properties thereby limiting the applicability of the material (*114*).

**Table 5. Zeta Potential of Cellulose Nanocrystals and PHB-CNC in Different Solvents**

| *Sample Name* | *Zeta Potential (mV)* | *Mobility (cm²/Vs)* |
|---|---|---|
| CNC in water | -55.32±3.01 | -1.584±0.2e-04 |
| CNC in acetone | -12.14±3.05 | -5.745±0.45e-05 |
| CNC in Chloroform | 49.61±5.34 | 3.745±0.96e-05 |
| PHB-CNC sol. | 169.95± 4.53 | 1.283±0.84e-04 |

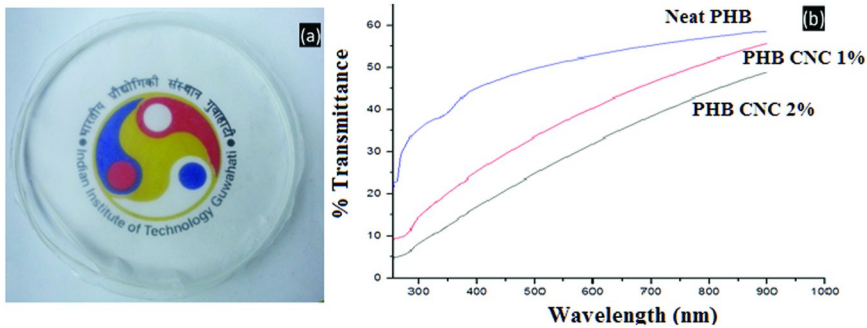


*Figure 7. (a) 1% PHB-CNC. (b) Transparency measurement of PHB-CNC films at different CNC loadings.*

We report on a cost effective process for the fabrication of CNC and its dispersion in PHB through solvent exchange cum solution casting technique which does not lead to degradation of the polymer chain. PHB is dissolved in chloroform at 90 °C under reflux conditions for 2 hours. Well-dispersed suspension of freeze dried CNC and PHB (in chloroform) is then prepared by using solvent exchange technique in combination with centrifugation at 10,000 rpm. In this method, CNC dispersed in water is centrifuged with acetone. After removing the remaining acetone, chloroform is added to the CNC precipitate and the mixture is again centrifuged at 2,000 rpm. The resulting CNC suspension (in chloroform) is finally added to PHB solution while being stirred at 60 °C for 20 min. Solvent casting technique is then used to fabricate films from PHB/CNC suspension using Teflon plates. This simple film casting technique does not

affect the morphology of the native PHB polymer chains in the presence of nanoparticles. The stability of the CNC solution dispersed in different phases was analyzed using zeta potential measurement. The magnitude of zeta potential indicates the degree of repulsion between adjacent similarly charged nanoparticles in the dispersion. Zeta potential studies, carried throughout the solvent exchange process, showed a transition from negative to positive passing through a region of instability (see Table 5). CNC dispersed in water had a stable zeta potential of –55mV. Upon solvent exchange with acetone, the solution zeta potential changed to –12 mV indicating a region of instability. On subsequent exchange with chloroform, the zeta potential value increased to +50 mV suggesting a better, stable dispersion of CNC. When CNC was mixed with PHB the stability rapidly increased to 170 mV, due to the presence of highly stable polymer solution in chloroform. Thus, a solvent exchange process helped in better dispersion of the CNCs in the melt state of polymer in chloroform solution. However it was found that increasing the CNC loading adversely affected the stability of PHB polymer solution presumably due to agglomeration of CNC nanoparticles. Figure 7a shows a transparent 1% PHB-CNC nanocomposite film fabricated using the above technique with IIT Guwahati logo in the background. The transparency of the film is analyzed in the 250–900 nm wavelength range using UV-Vis spectrometer and the results are shown in Figure 7b. Transparency of PHB films decreased with increase in CNC loadings due to agglomeration of CNCs. At optimum CNC loadings (~1 to 2%), when nanofiller dispersion in the polymer matrix is good, the transparency of PHB-CNC films was comparable, albeit somewhat lower, to that of native PHB films. The morphological, physical, mechanical and rheological properties of PHB-CNC nanocomposite films fabricated with different CNC loadings are discussed next.

**Hydrogen-Bonded Network of CNC with PHB Matrix: An FTIR and XRD Study**

The FTIR spectra of pure PHB and PHB/CNC nanocomposites at different CNC loadings are shown in Figure 8a. Addition of CNC to PHB has an effect on C=O bond stretching at 1750 $cm^{-1}$. Maximum stretching has been observed for 1% and 2% CNC loading indicating better networking at these loadings than at 5%. Moreover, the OH peak at 3656 $cm^{-1}$ in PHB is absent in the PHB-CNC nanocomposite. Stretching of C=O bond and absence of OH peak in the nanocomposites indicate the presence of hydrogen bonding between CNC and PHB. Presence of hydroxyl groups in cellulose and carboxyl and hydroxyl groups in PHB is expected to lead to strong intermolecular as well as intramolecular hydrogen bonding. Various possible bonding schemes are illustrated in Figure 8b. For example, hydroxyl (OH) at C6 and C3 of cellulose can bond with carbonyl (C=O) and C-O-C of PHB as shown in scheme I. However, OH at C6 also participates in intramolecular hydrogen bonding with OH at C2 within cellobiose. Scheme II illustrates the hydrogen bonding between PHB and cellulose nanocrystals end units, where C2 and C6 hydroxyl of cellulose bond with C=O and C-O-C of PHB. Another possibility of hydrogen bonding is shown in Scheme III where the end hydroxyl groups of PHB chains attach to C3 of

cellulose. Moreover, C3 hydroxyl may participate in intramolecular (with cyclic C-O-C of cellulose ) and intermolecular (with cellulose and PHB) hydrogen bonding or it may only have intermolecular bonding with PHB.

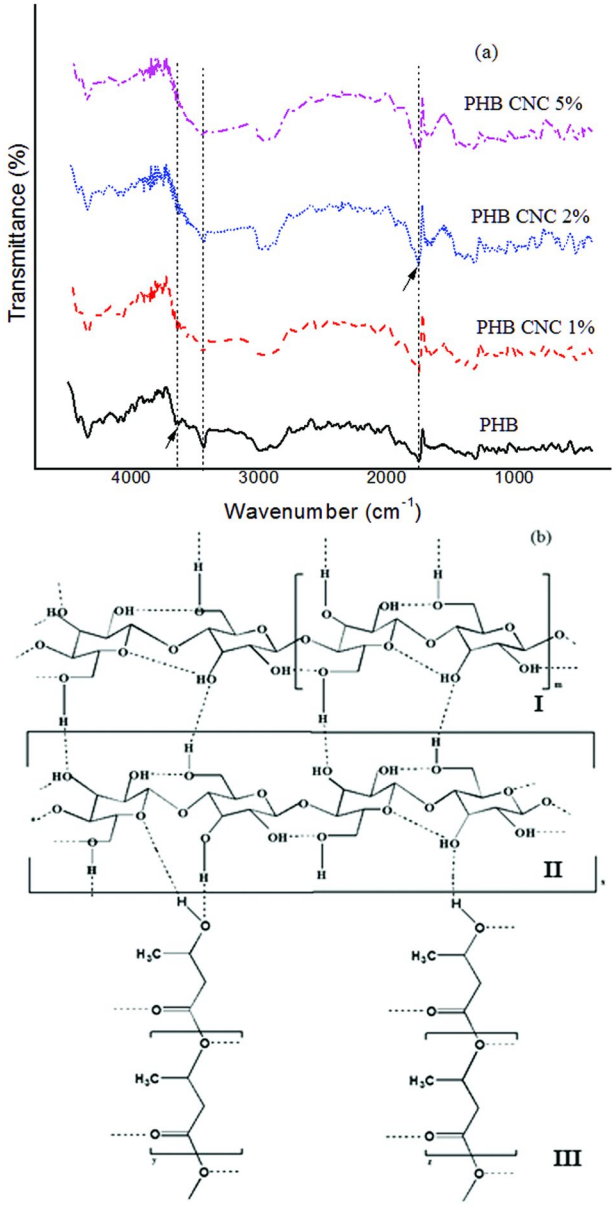


*Figure 8. (a) FTIR comparison of PHB film with PHB/CNC nanocomposite films. (b) Plausible mechanism for intermolecular and intramolecular H- bonding.*

The cyclic C-O-C of cellulose may also involve in intermolecular hydrogen bonding as suggested in Scheme III. Intramolecular hydrogen bonding in cellulose is observed primarily between hydroxyls at C6 and C2 whereas intermolecular hydrogen bonding possibly occurs between C6 and C3 hydroxyls.

XRD studies were carried out to further investigate the interactions between CNC nanoparticles and PHB polymer matrix. XRD patterns of pure PHB film and PHB/CNC nanocomposites are shown in Figure 9. Lattice planes of both CNC and PHB are clearly observed in the diffractograms. Broadening of the peak at 13.5° is correlated with the crystallite size in the nanobiocomposite using Scherrer's equation

$$D_k = \frac{0.9\lambda}{\beta \cos\theta}$$

where $D_k$ is the mean size of crystalline domains, 0.9 is the dimensionless shape factor, $\lambda$ is the X-ray wavelength, $\beta$ is the line broadening at half the maximum intensity in radians and $\theta$ is the Bragg angle.

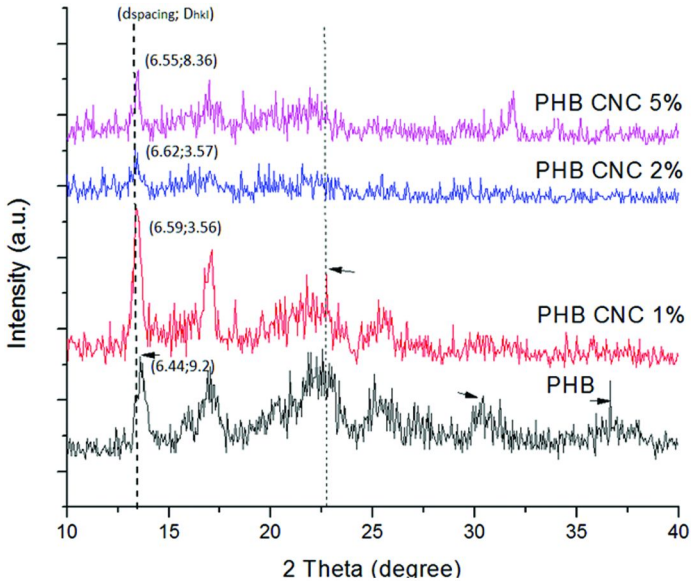


*Figure 9. XRD comparison of $d_{spacing}$ and $D_{hkl}$ of pure PHB film with different CNC loaded films.*

Lattice spacing and mean size of the crystalline domains have been calculated at various CNC loadings (1%, 2% and 5%) and are shown in Figure 10. $D_{hkl}$ values in pure PHB, and at 1%, 2% and 5% CNC loadings are 9.2 Å, 3.56 Å, 3.57 Å and 8.36 Å respectively. Low $D_{hkl}$ values indicate better intercalation of CNC into PHB matrix at lower loading levels. The CNCs intercalated into the PHB crystals through hydrogen bonding which led to shifting of the XRD peak at 2θ = 13.5° towards lower angles. CNCs predominantly occur as cellulose Iα

which has a triclinic unit cell. Two of the four crystal faces, (100) and (010), present relatively large, hydrophilic surfaces rich in OH groups. The XRD peaks at 14.7° and 16.2°, which represent (110) and (1–10) faces, are sharp corners with essentially only one surface-exposed chain with rounded corners because the chains with fewest interactions with the underlying body of the crystal are easily dissociated. But due to presence of large hydrophilic surface of CNCs there is relative more hindrance in better intermixing (*115*, *116*). Similar type of results were also reported by Ten et al. (*111*) who analyzed the $D_{hkl}$ values of diffraction peaks corresponding to 30.5°. They found that at lower CNC loadings the PHB crystallite size decreased due to hindrances in folding as CNCs exhibited confinement effects due to intercalation. Thus at lower loading levels (~ 2%), CNC was homogenously dispersed into the polymer matrix which was further revealed through field emission scanning electron microscopy (FESEM) based morphology study.

Field emission scanning electron microscopy (FESEM) images of nanobiocomposite films having different CNC loadings are shown in Figure 10. From the micrographs it was observed that well dispersed cellulose nanocrystals were present in all the films. However, better dispersion is found in films with 1% and 2% CNC loading than in films at 5% loading. Better dispersion is indicated by multidirectional orientation of CNCs in the film indicating better 3D networking. This networking is due to hydrogen bonding between CNC and PHB, possibly through one of the schemes shown in Figure 8b. Strong hydrogen bonding between cellulose and PHB is expected to improve the physical and mechanical properties of PHB.

**Mechanical Properties of PHB-CNC Films**

CNC is derived from natural biomass and possesses exceptional mechanical strength of 500 MPa and stiffness of 140-220 GPa (*115*). Incorporation of CNCs in biopolymers is expected to enhance the mechanical strength of the polymer while keeping the complete biodegradability of the biopolymers intact. The dispersion of hydrophilic CNCs into hydrophobic PHB is a challenging task. Though a solvent exchange method is useful for uniform dispersion of CNCs in PHB up to a threshold concentration of 2–3%, higher CNC concentrations lead to agglomeration of nanoparticles which is not desirable. At a CNC loading of 2% (by weight) the ultimate tensile strength improved considerably to 57.1 MPa from 36.0 MPa for the native PHB as did the elongation at break (see Table 6). This is due to better intercalation of the CNCs in to the PHB matrix at small CNC loadings, a fact also supported by XRD data (in Figure 9). Change in ultimate tensile strength and elongation at break with CNC loading is shown in Figure 11a while the effect of d-spacing on ultimate tensile strength and elongation at break is shown in Figure 11b. Decreased d-spacing indicates improved dispersion of CNCs into polymer matrix which leads to enhancement in the mechanical properties of the nanocomposites. At higher CNC loading the nanoparticles tend agglomerate within the polymer matrix.

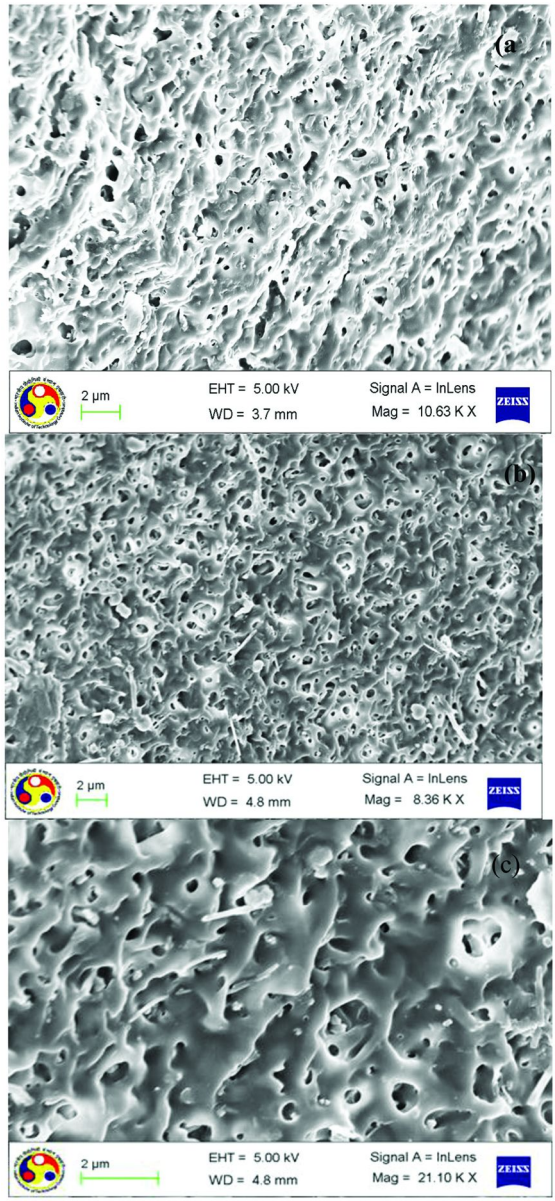


*Figure 10. (a) FESEM of PHB film with 1% CNC. (b) FESEM of PHB film with 2% CNC. (c) FESEM of PHB film with 5% CNC.*

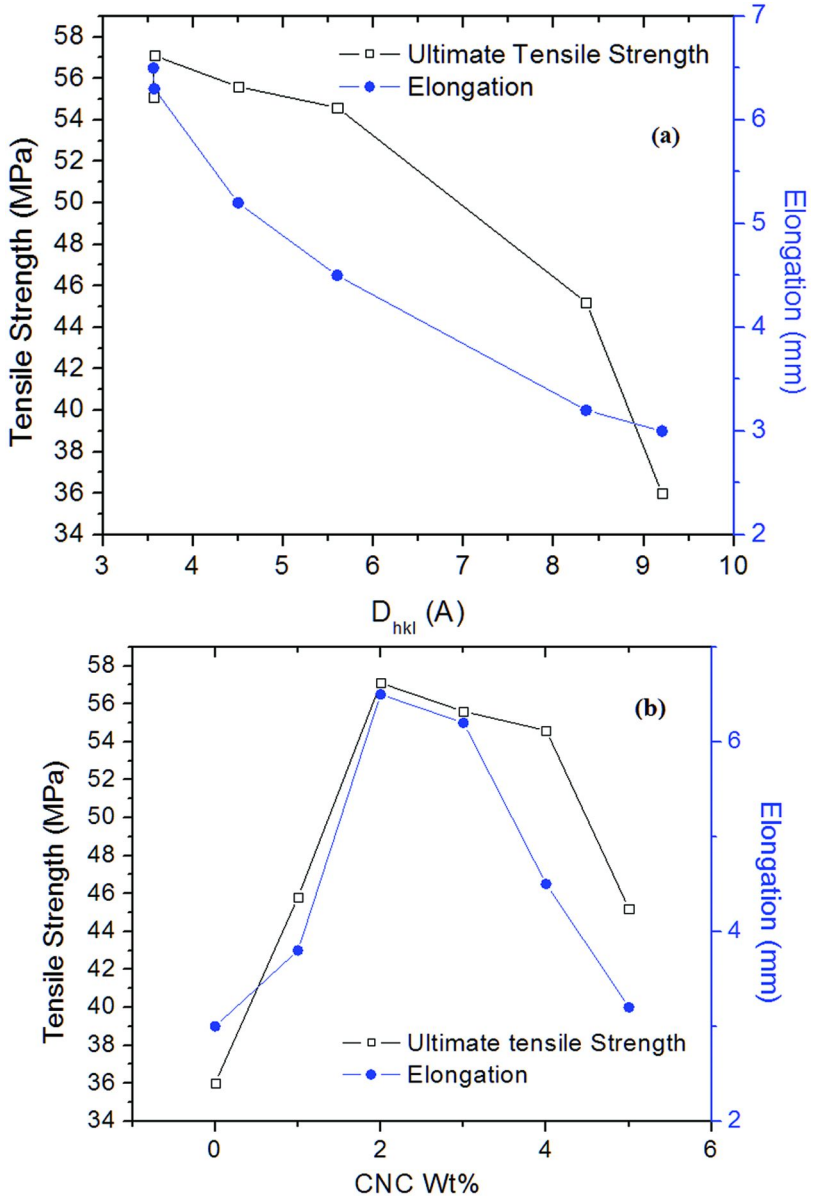


*Figure 11. (a) Effect of the d-spacing on the ultimate tensile strength and elongation of PHB-CNC films. (b) Effect of different loading of CNCs on ultimate tensile strength and elongation of films.*

**Table 6. Mechanical Properties of the PHB-CNC Films at Different CNC Loading**

| *Sample Name* | *Tensile Force (N)* | *Thickness (Mm)* | *Young Modulus (Gpa)* | *Area ($Mm^2$)* | *Uts ($N/Mm^2$)/ (Mpa)* | *Elongation At Peak Load (%)* |
|---|---|---|---|---|---|---|
| Phb Neat | 5.4 | 0.015 | 1.321 | 0.15 | 36.0±1.4 | 3.00 |
| Phb-Cnc 1% | 6.87 | 0.03 | 1.226 | 0.15 | 55.8±2.5 | 6.30 |
| Phb-Cnc 2% | 8.55 | 0.031 | 0.878 | 0.155 | 57.1±1.2 | 6.50 |
| Phb-Cnc 3% | 6.95 | 0.026 | 0.896 | 0.13 | 55.6±2.3 | 5.2 |
| Phb-Cnc 4% | 8.2 | 0.030 | 1.213 | 0.15 | 54.6±2.2 | 4.5 |
| Phb-Cnc 5% | 6.8 | 0.035 | 1.412 | 0.15 | 45.2±1.8 | 3.20 |

The Young's modulus of the PHB-CNC films with 2-3% CNC loading is much lower than that of unfilled PHB. The lower Young's modulus indicates more elastic behavior and higher flexibility of the films. PHB-CNC films were more flexible and transparent at 2-3% loading due to a decrease in the crystallinity of PHB as shown by DSC measurements (discussed later). However, at higher CNC loadings the crystallinity of PHB films remained unchanged from that of neat PHB films due to which they had higher Young's Modulus and were more brittle in nature. The tensile strength of the PHB-CNC films, at 55-70 MPa, is comparable to that of polyethylene terephthalate (PET) but the Young Modulus, at 2.8-3.1 GPa, is lower than PET due to brittle nature of PHAs (*117*). Results presented here are not in good agreement with observations by Ten et al. and Srithep et al (*113*). This is because, in the work of Ten et al. and Srithep et al. the polymer degraded during processing which resulted in reduction in mechanical strength. PHB degradation took place during melt extrusion at high temperature through the action of charged sulfate groups present on CNCs. Hence, the cost effective solvent exchange process outlined here leads to fabrication of bionanocomposite films without any alterations or degradation of the polymer chains.

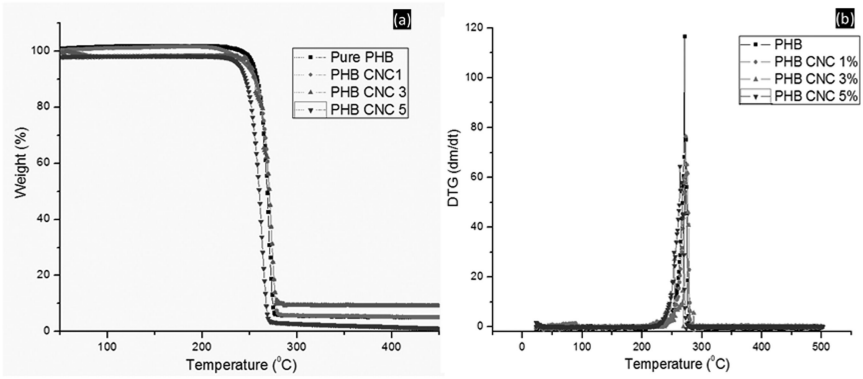


*Figure 12. (a) TGA graphs for the PHB-CNC nanocomposites at different loadings. (b) DTA graphs for the PHB-CNC nanocomposites.*

**Thermogravimetric Studies of PHB-CNC Films**

TGA and differential thermal analysis (DTA) curves for thermal degradation of PHB-CNC at different CNC loadings, namely 1%, 3% and 5%, were obtained at a constant heating rate of 10 °C/min from 25 °C to 500 °C. Smooth changes in the TGA curves and single peak in each DTA curve were observed indicating that the degradation mechanism remained unchanged throughout the degradation process (Figure 12a and 12b). The TGA curves decreased monotonously with temperature and no carbonaceous residue was left. A single peak observed in each DTA curve indicates a single step degradation reaction of PHB chains even in the presence of CNC nanofiller. With increased CNC loading, a decrease in the degradation

onset temperature of composites from 246 °C to 232°C was observed (see Table 7), which can be attributed to enhanced degradation of PHB in the presence of CNC. The presence of pendant sulphate groups at CNC ends presumably enhances chain scission of PHB at high temperatures. CNC induced enhancement in the polymer degradation rate is advantageous from environmental point of view. PHB-CNC samples at 1 and 2% CNC loading showed least conversion at maximum decomposition temperature (23.49 and 24.21 wt% respectively). This is because hydrogen bonded interactions between the nanofiller and polymer at low CNC loadings hindered the degradation of polymer.

**Table 7. Thermal Properties of the PHB-CNC Nanocomposites at Different Loading Percentages at Constant Heating Rate of 10 °C/min**

| *Sample* | $T_{on}$ *(°C)* | $T_p$ *(°C)* | $T_{1/2}$*(°C)* | $\alpha_p$ *(%)* | *RM (%)* |
|---|---|---|---|---|---|
| *PHB Neat* | 246.01 | 270.90 | 267.10 | 57.30 | 4.80 |
| *PHB-CNC 1%* | 240.50 | 272.53 | 276.51 | 23.49 | 5.27 |
| *PHB-CNC 3%* | 236.59 | 271.68 | 269.65 | 24.21 | 8.65 |
| *PHB-CNC 5%* | 232.20 | 262.02 | 261.06 | 35.60 | 6.25 |

$T_{on}$: extrapolated onset temperature. $T_p$: temperature at maximum thermal decomposition. $T_{1/2}$: temperature at 50% conversion. $\alpha_p$: conversion at $T_p$. *RM*: residual mass at 500 °C.

**Calorimetric Studies on PHB-CNC Films**

DSC thermograms showing the $T_g$ of pure PHB and PHB/CNC nanocomposites films are presented in Figures 13a and b. Double heating and cooling cycles have been carried for the films up to 200 °C. Melting peak of PHB during second heating is observed at 178 °C. PHB with 1% and 2% CNC loading have sharp melting peaks indicating uniform crystallites whereas 5% CNC sample exhibits a broad peak (see Figure 13a). During cooling, a crystallization peak is observed (see Figure 13b) and a trend in the crystallization temperature as well as in the degree of crystallinity is observed. At low CNC loading levels (1% and 2%) a lowering of the crystallization temperature is observed whereas at 5% CNC loading the crystallization temperature obtained is similar to PHB. Percentage crystallinity decreased marginally (51% to 47%) at low CNC loading (up to 2%). As pure PHB is brittle and highly crystalline, decrease in crystallinity is important in fabrication of films from PHB (*118*). Results obtained from crystallization and thermal degradation studies were similar to the results reported by Ten et al. and Srithep et al (*111*, *113*).

**Rheological Studies on PHB-CNC Films**

The linear viscoelastic region (LVE) of PHB-CNC, as determined from dynamic strain sweep, was found to be around 1% strain. Figure 14a shows the plot of the storage ($G'$) and loss ($G''$) modulus over a range of strain amplitude.

The region where the storage and loss moduli were found to be independent of the strain amplitude was selected as LVE. Linear range of strain (1%) and non-linear range of strain (50%) were chosen for further investigation of rheology parameters.

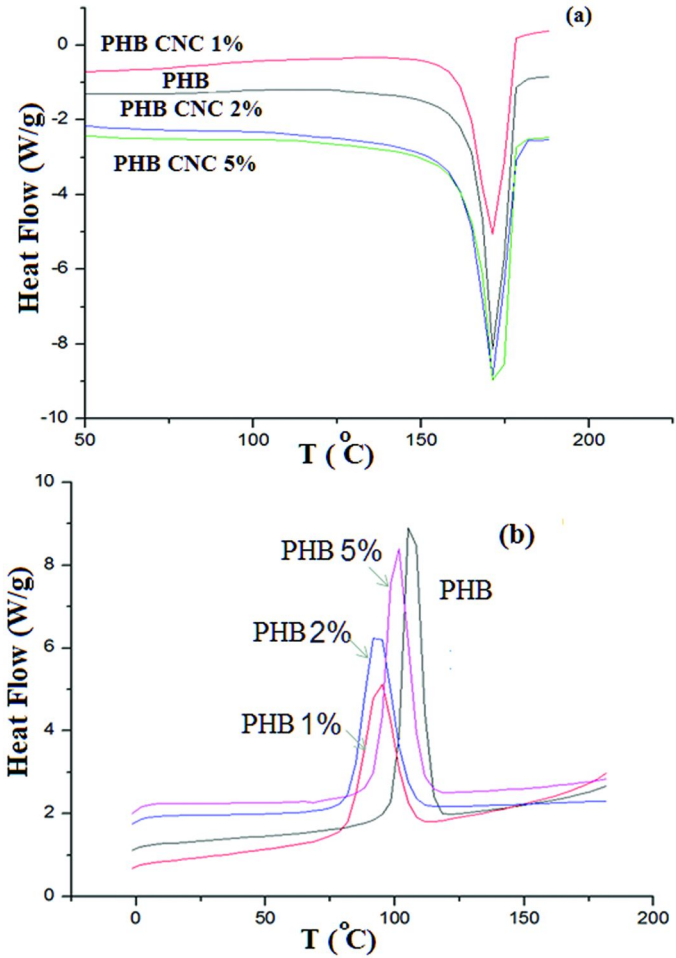


*Figure 13. (a) Second heating cycle during DSC for PHB and CNC loaded thin films. (b) First Cooling Cycle: Crystallization peaks for PHB and CNC loaded thin films.*

The linear viscoelastic region of the PHB-CNC with strain 1% was selected for measurement of the storage and loss moduli of neat PHB and PHB-CNC films under small-amplitude oscillatory shear (SAOS) conditions as shown in Figure 14b. The magnitude of the storage modulus (G′) of PHB-CNC films monotonically increased with CNC loading, especially at higher frequencies. This suggests that the filler strongly affects the relaxation spectrum of the composite samples. The effect of relaxation dynamics of composites at higher frequencies was more pronounced on G′, with a vertical shift of both the moduli by an amount proportional to the volume fraction of filler (φ) (*119*). With increase in the loading percentage of CNC, the dependence of G′ on frequency decreases sharply in the terminal zone and G′ curves exhibit a plateau. The relaxation dynamics of nanocomposites with high CNC loading at low frequencies is similar to that of neat PHB matrix, which indicates that the dispersion of CNC in the polymer matrix is hindered at high CNC concentrations.

To study the effect of CNC loading on rheological behavior of PHB-CNCs, the dependence of the enhancement in low-frequency G′ on CNC loading is probed. Figure 14c shows the low frequency G′ measured at frequency of 0.1 Hz as a function of CNC loading. Compared to that of neat PHB matrix, the low frequency G′ and complex viscosity of PHB-CNC increased by about 2 orders as CNC loading was increased to 5%. It indicates that the viscoelastic properties of PHB-CNC nanocomposites at low CNC loading levels (< 2wt% ) are still dominated by the PHB matrix, and with increase in CNC loading, the PHB-CNC composite may experience a transition from liquid to solid-like state. Therefore the percolation threshold is selected to be around 2 wt %. In case of aggregating nanoparticles, however, hydrodynamic interactions only emerge at higher frequencies, where the behavior of polymer matrix governs the rheological response (*120*). Over longer time scales the presence of the filler results in a gradual slowing down of the storage modulus dynamics, eventually resulting in arrest of the relaxation process above a critical filler volume fraction $\varphi_c$ (*121*). The formation of the low frequency plateau of $G'$ is expected in nanofilled polymers, and is primarily attributed to the formation of a three dimensional percolating network that spans the whole sample. Figure 14d shows that the $G'$ curve of 2% PHB-CNC starts to display a plateau region, which was absent in neat PHB and 1% PHB-CNC composite. Thus the critical volume fraction of the nanofiller falls in the composition range 1 %$\leq \varphi_c \leq$ 2 %, within which it predominantly exhibits elastic behavior. Such a conclusion is supported by the analysis of the low frequency log-log slope of $G'(\varphi)$ normalized to that of pure polymer (*122*), the amplifying factor $\alpha(\varphi)$. The amplifying factor monotonically decreases with $\varphi$ reaching a value of 0.5 at about 2 wt% CNC loading as shown in Figure 14f. We therefore conclude that for the PHB-CNC composites, the percolation threshold φc ~2wt%. However, further analysis of the storage and loss modulus of the 2% PHB-CNC composite showed that at higher frequencies there was an intersection point between G′ and G″ curve (see Figure 14d) suggesting that rod like CNCs form a percolation network structure (*120*). Figure 15 presents a schematic explanation of the aggregation phenomenon taking place inside the polymer matrix and between the nanoparticles. This phenomenon could be explained by the initiation of aggregation of nanoparticles, which may change

the viscoelastic behavior to solid-like behavior. On further increasing the CNC loading, the percolation network disappeared suggesting that the composite forms full solid-like material due to the aggregation of CNCs induced by the dominating effect of intermolecular hydrogen bondings.

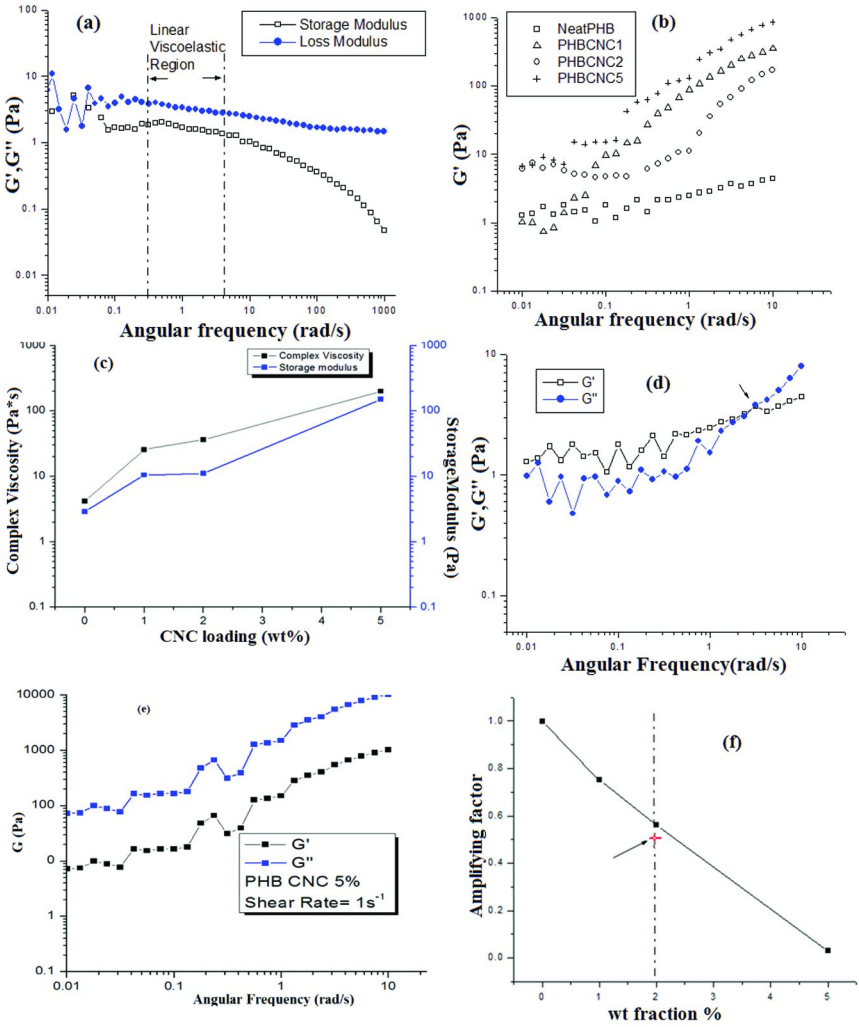


*Figure 14. (a) The Linear Viscoelastic Region of the PHB-CNC composite films at 188 °C. (b) The dynamic storage modulus for the PHB-CNC samples in SAOS measurements. (c) The dependence of low frequency storage modulus and Complex Viscosity measured at 0.1 Hz on different CNC loadings. (d) Comparison of the dynamic storage modulus for PHB-CNC 2 percent. (e) Comparison of the dynamic storage modulus for PHB-CNC 5 percent loadings. (f) Low frequency (0.1 Hz) log-log slope of G' (φ) normalized to that of pure polymer, α (φ).*

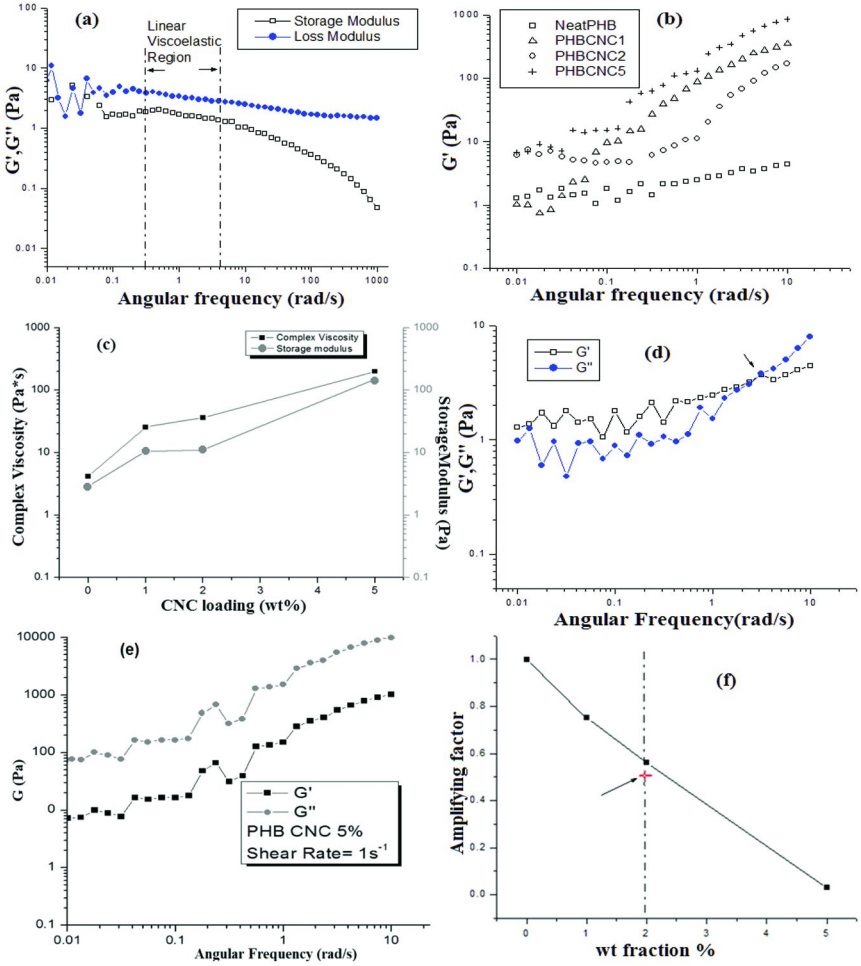


*Figure 15. (a) The Linear Viscoelastic Region of the PHB-CNC composite films at 188 °C. (b) The dynamic storage modulus for the PHB-CNC samples in SAOS measurements. (c) The dependence of low frequency storage modulus and Complex Viscosity measured at 0.1 Hz on different CNC loadings. (d) Comparison of the dynamic storage modulus for PHB-CNC 2 percent. (e) Comparison of the dynamic storage modulus for PHB-CNC 5 percent loadings. (f) Low frequency (0.1 Hz) log-log slope of G' (φ) normalized to that of pure polymer, α (φ).*

The polymer melt was subjected to the heating-cooling cycles of DSC at a heating rate of 5 °C/min throughout the temperature range of 40 °C to 190 °C. Storage and loss moduli for neat PHB for cooling and heating scans are shown in Figures 16a and 16b respectively. There is a slight difference in the *G′* and *G″* before and after the intersection point. At low temperatures the polymer displayed solid-like characteristics with the plateau modulus having a value greater than

10000 Pa. Moreover, at higher temperatures there is no significant difference between the two moduli whereas at low temperatures $G'$ is more than an order of magnitude larger than $G''$. Figures 16c and 16d show the variation of $G'$ and $G''$ with temperature for PHB-CNC nanocomposites at different CNC loadings under heating and cooling scan rates of 5 °C/min. The decay in modulus is highest for the 5% PHB-CNC and least for 1% PHB-CNC. This could be due to the formation of PHB crystallites in 1% PHB-CNC. Similarly, the excessive loss in modulus of 5% PHB-CNC could be attributed to the disruption or breaking of the PHB crystallites (*123*).

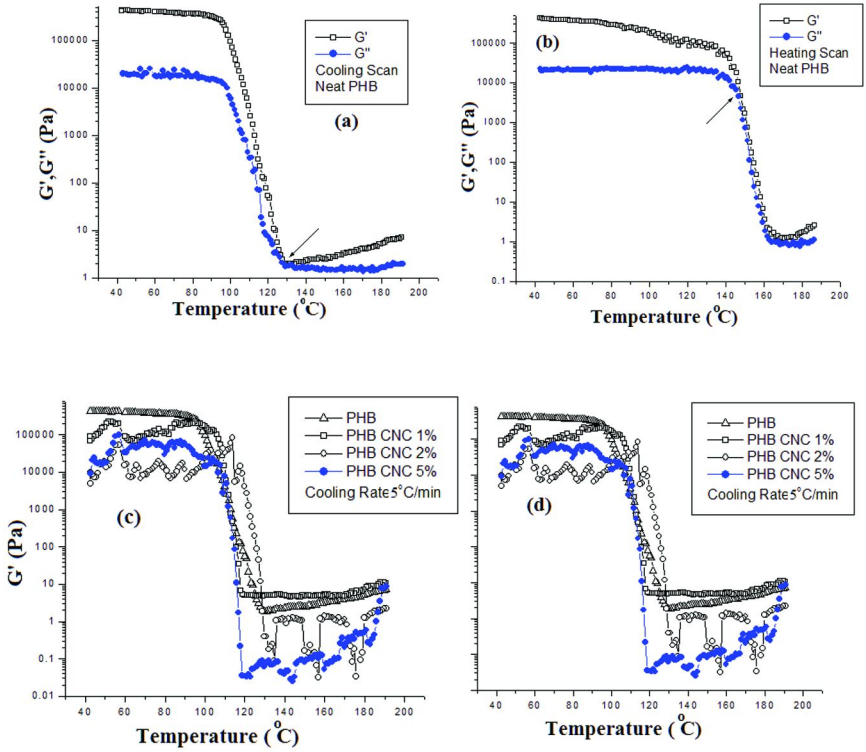


*Figure 16. (a) Variation of the storage and loss modulus of neat PHB under cooling scan rate of 5 °C/min. (b) Variation of the storage and loss modulus of neat PHB under heating scan rate of 5 °C/min. (c) Variation of the storage and loss modulus of PHB-CNC samples with different CNC loadings under cooling scan rate of 5 °C/min. (d) Variation of the storage and loss modulus of PHB-CNC samples with different CNC loadings under heating scan rate of 5°C/min.*

## Conclusions

Bionanocomposites from PHB and CNCs (obtained from lignocellulosic biomass) have been prepared and characterized for mechanical, thermochemical, rheological and colorimetric properties. CNC loadings of 1%, 2% and 5% have been used to fabricate nanobiocomposite films of thickness 80±10 μm. The thermal properties were studied using TGA and DSC. TGA and DTA showed enhancement of onset temperature and decrease in degradation rate with increase in CNC loading. Decrease in the crystallinity due to CNC loading helps in the fabrication of better thin films which exhibit a lowering of transparency with increase in CNC loading. FTIR study shows that hydrogen bonding between CNC and PHB enables the formation of well dispersed nanobiocomposite. From an X-ray diffraction study it was confirmed that lower values of Dhkl at 1% and 2% CNC loading (3.56 Å and 3.57 Å respectively) as compared to values at 5% CNC loading and for neat PHB (8.36 Å and 9.2 Å respectively) indicate better intermixing of CNC in PHB matrix at lower loading. At 1% and 2% CNC loading, crossover of storage modulus and loss modulus at high angular frequency, as revealed by rheological studies, indicates better CNC-PHB interactions. Optimum properties for the PHB-CNC nanocomposites are achieved at low CNC loadings (up to 2% by weight). Significant property enhancements are observed upon incorporation of small amounts of CNC in the PHB matrix. The attractive properties of the PHB-CNC nanocomposites, coupled with their biodegradability and non-toxicity, make them promising materials for application in food packaging.

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