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REVIEW ARTICLE

Stability of Pharmaceuticals

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CONTENTS

Many in-depth articles and seminar proceedings have appeared in the past 2 decades on various aspects of stability (1–10), but no single report has treated the overall subject in an integrated fashion. Investigations into the stability of pharmaceuticals have ranged from fundamental studies on the rates and mechanisms of reactions of the active substance, through evaluation of the influence of the formulation and production processes on the drug and drug product, to, finally, the role of the container and the effect of storage and distribution of the finished packaged article on the integrity of the product.

The objectives of this article are to review the many facets of stability and to outline what a present-day stability program does and should include. We hope to interrelate scientific considerations with regulatory requirements.

It has been recognized that there are legal, moral, economic, and competitive reasons, as well as those of safety and efficacy, to monitor, predict, and evaluate drug product stability (7). However, stability can and does mean different things to different people or to the same people at different times, even those in pharmaceutical science and industry. Although unified nomenclature has been proposed, various terminology is still employed to encompass the what and the how and the why of stability: stability study, kinetic study, compatibility study, stability evaluation, stability-indicating assay, expiration dating, outdating, shelflife, storage legend, preformulation studies, failures of a batch to meet specifications, microbiological stability, stability of the active ingredient, stability of the formulation, stability in the marketed package, stability in sample packages, stability in the dispensing package, and stability in the hands of the consumer. All of these areas have been referred to as stability.

In the pharmaceutical industry, the disciplines primarily involved with stability are pharmaceutical analysis and product development. However, physical and organic chemistry, mathematics, physics, microbiology, toxicology, production, packaging, engineering, quality control, and distribution are all included. Basic subjects for consideration are physical organic chemistry—the evaluation of rates and mechanisms of reactions, kinetics and thermodynamics, and, importantly, organic analysis.

One cannot monitor stability, determine the reaction rate, or investigate any mechanism without an analytical measurement. Hence, the pharmaceutical analyst is pri-

marily involved in stability, because he or she must develop a method that will quantitatively determine the drug in the presence of, or separate from, the transformation product(s). This determination is required to assure that the drug has not undergone change. To select the appropriate method(s), the analyst should have a thorough knowledge of the physicochemical properties of the drug, including an understanding of the routes by which a drug can be degraded or transformed.

The knowledge of the physicochemical properties of the drug is equally important to the development pharmacist in efforts to achieve the optimum drug formulation. Likewise, this knowledge is needed by the package development group so that an appropriate container can be provided.

The stability of this resultant product in various channels of commerce is of concern to the marketing and distribution departments and to the physician, pharmacist, and patient. This concern is manifested by the use of storage legends, expiration dates, protective packaging, and dispensing directions. Furthermore, from a regulatory viewpoint, one should assure that the product is of the "quality, strength, purity, and identity" that it is purported to be throughout the time it is held or offered for sale.

An in-depth discussion on all aspects of this topic is beyond the scope of this review. We intend, however, to highlight the areas involved, with particular attention to recent literature, and to present an integrated overview of a total stability program.

RATES, MECHANISMS, AND PATHWAYS OF DEGRADATION

Kinetics—Two of the main contributors to an understanding of kinetic principles as applied to drug development are T. Higuchi and Garrett (7, 11–13); they brought the principles of chemical kinetics to the evaluation of drug stability. Although the theory was well understood and groundwork in chemical reaction kinetics was underway, only a few papers on drugs appeared in the literature through the 1940's. Detailed studies on drugs were not undertaken until the 1950's. The classical concepts brought to bear were the consideration of factors influencing reactions in solution (14–19), as summarized below.

Most degradation reactions of pharmaceuticals occur at finite rates and are chemical in nature. These reactions are affected by conditions such as solvent, concentration of reactants, temperature, pH of the medium, radiation energy, and presence of catalysts. The manner in which the reaction rate depends on the concentration of reactants describes the order of the reaction. The degradation of most pharmaceuticals can be classified as zero order, first order, or pseudo-first order, even though they may degrade by complicated mechanisms and the true expression may be of higher order or be complex and noninteger.

The quantitative relationship of the specific reaction rate and temperature is the Arrhenius expression:

$$k = Ae^{-\Delta H_a/RT} \qquad \text{(Eq. 1)}$$

where k is the specific rate constant; T is temperature in degrees Kelvin; R is the gas constant; A, the preexponential factor, is a constant associated with the entropy of the reaction and/or collision factors; and ΔH_a is defined as the heat of activation. The equation is usually employed in its logarithmic form:

$$\log k = -(\Delta H_a/2.303RT) + \log A \qquad \text{(Eq. 2)}$$

The slope of a plot of log k against $1/T$ yields the activation energy. This equation provides the underlying basis which allows prediction of stability of pharmaceuticals by extrapolation of rate data obtained at higher temperatures.

An understanding of the limitations of the experimentally obtained heat of activation values is critical in stability prediction; the pitfalls of extrapolation of kinetic data were described (20–22). For example, the apparent heat of activation at a pH value where two or more mechanisms of degradation are involved is not necessarily constant with temperature. Also, the ion product of water, pKw, is temperature dependent, and $-\Delta H_a$ is approximately 12 kcal, a frequently overlooked factor that must be considered when calculating the hydroxide-ion concentration. Therefore, it is necessary to obtain the heats of activation for all bimolecular rate constants involved in a rate–pH profile to predict degradation rates at all pH values for various temperatures.

If photolysis is the rate-determining step of the reaction, most often no predictive advantage is gained by higher temperature studies because the ΔH_a is small and, hence, the effect of temperature is small. Conversely, the heat of activation may be high for pyrolytic reactions, but the degradation rates obtained at elevated temperatures may be of little practical value when extrapolated to room temperature.

Complex reactions, including reversible reactions, consecutive reactions, and parallel reactions, are occasionally encountered in the decomposition of pharmaceuticals. Some of these reactions are discussed under *Physical Organic Chemistry*. A recent review (23) dealt with the kinetics of the most frequently encountered complex drug degradation reactions.

Many drugs are derivatives of carboxylic acids or contain the functional group based on this moiety, *e.g.*, esters, amides, lactones, lactams, imides, and carbamates. The members of this class include many important drugs such as aspirin, penicillin, ascorbic acid, procaine, meperidine, and atropine. This class can illustrate the basic factors affecting the rates of all reactions (24).

The study of hydrolytic reactions as a function of pH yields a rate–pH profile. For an ester, the overall hydrolysis rate of a drug, D, may be expressed as follows:

$$-\frac{dD}{dt} = K_U + K_{H^+}[H^+] + K_{OH^-}[OH^-] + K_N[N] + K_{GB}[GB] + K_{GA}[GA] \qquad \text{(Eq. 3)}$$

where K_U is the rate constant for the uncatalyzed or water-catalyzed reaction, K_{H^+} is the rate constant for the hydrogen-ion-catalyzed hydrolysis, K_{OH^-} is the rate constant for the hydroxide-ion-catalyzed hydrolysis, K_N is the rate constant for nucleophilic catalysis, K_{GB} is the rate constant for general base catalysis, and K_{GA} is the rate constant for general acid catalysis.

The hydrolysis of a compound may be subject to some or all of these terms; however, at any given pH, only one or two terms are significant. The simplest profile is observed when a compound is subjected to only hydrogen-ion

or hydroxide-ion catalysis. The effects of other nucleophiles or general acids or bases are usually studied by varying their concentrations while maintaining the pH constant.

Solvent has a significant effect on the reaction rate. A simplified treatment of solvent effects is presented here. When both reactants are ions in a solvent medium or a continuous dielectric, absolute rate theory gives the following equation:

$$\ln k = \ln k_0 - \frac{N}{RT}\frac{Z_A Z_B e^2}{\epsilon\gamma} \qquad \text{(Eq. 4)}$$

where $\ln k$ is the rate constant at the dielectric constant ϵ, $\ln k_0$ is the rate constant in the medium of infinite dielectric constant, N is Avogadro's number, Z_A is the charge on ion A, Z_B is the charge on ion B, e is the electronic charge, T is absolute temperature, R is the gas constant, ϵ is the dielectric constant, and γ is proportional to the interatomic distance in the activated complex.

This equation predicts a linear relationship between $\ln k$ and $1/\epsilon$. No effect of the dielectric constant would be noted if one of the molecules were neutral because Z_A or Z_B would be zero. The effect of the dielectric constant on the reaction rate between an ion and a neutral molecule is expressed as:

$$\ln k = \ln k_0 + \frac{NZ^2e^2}{2\epsilon RT}\left(\frac{1}{\gamma_1} - \frac{1}{\gamma_2}\right) \qquad \text{(Eq. 5)}$$

where γ is the radius of the reactant ions and the other symbols are as defined in Eq. 4.

Equation 5 predicts that the logarithm of the rate constant will vary linearly with the reciprocal of the dielectric constant. However, many drugs are quite complex and often do not appear to follow theory; *e.g.*, the solvolysis rate of the aspirin anion increases with an increasing ethanol content, but the rates are relatively constant with an increasing dioxane content. Both of these solvents should have produced a decrease in the overall rate. However, based on this type of information, it was concluded that a possible rate-determining step was the attack of water or ethanol on an uncharged cyclic intermediate (25, 26).

For reactions involving two ionic species, the rate constant is dependent on the ionic strength, μ. For aqueous solutions at 25°, Eq. 6 expresses the variation of the rate constant with ionic strength:

$$\log k = \log k_0 + 1.02 Z_A Z_B \sqrt{\mu} \qquad \text{(Eq. 6)}$$

A straight line with a slope equal to $1.02Z_AZ_B$ is obtained when one plots $\log k$ *versus* $\sqrt{\mu}$. Equation 6 would predict no effect on a reaction when one reactant is neutral; but the activity coefficient of a neutral molecule is affected by ionic strength, and one can observe a linear relationship between the logarithm of the rate constant and ionic strength:

$$\ln k = \ln k_0 + b\mu \qquad \text{(Eq. 7)}$$

where b is an empirical constant.

These two ionic effects are commonly called the primary salt effect. In addition, one observes what is called the secondary salt effect, which is the effect of ionic strength on the dissociation constant of a buffer species.

Many pharmaceuticals are subject to general acid, general base, or nucleophilic catalysis in addition to hydrogen-ion or hydroxide-ion catalysis. Several linear free energy relationships quantitate the catalytic rate constant with a property of the species and relate the rate constant for a series of reactions. For acid–base catalysis, this free energy relationship is the Brönsted catalysis law and can be expressed as:

$$k_{GA} = G_A K_A^{\alpha} \qquad \text{(Eq. 8)}$$

and:

$$k_{GB} = G_B K_B^{\beta} \qquad \text{(Eq. 9)}$$

where K_A and K_B are acid and base dissociation constants, respectively; and G_A, G_B, α, and β are constants characteristic of the solvent, temperature, and reaction.

Many drugs have ionizable groups, and the reactions may proceed differently for the ionized and unionized forms. However, analytically one usually measures the total drug concentration, D_T. For a weak base, the contribution of the ionized, D_{H^+}, and unionized, D, drug are related through the pKa of the drug and the pH of the medium; thus:

$$D_T = D + D_{H^+} \qquad \text{(Eq. 10)}$$

The overall reaction rate observed is the sum of both reactions. Two examples, aspirin and barbiturates, that demonstrate the effect of ionization on the rate constant and the mode of degradation are provided in the next section.

The basic kinetic effects are important to an understanding of the reaction and of possible adverse, practical effects. For example, addition of an inert salt such as sodium chloride to adjust isotonicity can affect the reaction rate as a primary salt effect. Buffers used to control pH are also ionic species and can exert a primary salt effect. In addition, they exert a secondary salt effect and also act as catalysts. Sulfite salts are frequently added as antioxidants, but they can form addition products with the active ingredient or act as catalysts.

Organic solvents such as alcohol are generally used for solubilization; the concentration of the organic solvent can affect the dielectric constant of the solvent and thus influence the degradation rate of the active ingredients. The preservatives used to inhibit bacterial growth or other pharmaceutical aids may decompose and their decomposition products may, in turn, influence the decomposition rate of the active ingredients by one or more of the means discussed previously.

Physical Organic Chemistry—The basic kinetic principles outlined are applicable to all chemical systems. However, relatively simple molecules have been used to elucidate a principle or to establish fundamental relationships. A generation ago, physical chemistry and organic chemistry were considered to be two separate nonrelated disciplines. But a number of standard textbooks in the field, ranging from Hammett's (27), through classic works by Bell (28) and Ingold (29), to more recent treatises, relate reaction mechanisms and catalysis to biochemical systems. Most modern textbooks in organic chemistry now integrate physicochemical principles (16–19, 30–36).

Since most modern pharmaceuticals are complex organic molecules, a firm understanding of mechanistic organic chemistry is vital to any detailed study of drug degradation; conversely, degradation studies of many classic drugs have added to an understanding of the mechanism

of many organic reactions. Most widely used drugs have been studied and provide good models for future studies. It is not within the scope of this article to review the myriad studies that have been conducted, but we shall illustrate the complexity and depth through review of two classic examples—aspirin and barbiturates—and highlight the types of reactions that drugs can undergo by a review primarily of the literature of the last few years.

Aspirin is an excellent example of a pharmaceutical compound on which in-depth kinetic studies have been performed and for which reaction mechanisms have been proposed (37–39). The first detailed studies on aspirin hydrolysis were published in 1950 by Edwards (40, 41), 42 years after the first study was reported (42). His work clearly demonstrated specific acid–base catalysis and pH-independent solvolysis of aspirin to salicylic acid. The rate constants for hydrogen-ion and hydroxide-ion catalyses were found to differ with the charge of the molecule. Edwards explained the relationship between the observed rate constant and pH on the assumption that aspirin hydrolysis occurs according to the six simultaneous reactions shown in Scheme I.

$$CH_3COO\cdot C_6H_4COOH + H_3O^+ \xrightarrow{k_1} HOC_6H_4COOH + CH_3COOH + H^+$$

$$CH_3COO\cdot C_6H_4COOH + H_2O \xrightarrow{k_2} HOC_6H_4COOH + CH_3COOH$$

$$CH_3COO\cdot C_6H_4COOH + OH^- \xrightarrow{k_3} HOC_6H_4COOH + CH_3COO^- \text{ or } HOC_6H_4COO^- + CH_3COOH$$

$$CH_3COO\cdot C_6H_4COO^- + H_3O^+ \xrightarrow{k_4} HOC_6H_4COOH + CH_3COOH$$

$$CH_3COO\cdot C_6H_4COO^- + H_2O \xrightarrow{k_5} HOC_6H_4COOH + CH_3COO^- \text{ or } HOC_6H_4COO^- + CH_3COOH$$

$$CH_3COO\cdot C_6H_4COO^- + OH^- \xrightarrow{k_6} HOC_6H_4COO^- + CH_3COO^-$$

Scheme I

The observed overall first-order rate constant, k, can be expressed as a function of the six second-order rate constants and the acid dissociation constant, K, of aspirin:

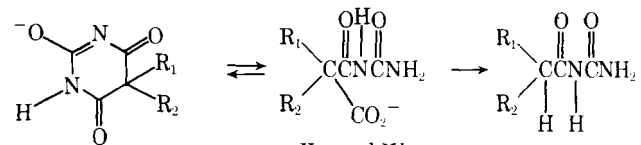
$$k = \frac{k_1[C_{H^+}] + k_2[C_{H_2O}] + k_3[C_{OH^-}]}{1 + K/[C_{H^+}]} + \frac{k_4[C_{H^+}] + k_5[C_{H_2O}] + k_6[C_{OH^-}]}{1 + [C_{H^+}]/K} \quad \text{(Eq. 11)}$$

Garrett (25, 26) also investigated the pH–rate profile for aspirin hydrolysis, particularly in the pH 4–8 range. Garrett's work pointed to intramolecular nucleophilic catalysis by the ionized carboxyl group. When the carboxylate ion is intramolecular, it catalyzes a number of ester reactions, although it is not a particularly strong nucleophile. As mentioned, the addition of alcohol increases the solvolysis rate, thus strongly suggesting the involvement of a solvent molecule in the transition state. On the basis of the kinetic and isotopic studies, aspirin hydrolysis was shown to be an intramolecular nucleophilic catalyzed hydrolysis involving an anhydride intermediate. It was assumed that the transition state of the reaction involved addition of the carboxylate ion to the carbonyl group of the ester, forming a tetrahedral addition intermediate.

Fersht and Kirby (43, 44) studied the reactivity of a series of substituted aspirins toward hydrolysis. The results show that the most likely mechanism for aspirin hydrolysis was one in which the carboxylate group acted not as a nucleophile but as a general base. The pH–rate profile for aspirin hydrolysis, as determined by Edwards (40, 41), showed that the transition state for hydrolysis in the pH-independent region involved the aspirin anion, either alone in a unimolecular reaction or together with one or more molecules of solvent.

Three mechanisms have been proposed on the basis of the kinetic results for the intramolecular catalytic hydrolysis of aspirin by the carboxyl group: (*a*) a unimolecular process in which the carboxylate group acts as a nucleophile, (*b*) a general acid catalysis in which the undissociated carboxylic acid group reacts with hydroxide ion, and (*c*) a general base catalysis in which the carboxylate anion reacts with a water molecule.

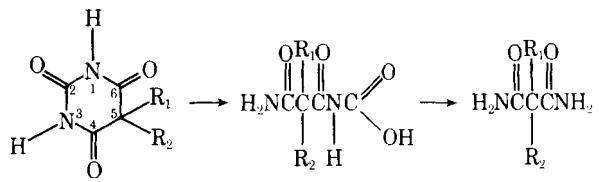
The barbiturates provide another excellent example of the complex mechanisms by which drugs degrade (Scheme II). Early workers (45, 46) assumed that the hydrolysis of barbiturates I*a* and I*b* to the corresponding malonuric acids was irreversible, and various degradation schemes were predicted on that assumption. Garrett *et al.* (47), in the process of further elucidating the hydrolysis kinetics of several important barbiturates, discovered that diethylmalonuric acid (II*a*) in basic solution may cyclize to form barbital (I*a*). Gardner and Goyan (48) confirmed the reversibility of hydrolysis of the barbituric acid nucleus and noted that it may have interesting biological ramifications. Furthermore, they rationalized previous findings (46) in the light of a similar reaction involved in the cyclization of 2-ureidobenzoic acid (49). Thus, the unionized barbiturate (III) could be cleaved at the 1,2-position, leading to production of the bisamide (IV), or at the 1,6- (3,4-) position, leading to the ureide (V); the ionized barbiturate would cleave only at the 1,6- (3,4-) position, leading to the ureide (or malonic acid) exclusively.



I*a*: $R_1 = R_2 = C_2H_5$
I*b*: $R_1 = C_2H_5$, $R_2 = C_6H_5$

II*a* and II*b*

V



III

IV

Scheme II

Recently, Khan and Khan (50) observed that earlier workers did not kinetically detect the existence of di- and trianionic tetrahedral addition intermediates in the base-catalyzed hydrolysis of barbituric acid because their alkali concentration range was low. At pH values higher than the pKa_2 of barbituric acid, the equilibrium concentration of undissociated barbituric acid was negligible compared to the concentration of mono- and dianionic

barbituric acids. The equations were developed for $k_{1,obs}$ and $k_{2,obs}$ for the following consecutive irreversible first-order reaction path: barbituric acid $\xrightarrow{k_{1,obs}}$ malonuric acid $\xrightarrow{k_{2,obs}}$ ammonia. The rate constants showed three regions of hydroxide-ion dependence:

1. The reciprocals of the rate constants were linearly related to the reciprocal of the hydroxide concentration at low concentration.

2. The rate constants were independent of the hydroxide-ion concentration at higher concentrations of hydroxide ion.

3. The rate constants observed the following relationships at even higher concentrations of hydroxide ion:

$$k_{obs} = a + b[OH^-] + c[OH^-]^2 \qquad \text{(Eq. 12)}$$

The empirical parameters a, b, and c were evaluated using the method of least squares. A trianionic tetrahedral intermediate was proposed to account for the second power of the hydroxide ion in Eq. 12.

Hydrolysis—One common pathway by which drugs degrade is hydrolysis; the two reactions already discussed exemplify this route. Several other examples of drug hydrolysis are included in Table I. Also included in this table are drugs containing other functional groups that can undergo various elimination or addition reactions in an aqueous medium frequently classified as hydrolysis, although the elements of water are not necessarily involved. This list was drawn primarily from the literature of the 1970's; the references listed earlier (1–19) give numerous other examples.

Oxidation—After hydrolysis, the next most common pathway for drug breakdown is oxidation. Many major drugs, such as narcotics, vitamins, antibiotics, and steroids, are prone to undergo this reaction, but there is a dearth of detailed studies on oxidation reactions.

The most common form of oxidative decomposition occurring in pharmaceuticals is autoxidation through a free radical chain process. The free radicals are produced by homolytic bond fission of a covalent bond: $A{:}B \rightarrow A\cdot + B\cdot$. The radicals readily remove electrons from other molecules, and this process is oxidation. The autoxidation of the free radical chain process can be described by the reactions in Scheme III.

$$RH \xrightarrow[\text{catalysis}]{\text{heat, light}} R\cdot + H\cdot$$

$$R\cdot + O_2 \rightarrow RO_2\cdot$$

$$RO_2\cdot + RH \rightarrow ROOH + R\cdot$$

$$ROOH \rightarrow RO\cdot + \cdot OH$$

$$RO_2\cdot + X \rightarrow \text{products}$$

$$RO_2\cdot + RO_2\cdot \rightarrow \text{products}$$

Scheme III

The heavy metals (copper, iron, cobalt, and nickel) catalyze oxidation by shortening the induction period and also affect the oxidation rate by promoting free radical formation.

Oxidations in solution are also subject to specific acid–base catalysis and generally follow first- or second-order kinetics. For example, the oxidative degradation of prednisolone is base catalyzed and exhibits first-order dependency (82). Other solvents may have a catalytic effect on reactions when used alone or in combination with water.

Table I—Hydrolytic Reactions

Compound	Reaction	Reference
Salicylamide	Amide hydrolysis	51
N-Haloacetylphthalimides	Substituted amide hydrolysis	52
1-Acyl-3,5-dimethylpyrazoles	Substituted amide hydrolysis	53
N-Acylphthalimides	Imide hydrolysis	52
Meperidine	Ester hydrolysis	24
Pyridoxine monooctanoate	Ester hydrolysis	54, 55
Trantelinium bromide	Ester hydrolysis	56
Salicylanilide *N*-methylcarbamate	Carbamate hydrolysis	57
4-Biphenyl-*N*-methylcarbamate	Carbamate hydrolysis	57
17α-Acetoxy-6α-methyl-4-pregnen-3,20-dione 3-oximino ester	Hydrolysis of oximino ester	58
Penicillins	Hydrolysis of β-lactam	59–61
Cephalosporins	Hydrolysis of β-lactam Intramolecular aminolysis	62, 63
Clindamycin	Dethiomethylation	64
5-Aminodibenzo[*a*,*d*]cycloheptane derivatives	Deamination	65
Cytarabine (arabinosylcytosine)	Deamination	66
Cytosine	Deamination	67
Cytidine	Deamination	67
5-Azacytidine	Deamination Scission of N–C bond	68
Chlordiazepoxide	Deamination Scission of C=N linkage	69
N-Chlorosuccinimide	Dechlorination	70
N-Chloroquinuclidinium ion	Dechlorination	70
N-Chloro-*N*-methylbenzenesulfonamide	Dechlorination	70
N-Chlorinated piperidines	Dechlorination	71
Iodocytosine	Deiodination Deamination	72
Δ^9-Tetrahydrocannabinol	Hydration and ether solvolysis	73
Antimycin A_1	Hydrolytic ring cleavage Loss of CHO group	74
Dexoxadrol	Hydrolysis of ketal group	75
Hydrochlorothiazide	Ring opening through hydration of free or cationic imine	76, 77
Mazindol	Scission of C=N linkage	78
Methaqualone	Ring cleavage	79
Coumarinic acid	Lactonization	80
Canrenone	Scission of C–S bond Lactonization	81

Ketones, aldehydes, and ethers may also influence free radical reactions, either directly or through trace impurities such as peroxides.

Many drugs are complex molecules and contain multiple functional groups subject to both hydrolysis and oxidation, *e.g.*, ascorbic acid, penicillins, and phenylbutazone. The studies conducted on the latter are summarized here.

The rates and degradation mechanisms of phenylbutazone were studied extensively (83–89). Phenylbutazone can undergo both hydrolysis and oxidation; the initial hydrolytic or oxidative products can be decarboxylated and/or further hydrolyzed or oxidized. On the basis of a detailed study, it was concluded that the equilibrium between phenylbutazone and the carboxylic acid resulting from hydrolysis of the pyrazolidine ring was dependent on solvent but practically independent of pH (86). Slingsby and Zuck (87) noted that oxidation at the C-4 position to produce 4-hydroxyphenylbutazone was the major decomposition route in the solvents they investigated. Awang *et al.* (89) proposed the hydroperoxide at C-4 as an intermediate en route to its formation. They also proposed a mechanism for formation of several other compounds on hydrolysis, decarboxylation, and oxidation of 4-hydroxyphenylbutazone.

Table II—Oxidation

Compound	Site of Oxidation	Reference
Vitamin A esters	Aliphatic chain	90
Amitriptyline hydrochloride	Dimethylamino side chain	91
Hydrocortisone	Dihydroxyacetone side chain	92
Dipyrone	Methanesulfonate	93
Dopa	Phenolic groups	94
Methyldopa	Phenolic groups	95
Ascorbic acid	Hydroxyl groups	96, 97
Methylprednisolone	Hydroxyl at C-21	98
Phenothiazine	5-S in the ring	99
Chloramphenicol	Combination of hydrolysis and oxidation	100

Recent references on oxidation of several drugs are included in Table II.

Miscellaneous Reactions—In addition to hydrolysis and oxidation, many other degradative reactions of drugs have been studied, including addition, elimination, isomerization and epimerization, polymerization, acylation, transesterification, and photolysis. (Most often, light catalysis provides energy to initiate an oxidation reaction.) Some examples of catalysis are included in Table III. Examples of miscellaneous reactions are summarized in Table IV.

DOSAGE FORMS

Preformulation—The excipients employed in pharmaceutical formulations are quite complex and are sometimes even heterogeneous mixtures. To determine the effect of these substances on the drug, it is necessary to conduct semiempirical studies with these excipients as an interface between basic physicochemical evaluation of the substance and final formulation. Preformulation studies are conducted to ascertain the compatibilities of the drug substance with excipients, including biological and chemical preservatives that may be necessary for a given formulation.

Since the selection of a type of dosage form is determined primarily by the preferred route(s) of drug administration, the development pharmacist must provide a relatively stable formulation within these constraints. Consideration also must be given at this time to potential packages for the drug product and their possible effects on stability.

Table III—Catalysis

Compound	Catalyst	Reference
Epinephrine	Sodium metabisulfite Sodium bisulfite Acetone bisulfite	101
Penicillins	Copper(II)–glycine chelate Copper(II)	102, 103
Penicillin G potassium	Monohydrogen and dihydrogen citrate ions	104
Cyclic anhydrides	Perchloric acid	105
Nalidixate sodium	Light	106
9-Aminomethylacridan	Light	107
Phenothiazine	Light	108
Dihydroergotamine mesylate	Light	109
Antipyrine (phenazone)	Light	110
Aminopyrine (aminophenazone)	Light	110
Dipyrone (noramidopyrine methanesulfonate)	Light	110
α-[(Dibutylamino)methyl]-6,8-dichloro-2-(3′,4′-dichlorophenyl)-4-quinolinemethanol	Light	111

Table IV—Miscellaneous Reactions

Compound	Type of Reaction	Reactant	Reference
Morphine	Addition	Sodium bisulfite	112
Dexamethasone phosphate	Addition	Sodium bisulfite	113
Fluorouracil	Addition	Sodium bisulfite	114
Benzylideneanilines	Addition	Diethyl phosphonate	115
Homatropine	Acylation	Aspirin	116
Morphine	Acylation	Aspirin	117
Aspirin	Transesterification	Polyethylene glycol	118, 119
Thiamine	Transamination	Aromatic amines	120
Epitetracycline	Dehydration	Acids	121
Tetracycline	Dehydration	Acids	122
Penicillin	Isomerization	Acids	123, 124
Lincomycin monoesters	Isomerization	Alkalis	125, 126
Tetracyclines	Epimerization	Alkalis	127
Pilocarpine	Epimerization	Alkalis	128
Prostaglandin E_1 and dinoprostone (E_2)	Epimerization	Acids	129
Ampicillin sodium	Dimerization	Self-aminolysis	130
Ampicillin–hetacillin	Interconversion	Acetone	131, 132
Acetaminophen	Complexation	Antipyrine	133
Heterocyclic compounds	Chelation	Copper, aluminum, and iron	134

Preformulation studies are conducted not only to determine the physical and chemical compatibility of the drug substance with other drug substances and several possible excipients, both individually and in combination, but also to forecast the effects of formulation on drug availability.

Akers (135) described the methodology, management, and evaluation of a systematic preformulation program for solid oral dosage forms. He indicated that the development of a stable and effective drug dosage form is determined by the type, quality, and organization of preformulation studies. The interactions among active components and additives, polymorphs, and micelle-forming agents were reviewed (136). Datt (137) also discussed changes that can occur in pharmaceutical preparations and the ways in which they can be circumvented.

The methods used to determine deterioration of drugs, with special attention to evaluating the stability of individual components, were reviewed (138). An approach to the determination of stability in solid pharmaceutical systems during preformulation studies was proposed (139), as were various other approaches for stability evaluations during preformulation studies (140–143). Results of such studies have been applied to proper formulation selection and should be used to design the appropriate subsequent studies for the selected formulation.

Solutions—Unlike heterogeneous systems such as solids and semisolids, the stability of drugs in a homogeneous solution can be predicted with a great degree of accuracy, and the data obtained through basic kinetic studies can often be applied directly to the formulation. However, "extrachemical" or additional reactions can occur to the drug formulated in solution, and they may be overlooked or not considered during the basic studies.

Effect of Additives—The effect of excipients and pharmaceutical aids on stability can be significant. The pH of solutions containing lidocaine hydrochloride changes

in the presence of 5% dextrose in saline solution, normal saline, and lactated Ringer solution (143). Sodium bisulfite can cause precipitation of imipramine hydrochloride. Other examples of the effects of various excipients and pharmaceutical aids on stability of pharmaceuticals in solution are summarized in Table V.

Many parenteral drugs are lyophilized or dry filled into ampuls since they have limited stability in aqueous solution. Therefore, on reconstitution with sterile water or other commonly used diluents or when added to intravenous fluids, degradation often occurs. Detailed stability studies on reconstituted preparations must be undertaken to evaluate the effect of time and storage conditions with commonly used vehicles.

Effect of Container—Branchi and Mecarelli (155) discussed the "chemical inertness" of glass containers from the standpoint of composition of various glasses, leaching of substances from glass by water and other solutions, and the mechanism of such reactions. Other investigators reported on the effect of water at high temperature on borosilicate, soda-lime-treated, and untreated pharmaceutical glass containers (156). For dispensing low pH liquids, surface-treated "parasolvex" flasks were found to provide a marked advantage over flasks of normal glass (157).

Glass was found to be a better container than polyethylene for storage of "cherry laurel" distilled water (158). Stability studies on normal saline solution stored in various glass containers revealed that the materials in certain glass types and stoppers caused a significant pH increase on storage due to material release from the stopper upon autoclaving (159). Sterilization also increased the pH of 5% ephedrine hydrochloride solution; this increase was caused by material leached from the ampul glass (160).

Materials used in production equipment, such as copper and brass, were found to accelerate the decomposition of propazine solutions (161). Interaction of phenylephrine hydrochloride with low density polyethylene containers was reported (162). This interaction was quite significant at room temperature, and the data indicated an apparent binding of phenylephrine hydrochloride to the low density polyethylene bottles. The extent of drug sorption by the plastic materials can be determined by standard methods (163).

Effect of Environment—Discoloration of dosage forms is frequently due to exposure to light and/or oxygen. Light- and oxygen-sensitive promethazine hydrochloride (164) was used to evaluate the effectiveness of plastic (polyethylene) containers compared to those made of glass. Colored plastic or brown glass gave better protection from light than white or clear containers. When light was excluded, a more rapid decrease of drug was observed in plastic than in glass because of oxygen permeation. By using oxygen permeation velocity constants, it could be shown that oxygen saturation was attained after 24 hr.

The use of light-resistant containers was recommended to alleviate the light instability problem for metaproterenol (orciprenaline) sulfate (165), reserpine (166), phenylbutazone sodium (167), and dexamethasone (168). Reserpine degradation can be minimized by using nitrogen atmosphere, an antioxidant such as thiourea, and a chelating agent. Incorporation of a UV absorber in the reserpine solution can also materially reduce degradation (166). The stability of phenylbutazone solution can be enhanced by the use of an antioxidant, sodium metabisulfite, and a chelating agent, diethylenetriaminepentaacetic acid (167).

Table V—Effect of Pharmaceutical Aids on Stability of Active Ingredient

Compound	Other Ingredients	Remarks	Reference
Cholecalciferol (vitamin D_3)	2% Polyoxyethylene ester[a]	Polysorbate 80 and pH mainly responsible for observed decomposition	144
	Surfactant[b]		
	Polysorbate 80[c]		
Pyridoxal 5-phosphate	Thiamine	Increased decomposition rate at pH 6	145
	Thiamine diphosphate	Increased decomposition rate at pH 6	
	Riboflavin phosphate	Increased decomposition rate at pH 6	
	Adenosylcobalamine	Increased decomposition rate at pH 6	
	Pyridoxal	Increased decomposition rate at pH 6	
	Pyridoxine	Increased decomposition rate at pH 6	
Kanamycin	Honey	Loss of activity at room temperature	146
	Sugar syrup	Loss of activity at room temperature	
Bacillin-3	Honey	Loss of activity at room temperature	146
	Sugar syrup	Loss of activity at room temperature	
Cephapirin sodium	Mannitol	Less stable with this component	147
Tetracycline	Calcium	Complexation	147
	Magnesium	Complexation	
Tetracyclines	Urea	Decreased epimerization	148
	Thiourea	Decreased epimerization	
	Polysorbate 20	Decreased epimerization	
	Polyethylene glycol 6000	Decreased epimerization	
Thimerosal	Bromide	Form difficultly soluble halides of cationic mercury compounds	149
	Chloride		
	Iodide		
Menadione	Sodium metabisulfite	Lower pH due to hydrolysis of sodium metabisulfite followed by oxidation of resulting sodium bisulfite	150
Apomorphine hydrochloride	Penicillamine at pH 3.6–4.0	Stabilization	151
Epinephrine (adrenaline)	Boric acid, povidone, erythorbic acid	Stabilization	152
Epinephrine	Sodium hydrogen sulfite	Stabilization	153
Tryptophan	Sodium pyrosulfite, oxygen	Discoloration, precipitation	154

[a] Cremophor EL, derivative of ricinoleic acid. [b] Dupasol X. [c] Tween.

Semisolids—In this category are included all dosage forms that are not true solutions or dry oral solids. Many drugs that would undergo significant degradation, if marketed as a solution, can be stabilized by formulating the active ingredient into a suspension or emulsion. Gels, ointments, suppositories, creams, and lotions are typical semisolid preparations.

In addition to chemical inactivation of the therapeutic agent, these items are subject to a wide variety of physical and chemical changes: separation, sedimentation, creaming, and cracking. Since many excipients are natural

products—fats, oils, waxes, flavoring agents, and perfumes—they are quite subject to oxidation (rancidity) and microbiological contamination.

Effect of Additives—The effect of additives on the kinetics of interconversion of succinylsulfathiazole crystal forms was investigated (169). These investigations showed that a physically stable aqueous pharmaceutical suspension may be achieved by including a suitable transformation retardant. Under these conditions, the suspension keeps its uniformity and ease of resuspension for the expected shelflife of the preparation.

The autoxidation of the oil phase of an oil-in-water emulsion during storage in light was dependent on the emulsifier used (170). The observed differences in the extent of autoxidation might have resulted from different solubilities of oxygen in various emulsifier solutions.

The rheological and penetrometric characteristics of seven vitamin A-containing ointments changed after 14 months of storage. The decrease in vitamin content was a function of the ointment base; the presence of polyethylene glycol and sodium lauryl sulfate in the base enhanced degradation (171). Vitamin A was also unstable in hydrocarbon gels and lipogels (172). Incorporation of an antioxidant, such as α-tocopherol acetate, improved stability.

The effect of different ointment bases on the stability of oxacillin sodium was reported recently (173). In zinc oxide paste, anthralin was converted rapidly into a therapeutically inactive compound (174). Added salicylic acid improved the stability by deactivation of the surface zinc oxide through formation of zinc salicylate.

Four decomposition products were isolated from aminophylline suppositories. Three were identified as amides resulting from reaction of ethylenediamine with constituents of the suppository bases (175). Drofenine (hexahydroadiphenine) hydrochloride in suppositories decomposes to yield the free base and its *N*-oxide. The mechanism of decomposition was independent of the type of suppository base (176).

Effect of Physicochemical Factors—Practical stability considerations of emulsions and suspensions may show an inverse temperature relationship; instead of increased stability under colder or refrigerated conditions, one may encounter irreversible phase changes. Coagulation of particles was observed during freezing–thawing of suspensions. The primary factor involved in the coagulation was the small size of suspended particles (177).

A technique for evaluating the stability of emulsion bases and active components contained within such emulsions was described recently (178). The method, diffuse reflectance spectroscopy, has the capability of detecting changes in particle size or surface properties of emulsions as functions of time without disturbing the system.

The decrease in the acid-consuming capacity of aluminum hydroxide gel during aging, as measured by the USP test, has been found to be due to a decrease in the reaction rate rather than a decrease in equilibrium reactivity. The reactivity profile has three phases related to the structure of the gel (179). Gels containing sorbitol lost less than 10% of their acid-consuming capacity during a 6-month aging period compared with a loss of more than 60% for an identical gel without sorbitol. The mechanism by which sorbitol stabilizes the gel appears to be through inhibition of the secondary polymerization reaction which takes place on aging (180).

The photostability of compounds can be improved by suitable selection of the dosage form. For example, the micellar forms of chlorpromazine, triflupromazine, and homofenazine show greater stability. It has been postulated that, in the colloidal state, these compounds receive better protection in a lyophilic environment than in a hydrophilic environment (181). Ong and Kostenbauder (182) studied the effect of micellar sodium lauryl sulfate on the cupric-ion-promoted hydrolysis of some dicarboxylic acid hemiesters to evaluate the potential of such association of colloids for increasing product lability to metal-ion-promoted hydrolysis. The rate of cupric-ion promoted hydrolysis of sodium *n*-decyl oxalate in the micellar phase is about 50 times as fast as that in bulk solution. However, the hydrolysis rates of hydrocortisone sodium 21-hemisuccinate and hydrocortisone sodium 21-hemi-(3,3-dimethylglutarate) were unaffected by the copper ion.

Solids—Although solid oral dosage forms constitute a large majority of pharmaceutical products, few detailed kinetic studies and studies on rates and mechanisms of drug degradation in the solid state have been published. Most fundamental works on matter in the solid state are on inorganic materials or are from fields other than the pharmaceutical industry. Heterogeneous systems encountered in pharmaceutical dosage forms are often difficult to study and are not as reproducible as a homogeneous solution.

In recent years, Carstensen (1) and coworkers have added a great deal to our understanding in this area. Drug stability in the presence of excipients can be significantly different from that of the neat active ingredient. In the absence of excipients and moisture, topochemical and nucleation-governed reactions occur; some of these approximate first-order reaction rates. The nucleation reactions give rise to sigmoid curves, which are not simple first- or zero-order reactions. In the presence of moisture, the decomposition kinetics should be dictated by the rates in saturated solution and should be zero order.

Carstensen and Pothisiri (183) recently showed that first-order decomposition patterns may be possible in the absence of moisture or when moisture content is low. This effect was demonstrated with *p*-aminosalicylic acid as a model system to approximate the situation encountered in dosage forms. Other recent publications of interest on solid-state decomposition are on *para*-substituted salicylic acids (184), aspirin (185), and digoxin (186).

Although there are not many detailed physical chemistry studies, numerous publications of an empirical or practical nature described incompatibilities or instabilities or other changes in the solid state (187–198). As with liquids and semisolids, numerous "extrachemical" parameters change as a function of time in tablets and capsules. Some of these are discussed here.

Effect of Additives—The discoloration of tablets containing a variety of pharmaceutical compounds, such as 8-hydroxyquinoline sulfate, aminopyrine (amidophenazone), papaverine, theobromine, and salicylamide, was reduced markedly by using carboxymethylcellulose sodium solution during granulation. Carboxymethylcellulose sodium was found to act as a scavenger for trace metals,

which are a causative factor frequently encountered in the discoloration of pharmaceutical tablets (199).

The moisture sorption and volume expansion of anhydrous α- and β-lactose tablets were examined under various relative humidity conditions (200). The moisture adsorption and tablet expansion occurred more readily with α-lactose tablets, leading to the formation of the monohydrate. Lactose was also found to induce the discoloration of several drugs in solid dosage forms (201).

The adsorption of some antirheumatics on antacids was investigated (202). Elution studies showed that salicylates and anthranilic acid derivatives were tenaciously held by magnesium oxide, while magnesium trisilicate showed intermediate retention power for antipyrine and aminopyrine. A marked reduction in the apparent partition coefficient was observed for all drugs tested in the presence of magnesium trisilicate or aluminum hydroxide.

Effect of Container—The intertablet migration of nitroglycerin could be related to the container used for storage. In certain containers, intertablet transfer among a set of nominally equivalent tablets occurred after several months and resulted in decreased content uniformity. The mechanism of intertablet migration involved capillary condensation (203).

Effect of Environment—The fading of colored tablets by light (high-pressure mercury vapor lamp) was tested (204). The intensity of UV rays contributed mainly to the fading, but visible light also was responsible. Eosin and light green SF had poor stability; tartrazine was very stable.

The influence of a protective coating of sunscreening agents on the photostability of FD&C Blue No. 1 and erythrosine sodium (FD&C Red No. 3) was investigated. The tablets were exposed to 1000 footcandles of light, and approximate shelflives of various colored tablets were calculated (205, 206).

The discoloration of reserpine (0.1-mg)–dihydralazine sulfate (10-mg) tablets on prolonged exposure to air was reported (207). Reserpine was responsible for the discoloration of these tablets, and it was recommended that discolored material should not be used clinically.

Alam and Parrott (208) found a close correspondence between the changes occurring in the dissolution rate of hydrochlorothiazide tablets at elevated temperatures and those occurring after prolonged storage at room temperature. The retardation of the dissolution rate also was reported for sodium salicylate tablets (209), and a possible effect with acetaminophen tablets was noted (210). A study on phenylbutazone tablets BP showed a progressive decrease in dissolution with age (211). This effect could be simulated in short periods at elevated temperatures.

STABILITY-INDICATING METHODS

A review article on stability-indicating methods for drugs and their dosage forms (212) concerned itself primarily with a functional group approach to stability evaluation. The functional group analyses discussed were those most commonly finished with a titrimetric or spectrophotometric determination. Siggia (213, 214) wrote two comprehensive texts on analysis *via* functional groups and demonstrated that functional group analysis can be finished with various instrumental methods.

The current trend in stability-indicating methods is based on direct chromatography or derivatization chromatography. These approaches are used extensively in stability evaluation of pharmaceutical products.

Mollica and Lin (215) discussed problems facing an analytical chemist while developing an analytical method that will quantitatively determine the intact drug molecule in the formulation. When it is not possible to determine the intact drug directly because of interfering substances, it is desirable to precede the analytical finish with a separation procedure. This step can be solvent extraction or chromatographic separation.

The USP and NF (216, 217) provide yet another approach to evaluating stability. It entails monitoring the content of a decomposition product, *e.g.*, salicylic acid in aspirin and disulfonamide in hydrochlorothiazide, while utilizing an assay for the drug itself that may not be totally stability indicating. In some ways, this approach provides a more rigorous control of product stability. In the examples cited, the presence of 1–4% of a decomposition product will make the item unsuitable; thus, much tighter limits are being applied to these products as compared to those that must meet the rubric limits for potency. These examples also illustrate that different standards are used to define product stability. Although at times it is necessary to use more rigorous controls, it would be desirable to develop more uniform criteria for stability evaluation.

To select an appropriate method, the analyst should have a thorough knowledge of the physicochemical properties of a drug, degradation products, degradation mechanisms, and degradation reaction rates. (See discussion under *Rates, Mechanisms, and Pathways of Degradation.*) One can then develop a specific method suitable for monitoring the stability of an active ingredient or formulation. The methodology used for kinetic studies (solid state or solution) can generally be considered scientifically suitable for monitoring stability of pharmaceutical formulations if similar modes of decomposition are encountered. However, the method may not satisfy regulatory or compendial needs.

The effect of drug–excipient interactions on analytical methodology cannot be ignored (218). Frequently, these interactions not only lead to low assay values but also affect drug availability to the patient.

A recent review dealt with drug decomposition and analytical methods for the determination of decomposition products (219). Other recent reviews covered the stability of ascorbic acid (vitamin C) tablets (220), shelflife studies on some oral liquid vitamin formulations (221), analysis of polyene antifungal antibiotics (222), study of free salicylic acid and acetylsalicylic anhydride in aspirin-containing drug specialties (223), analytical methods for prostaglandins (224), and stability of stabilized nitroglycerin tablets in typical distribution and administration systems (225).

For the purpose of this article, stability-indicating methods are classified as electrometric methods, solvent extraction methods, spectrophotometric methods, and chromatographic methods.

Electrometric Methods—Titrimetric methods (aqueous or nonaqueous) that can be used for the precise analysis of the active ingredient most often do not offer the desired specificity for the analysis of pharmaceutical

products. However, if the decomposition products do not interfere with the titration, *e.g.*, formation of nonbasic degradation products of an organic amine or amine hydrochloride, then one may be able to utilize titrimetry. Alternatively, by employing suitable extraction procedures for eliminating possible interferences from excipients and/or decomposition products, one can use titrimetry for monitoring the stability of products.

Organic polarography has been used for the analysis of pharmaceutical products because it offers the desired specificity, but its use has been limited by several technical disadvantages (226). The advantage of polarography for the determination of ethacrynic acid in the presence of its principal degradation product, a dimer, was demonstrated (227). Polarography was also found useful for studying acid and base hydrolyses and β-lactamase degradation of several cephalosporins (228). The compounds were polarographically reduced in the acidic medium. A wave believed to be due to two-electron reductive elimination of the C-3 position substituent was found suitable for stability evaluation.

Solvent Extraction Methods—It is possible to extract acidic, neutral, or basic compounds selectively into organic solvents on the basis of the partition behavior of their ionized and unionized species. The compendia (216, 217) utilize a double-extraction procedure as the preferred method of analysis for organic nitrogenous bases. This approach provides some degree of specificity, because it is possible to remove compounds that are neutral or acidic or have more polar substituents that could arise upon degradation. It does not, however, eliminate isomers or other closely related basic substances. Therefore, the validity of this approach for monitoring stability should be demonstrated prior to its utilization.

Spectrophotometric Methods—Direct spectrophotometric determination is widely used in pharmaceutical analysis but generally lacks selectivity. The selectivity or specificity can be improved through separation or by reaction of an appropriate functional group. For example, the reactions that produce a colored product are generally measured in the visible region of the spectrum. Other reactions increase conjugation to permit measurements in the UV region.

Due to its limited sensitivity, IR analysis is primarily used for identification of decomposition products and has found very few quantitative applications in stability evaluation. NMR spectroscopy is finding an increasing number of applications since it offers specificity along with simplicity of operation. But it, too, lacks sensitivity and precision.

Colorimetric Analysis—Carboxylic acid derivatives (anhydrides, halides, lactams, lactones, amides, and esters) are converted to the corresponding hydroxamic acid by reacting with hydroxylamine hydrochloride in an alkaline medium. The hydroxamic acid is then allowed to react with ferric chloride in the presence of dilute acid to produce red-violet ferric hydroxamate (229).

Soloway and Lipschitz (230) reported observations on amides. The hydroxylaminolysis of amides and the formation of colored complexes of the hydroxamic acids so derived with ferric ion afforded a convenient means of determining amides in the presence of their amino compounds and acid constituents. These reactions were used for stability-indicating colorimetric analyses of N^1-acetylsulfanilamide and N^1-acetylsulfisoxazole since their hydrolysis products did not interfere in the analysis (231, 232). A hydroxylamine colorimetric method has been included in the *Code of Federal Regulations* for the analysis of various cephalosporins (233). This method is stability indicating for decomposition of the β-lactam ring.

Le Pedriel *et al.* (234) reported that acetaminophen and nitrous acid react under mild conditions to form 2-nitro-4-acetamidophenol, which can be analyzed by its color in alkaline solution. Furthermore, they found no interference from acetanilide or phenacetin, a hydrolysis product. Inamdar and Kaji (235) used this reaction for dosage form assay but utilized the yellow color of the nitroso derivative in acid solution for measurement instead of the orange-red color of the phenolate ion. A suitable modification of the colorimetric method was utilized for acetaminophen analysis of conventional and sustained-release tablet formulations containing phenacetin, phenylpropanolamine hydrochloride, and phenyltoloxamine dihydrogen citrate (236).

The reaction between homatropine methylbromide and Dragendorff reagent was stabilized so this colorimetric reaction could be employed for stability evaluation of pharmaceutical formulations (237, 238). The method was specific for the parent compound in the presence of its major decomposition product, tropine methylbromide.

A specific method for the determination of ascorbic acid in the presence of dehydroascorbic acid and 2,3-dioxo-*l*-gluconic acid was reported (239). The method is based on the colorimetric reaction of phenylhydrazinium chloride with ascorbic acid in an acidic medium. The reaction of isoproterenol (isoprenaline) with thiosemicarbazide in alkaline medium was utilized as a specific colorimetric reaction for the analysis of pharmaceutical formulations (240). The picric acid reaction was useful for the determination of testosterone in oily injections (241).

UV Analysis—The spectrophotometric determination of ephedrine and other phenylalkanolamine drugs as benzaldehydes after periodate oxidation was specific for compounds with the general structure Ar–CHOHCH(NHR_1)–R_2 (242). Aspirin stability in methoxypolyethylene glycol, polyethylene glycol acetate, or a mixture of polyethylene glycols was monitored by simultaneous UV spectrophotometric determinations (243). The decomposition was primarily transesterification with the vehicle. Isoniazid (isonicotinic acid hydrazide) could be determined spectrophotometrically by formation of the hydroxamic acid in the presence of nickel(II) (244).

A procedure for the measurement of small amounts of ampicillin in hetacillin was reported; the former molecule yields a UV-absorbing compound with nickel(II) in dimethyl sulfoxide (245). Cephalosporin was analyzed by differential UV spectrophotometry after employing β-lactamase for hydrolysis of the β-lactam ring (246).

Mixtures of tetracycline and 4-epitetracycline were assayed utilizing the large difference in their circular dichroism spectra in the UV region (247). The proposed method was specific and required no prior separation of degradation products. The assay was not useful, however, for the determination of anhydrotetracycline; the latter, even at levels of 10%, affects the assay for tetracycline by only 1%.

Fluorometric Analysis—A stability-indicating spectrophotofluorometric method for epinephrine was reported by Prasad *et al.* (248). In this procedure, the drug was oxidized *via* iodine to "adrenochrome" and then cyclized with alkali to "adrenolutin," which is responsible for the fluorescence. The main decomposition product of epinephrine from simulated formulations containing sodium bisulfite, epinephrinesulfonic acid, was found not to interfere in the assay. A similar method was also described for the analysis of partially decomposed isoproterenol solutions (249).

NMR Analysis—Rackham (250) recently reviewed the applications of quantitative NMR spectroscopy in pharmaceutical research. Several other recent publications emphasized the importance of this technique in stability evaluations. A stability assay for amyl nitrite ampuls was proposed by Schirmer *et al.* (251). The percentage of amyl nitrite in an amyl nitrite ampul is determined from the ratio of the area under the CH_2ONO band to the area under the CH_3 band in the NMR spectrum of the sample. Decomposition of amyl nitrite in ampuls produces N_2O, NO, CO, CO_2, and at least 12 liquid components including water, amyl alcohol, isovaleric acid, valeraldehyde, amyl isovalerate, and amyl nitrate.

Recently, Turczan and Medwick (252) observed that this method yielded relative stability information because the α-methylene group is affected by instability whereas the methyl group is not. They recommended a choice of solvent and internal standards to provide absolute results.

NMR spectroscopy also was found useful for the stability evaluation of cocaine hydrochloride in aqueous solution (253).

Chromatographic Methods—A large number of stability-indicating methods entail some form of chromatography: paper, thin-layer (TLC), column, gas (GLC), and liquid (HPLC). The latter two techniques not only offer separation but provide precise methods of quantitation. Recent reviews (254–264) summarized advances in this area, and several examples of these techniques have been included in this review.

Less than a decade ago, paper chromatography was used extensively in pharmaceutical analysis. This technique has rapidly given way to TLC, GLC, and HPLC. Of these three techniques, HPLC is finding the most widespread application today. Instrumentation for HPLC became commercially available approximately 7 years ago, and the rapid growth of this technique is apparent from the literature; it is already included in several USP XIX monographs.

With this technique, a compound can be chromatographed in several ways and, importantly, the volatility that is required for GLC is not a limitation. The problems due to thermal instability are not encountered because most separations are carried out at ambient or low temperatures. Of various chromatographic techniques employed in stability evaluations, GLC and HPLC provide the most useful quantitative information.

Paper Chromatography—An excellent review on applications of paper chromatography was published recently (265). The following discussion is limited to a few recent examples. A paper chromatographic method was found useful for monitoring the stability of tetracycline and its hydrochloride salt (266). The method is based on the complexation of the antibiotic with a mixture of urea and edetate disodium on chromatographic paper at pH 7.4.

Both paper chromatography and TLC were used for stability evaluation of chlorpromazine (aminazine) solution (267). Several degradation products were observed with both techniques; however, paper chromatography showed better resolution.

TLC—The applications of quantitative TLC in pharmaceutical analysis were reviewed recently (268, 269). A few examples from recent literature are provided here to illustrate the usefulness of this technique. Quantitative TLC by direct fluorometry was rapid and specific for the determination of tetracycline hydrochloride and its degradation product or impurities (270). This method was used for stability investigation of liquid pharmaceutical preparations containing tetracycline.

Other applications include the determination of atropine sulfate in the presence of its hydrolytic degradation products (271) and stability studies on androgenic hormones (272).

Column Chromatography—This technique is being replaced by high-pressure liquid chromatography (HPLC). A few recently published methods for stability evaluation (273–276) utilized separation on ion-exchange columns. A partition column chromatographic procedure was found useful for the determination of tetracyclines (277).

GLC—Derivatization in GLC for pharmaceutical analysis was reviewed recently (260). Derivatization provides an additional approach to selectivity if the appropriate derivatization technique is selected. A few recent examples are described here.

A rapid GLC method was proposed for the simultaneous analysis of salicylic acid in aspirin tablets and in codeine–propoxyphene-type capsules and tablets (278, 279). The procedure involved formation of the methyl ester of salicylic acid with diazomethane. Derivatization with diazomethane also was used for analysis of reserpine and rescinnamine (280).

The degradation of terbutaline under oxidative conditions was investigated by GLC of its trimethylsilyl derivative (281). Silylation was found useful for GLC of iodochlorhydroxyquin and related 8-hydroxyquinolines (282) and in analysis of scopolamine in the presence of its degradation products (283). Patel *et al.* (284) noted that silylation provided advantages for aspirin determination over previously published methods. Silylation also was found useful for analysis of levodopa (285), cycloserine (286), and phenylephrine (287).

Direct GLC of several basic drugs was reported in 1974 (288). GLC was found suitable for stability determination of new potential drugs such as *dl*-3-(*p*-trifluoromethylphenoxy)-*N*-methyl-3-phenylpropylamine and *dl*-3-(*o*-methoxyphenoxy)-*N*-methyl-3-phenylpropylamine (289), aprindine [*N*,*N*-diethyl-*N*′-(2-indanyl)-*N*′-phenyl-1,3-propanediamine] (290), and promethazine hydrochloride (291). This technique was also found useful for the determination of meprobamate and related carbamates (292), cyclophosphamide (293), carbamazepine (294), 9-acridine derivatives (295), and ephedrine and phenylalkylamines (296).

The combination of GLC–mass spectrometry provides a valuable tool for the determination of low levels of degradation products. An example of the low sensitivity (200–800 pg) and selectivity possible with this technique

Table VI—HPLC of Pharmaceuticals

Compound	Mode of HPLC	Reference
Prostaglandins A_2 and B_2	Anion exchange	301
Barbiturates	Anion exchange	302
Penicillin G potassium	Anion exchange	303
Ampicillin	Anion exchange	304
Tetracyclines	Cation exchange	305
Xanthines	Cation exchange	306
Trisulfapyrimidines	Cation exchange	307
Sulfa drugs	Cation exchange	308
Imidazolines	Cation exchange	309
Benzodiazepines	Adsorption	310
Riboflavin	Adsorption	311
Sulfacetamide sodium	Adsorption	312
Cholecalciferol	Adsorption	313
Canrenone	Adsorption	314
Carbamazepine	Adsorption	315
Phenytoin (diphenylhydantoin)	Adsorption	316
Phenobarbital	Adsorption	316
Aspirin, phenacetin, and caffeine	Adsorption	317
Corticosteroids	Partition	318
Hydroxysteroids (derivatized)	Partition (reversed phase)	319
Sulfasalazine (salicylazosulfapyridine)	Partition (reversed phase)	320
Procaine	Partition (reversed phase)	321
Tetracyclines	Partition (reversed phase)	322
Synthetic estrogens	Partition (reversed phase)	323
Phenol	Partition (reversed phase)	324
Ergotamine	Partition (reversed phase)	325
Nortriptyline	Partition (reversed phase)	326
Androsterone (derivatized)	Ion exchange and partition (reversed phase)	327
Dehydroepiandrosterone (derivatized)	Ion exchange and partition (reversed phase)	327
Vitamins (water soluble)	Anion and cation exchange	328
Analgesics (aspirin, caffeine, acetaminophen, and salicylamide)	Anion and cation exchange	329

is seen in the analysis of prostaglandin analogs (297).

HPLC—The theory of HPLC was reviewed recently (298). The importance of this technique to the pharmaceutical analyst can be measured by the large number of recent reviews on the applications in pharmaceutical analysis (259, 261–264, 299, 300). Several applications of recent interest are summarized in Table VI.

MARKETED PRODUCT STABILITY

In the section on *Dosage Forms*, factors influencing stability of common formulations—solutions, semisolids, and solids—were considered. However, a modern pharmaceutical product is not only the optimum formulation, it is also the optimum formulation/package combination. Formulations are designed to maintain or enhance the stability of the active ingredient or other component(s) subject to deterioration and to ensure the pharmaceutical elegance of the product. The design is based on physicochemical properties of the active substance(s) and its compatibility with excipients. This information can be derived from kinetic and preformulation studies. Information on the stability of specific compounds is available in the general pharmaceutical literature (2, 330, 331).

Many approaches are used to stabilize or protect formulations, including lyophilization; microencapsulation (332, 333); control of surface area (334); addition of chelating agents, preservatives, and antioxidants; physical separation of incompatible ingredients; coatings (335); and opaque coverings. The need and use of these devices are intrinsic to the dosage form under consideration.

Extrinsic to the stabilization of the dosage form is the stability of the dosage form–container combination. The container is an integral part of such products as topicals and parenterals. Wood (336) indicated that in addition to physical, chemical, bioavailability, and microbiological criteria, container interactions should be monitored in the evaluation of the stability of topicals. In many cases, it is not feasible for evaluation purposes to isolate the dosage form from the intended container. The container becomes an integral part of the drug product. Even though extensive studies have been conducted on the dosage form, additional studies in the container(s) of choice are necessary to obtain a total stability characterization of the product.

During the design of a product, it is essential that storage and end use be considered. "Use tests" may be indicated where the immediate container will be continually disturbed during use, *e.g.*, an elixir or syrup where, upon use, the headspace will increase or the surface to volume ratio will change.

In addition, consideration has to be given to possible requirements or restrictions on storage of the product–container combination to provide adequate assurance that product performance is satisfactory throughout the determined shelflife. Establishment of packages, storage legends, and shelflife are as (or more) important as the basic efforts taken to determine the stability of the active ingredient as presented in previous sections.

Manufacturer's Container: Selection—Esthetics, economics, stability, safety, law, production, and quality control requirements should be considered in the selection of the appropriate container for a pharmaceutical product (6). Stability cannot be separated from any one of the other factors. Poor product stability in a chosen container affects all of the stated factors; hence, it is of primary significance. For purposes of marketed product stability, the manufacturer's container is defined to be all package components in intimate contact with the product or that provide a degree of protection, *e.g.*, closure, seal, or overwrap.

The *Code of Federal Regulations*, Title 21, Section 314.1, requires that, for any New Drug Application, stability data for the dosage form be provided "in the container in which it is to be marketed." The significance of this requirement is highlighted by the problems experienced with nitroglycerin tablets that led to very explicit federal regulations (337) for the packaging and handling of that product. The enactment of this regulation followed the discovery that appreciable evaporation of nitroglycerin from tablets occurred when stored in plastic containers and certain strip packages. Much has been published on the stability of nitroglycerin tablets relative to the container in which they are stored or dispensed (225, 338–341).

The compendia (342, 343) have provided definitions for various types of containers based on their capability to provide protection:

"Light-resistant Container—A light-resistant container protects the contents from the effects of light by virtue of the specific properties of the material of which it is composed, including any coating applied to it. Alternatively, a clear and colorless or a translucent container may be made light-resistant by means of an opaque covering, in which case the label of the container bears a statement that the opaque covering is needed until the contents have been used. Where it is directed to 'pro-

tect from light' in an individual monograph, storage in a light-resistant container is intended.

Well-closed Container—A well-closed container protects the contents from extraneous solids and from loss of the drug under the ordinary or customary conditions of handling, shipment, storage, and distribution.

Tight Container—A tight container protects the contents from contamination by extraneous liquids, solids, or vapors, from loss of the drug, and from efflorescence, deliquescence, or evaporation under the ordinary or customary conditions of handling, shipment, storage, and distribution, and is capable of tight reclosure. Where a tight container is specified, it may be replaced by a hermetic container for a single dose of a drug.

Hermetic Container—A hermetic container is impervious to air or any other gas under the ordinary or customary conditions of handling, shipment, storage, and distribution."

A quantitative test to measure the permeation of a container closure system was included in USP XIX and NF XIV (344, 345). The limits established to define tight and well-closed containers became official on April 1, 1977 (346). These limits supplement the requirements and tests for light-resistant containers that have been in effect for several editions of the USP and NF.

The various properties one should consider in evaluating pharmaceutical containers were summarized by Krueger (347). Pharmaceutical packages are designed to provide not only a means of transport and brand identification but to serve more significant functions: to provide adequate protection and to ensure the stability of the product while in distribution and storage. A necessary prerequisite to the determination of the degree of protection afforded by a package must be the accumulation of information on the drug product itself. Products subject to hydrolysis or deleterious physical changes caused by moisture require containers that restrict moisture transmission. Light-sensitive dosage forms require barriers that screen out the harmful wavelengths. Formulations subject to oxidation that are single-dose sealed units require blanketing with an inert atmosphere prior to sealing, *e.g.*, nitrogen or carbon dioxide flushing.

When the types of protection that the product requires from the package are established, a basic understanding of the properties of package components, the container and closure, is needed for appropriate package selection. Many publications have dealt with the properties of packaging materials, and several conferences have covered the special requirements for containers needed by the pharmaceutical industry. One such conference, "Pharmaceutical Parameters in Container Selection," in 1969, was sponsored by the University of Wisconsin, Extension Services in Pharmacy.

A comprehensive summary of recent advances in packaging pharmaceuticals, including the relationship of the product and the package, packaging materials, and packaging technology, was prepared by Dean (348). Heubner (349), Ross (350), and Spingler (351) also provided extensive information on the relation of the package to the pharmaceutical product.

Information on the properties and applications of various types of package materials, glass (155–157, 352, 353), aluminum tubes (354), aerosols (355, 356), blisters (357), unit packaging (358, 359), elastomeric closures (360), and plastic (163, 361–369), is also available.

In recent years, polymeric materials have become widely used for pharmaceutical packages. Cooper (370) prepared a useful reference on plastic containers which defines the areas of potential problems, discusses the relationship of the containers to dosage forms, reviews the regulatory requirements around the world, and presents the existing standards for plastic containers.

Specialized test procedures have been developed to determine properties of plastic containers such as oxygen permeation (371). A procedure to determine sorption of drugs on low density polyethylene was reported (372). The permeability of packages made from various resins to gas, water vapor, radiation, bacterial penetration, and sorption phenomena was reviewed (373). The numerous publications dealing with plastic packaging components tend to indicate its utility and growing popularity in drug packaging.

The ultimate criterion for the suitability of a particular container is testing of the drug in the container under normal and stress conditions for extended periods. The scope and nature of this testing as it relates to the product were reviewed earlier. Special consideration must be given to interactions between the product and the container that might not be ascertained by the normal testing of the product. These interactions could include migration of one or more components (additives) of the package into the drug, absorption or adsorption of the drug into or onto the package, esthetic changes of the package or drug, actual physical deterioration of the package, and formation of a reaction product at the drug–container interface.

Package Stability: Dispensing and Repackaging—The USP (374) now includes an entire section entitled "Stability Considerations in Dispensing Practice." Included in this section are: an overall definition of stability; various aspects of stability including chemical, physical, microbiological, therapeutic, and toxicological; factors affecting stability including the nature of the container; the process by which manufacturers select the optimum formulation and container; and the responsibilities of the pharmacist with regard to stability. Furthermore, this section directs the pharmacist to dispense pharmaceutical products in the proper container and closure.

If repackaging is necessary, the following guidelines for the pharmacist have been provided by the USP:

"Repackaging—In general, repackaging is inadvisable. However, if repackaging is necessary, the manufacturer should be consulted concerning potential problems. In the filling of prescriptions, it is essential that suitable containers be used. Appropriate storage conditions and an appropriate expiration date should be indicated on the label of the prescription container. Single-unit packaging calls for care and judgment, and for strict observance of the following guidelines: (1) use moisture-proof packaging materials for solids; (2) where stability data on the new package are not available, repackage at any one time only sufficient stock for a limited time (e.g., 30 days); (3) include on the unit-dose label a lot number or the date of repackaging and an appropriate expiration date; (4) where a sterile product is repackaged from a multiple-dose vial into unit-dose (disposable) syringes, discard the latter if not used within 24 hours, unless data are available to support longer storage; (5) where quantities are repackaged in advance of immediate needs, maintain suitable repackaging records showing name of manufacturer, lot number, date, and designation of persons responsible for repackaging and for checking; (6) where safety closures are required, use container closure systems that ensure compliance with compendial and regulatory standards for storage."

At the time of publication of USP XIX, it was indicated that, upon repackaging, the pharmacist might become responsible for the stability of a product. The Second Interim Revision Announcement to USP XIX and NF XIV indicated that as of April 1, 1977, the monograph requirements for tight or well-closed containers for official products must be adhered to upon dispensing of a prescription. Hence, the responsibility for proper repackaging has been placed on the pharmacist.

As mentioned previously, a serious problem in the dispensing of pharmaceuticals concerns nitroglycerin tablets. The problems encountered in repackaging this product were discussed by Shangraw and Contractor (375). Specific regulations (376) issued by the Food and Drug Administration (FDA) restrict the dispensing of nitroglycerin preparations to the original, unopened container. This regulation was based on the stability of the nitroglycerin preparation relative to its container.

Studies have been conducted to determine the stability of pharmaceutical products under prescription (dispensing) conditions. Russell *et al.* (377) reported on the effects of storage on the glyceryl trinitrate content of nitroglycerin tablets in British dispensing containers. Adamski and Socha (378) showed that chloramphenicol decomposed when stored in frequently opened vessels.

Over the past few years, there has been growing concern about the stability of pharmaceuticals repackaged for hospital use. Several recent publications demonstrate the variety of investigations conducted on the stability of product–container combinations prepared for hospital use. These include investigations on amphotericin B in infusion bottles (379), syrups in single-unit polypropene and polythene cups (380), tobramycin sulfate in bottles and bags (381), and sodium bicarbonate injection in disposable polypropylene syringes (382).

Pharmaceutical stability is acknowledged as a complex area that is further complicated by the relationship of the product to the container and the effects on stability, both positive and negative, of the product–container combination. As already indicated, the USP recommends against repackaging pharmaceuticals and advises the pharmacist to take every precaution if repackaging is necessary. Generally, products repackaged at the dispensing level are stored for relatively short periods; but during that interval, the product can be exposed to harsh storage conditions in the hands of the patient (*e.g.*, the proverbial windowsill). The effects of storage in the prescription container are usually not well defined since the bulk of stability testing is done at the manufacturer's level in the manufacturer's container(s). Hence, repackaging of pharmaceuticals at the dispensing level becomes the weakest link in the protection of the product between the manufacturer and the patient.

Storage Conditions—One aspect of stability evaluation is the determination of effects of environmental conditions on the product. The factors most commonly tested are heat, humidity, light, and air. Temperature tends to accelerate all reactions according to rate theory (see *Rates, Mechanisms, and Pathways of Degradation*), and the other factors accelerate, catalyze, or mediate hydrolytic, photolytic, oxidative, *etc.*, reactions (see *Dosage Forms*).

These data also form the basis for establishment of an expiration date. Expiration dates, however, have real significance only when they can be related to specific storage conditions. That a label bears a 5-year expiration date does not ensure that the product will be suitable after storage for 5 years under any conditions. As previously described, packages can provide a degree of protection from some of the elements, but it is still advisable to relate the expiration date to specific storage conditions.

Levi and Benney (383) pointed out that storage of pharmaceutical products can be defined and controlled by the manufacturer through distribution to the wholesale and pharmacy level. Thereafter, the only means of control is the expiration date and storage legend appearing on the product label. These investigators (383) provided a summary of temperatures experienced in some U.S. cities to define the average climate and to relate storage of laboratory samples to actual field conditions.

Haynes (384) proposed that a "virtual temperature" be determined for laboratory storage of stability samples which relates to actual market conditions. This temperature is determined from the Arrhenius relationship and rate constant for degradation at various temperatures. He provided the virtual temperature for several cities in the United States and abroad. Other publications (385–387) discussed actual market conditions for pharmaceuticals and their relation to laboratory testing.

To obtain laboratory data more representative of field conditions, cycling storage units can be used to simulate actual conditions. Feinberg (388) described the Defense Personnel Support Center "Accelerated Aging Test" which employed cycling conditions. Normally one assumes that the greater part of the storage in the field will be at average climatic conditions with limited exposure to stress conditions. A significant number of locations that provide storage for pharmaceutical products, such as hospitals, pharmacies, and wholesalers, are air-conditioned (383). This fact tends to reinforce the position that long-term storage will generally not be at stress conditions. Levi and Benney (383) proposed a scheme for testing and labeling that takes into account the potential for storage at varying conditions.

Compendial monographs (216, 217) provide directions for "Packaging and Storage"; the storage conditions stipulated in the individual monographs are defined as:

> *"Cold—Any temperature not exceeding 8° (46°F). A refrigerator is a cold place in which the temperature is maintained thermostatically between 2° and 8° (36° and 46°F). A freezer is a cold place in which the temperature is maintained thermostatically between −20° and −10° (−4° and 14°F).*
>
> *Cool—Any temperature between 8° and 15° (46° and 59°F). An article for which storage in a cool place is directed may, alternatively, be stored in a refrigerator, unless otherwise specified in the individual monograph.*
>
> *Room Temperature—The temperature prevailing in a working area. Controlled room temperature is a temperature maintained thermostatically between 15° and 30° (59° and 86°F).*
>
> *Warm—Any temperature between 30° and 40° (86° and 104°F).*
>
> *Excessive Heat—Any temperature above 40° (104°F).*
>
> *Protection from Freezing—Where, in addition to the risk of breakage of the container, freezing subjects a product to loss of strength or potency, or to destructive alteration of the dosage form, the container label bears an appropriate instruction to protect the product from freezing."*

In addition, USP XIX and NF XIV require that:

"Where no specific storage directions or limitations are provided in the individual monograph, it is to be understood that the storage conditions include protection from moisture, freezing, and excessive heat."

If no storage legend appears on a product label, can it be assumed that the product is resistant, within acceptable levels, to the deleterious effects of storage for the entire expiration period at the most severe condition allowed, *e.g.*, 40° (excessive heat)? With the vast majority of pharmaceutical dosage forms, this stability cannot be assumed. It would be necessary to test the material for the full expiration period at the most severe condition to provide such assurance.

The Defense Personnel Support Center has acknowledged that at times certain products may not be stored in accordance with required storage legends. They provide guidelines (388) on the maximum number of days an article requiring refrigeration may be stored out of refrigeration.

Kiger (389, 390) and Schumacher (391) discussed the results of environmental factors on the stability of drugs and listed the storage requirements of some prime drug products for maximum efficiency and the most reliable storage time.

Predictive (Accelerated Testing)—To determine the stability of a pharmaceutical product, samples are stored under the anticipated marketed storage conditions, usually ambient temperature, and either the content of intact drug or the content of degradation product is monitored by specific analytical, microbiological, or physical methods at predetermined intervals. The point at which the product degrades to the lower limit of its specifications is considered the shelflife (392). Depending on the stability of the item, the time involved for this test could be very short or quite long. Obviously, it would be to the investigator's advantage to be able, in a relatively short time, to predict the shelflife of the product. Toward this end, various approaches for predicting shelflife have been developed and applied.

Garrett (5) pointed out that predictive methods may be applied to evaluate degradation rates as functions of several magnitudes of stress. Stability at ambient conditions may be predicted from data at higher temperatures by the application of physicochemical laws utilizing the appropriate statistical evaluation. This approach enables one to estimate stability over short test periods, to evaluate quickly variations in batches or components, to select rapidly the optimum formulations from a series, to apply statistical methods, and to minimize the error component in the variation in analytical results.

Carstensen (393) outlined some procedures that can be used in stability extrapolations and predictions and discussed the application of Arrhenius plotting and applicable confidence limits. Graham (394) pointed out that predictive studies require that the degradation rate be studied as a function of an applied stress such as a change in pH, temperature, or ionic strength. Kinetic experiments are much more easily applied to the drug itself than to formulations; for the latter, such studies are more easily interpreted for solution-type products than for solid dosage forms.

The use of a nomographic chart to facilitate the analysis of data from accelerated testing for predictive purpose was presented by Lordi and Scott (395). An optimized stability testing program was outlined. More recently, a new nomogram was proposed (396) that is capable of predicting stability based on two analytical determinations of samples kept at two definite temperatures above room temperature for certain time periods. This method may be applied to zero-, first-, and second-order kinetics.

Several predictive approaches utilizing isothermal and/or nonisothermal methods have been described. Matsuura and Kawamata (197) developed a method for prediction of shelflife under nonisothermal shelf conditions through the use of an analog computer; the calculated results were in good agreement with experimental values. Zoglio *et al.* (196) reported on a continuous nonisothermal–isothermal method for stability prediction. A simple experimental procedure utilizing nonisothermal estimation of the activation energy and preexponential factor for drug decomposition from a single experiment was described by Madsen *et al.* (194). A computer program was written to assist in the computational aspects. Flexible nonisothermal stability studies that eliminate the need for a fixed time–temperature profile were described by Maulding and Zoglio (191).

Through the use of the Arrhenius equation to extrapolate observed changes, an 8-week testing program for predicting tablet stability was described (397). Carstensen and Su (398) presented the statistical aspects of Arrhenius plotting for predictive purposes. The application of the Gauss–Newton method to accelerated data was demonstrated. A scheme was suggested (399) based on short-term stability data at room temperature for obtaining reliable expiration dates.

Prediction of physical changes of drug preparations such as color stability of a liquid multisulfa preparation (400) and of tablet formulations (401) were reported. The disadvantages of accelerated testing of perfume components of products were reported (402), showing a possible lack of correlation between results and actual market performance. A rapid method for accelerated determination of oxidation of cosmetics was described (403). The test material was heated to 120°, and then oxygen was blown into the container; correlation with other methods was very good.

Several other approaches and review articles on accelerated aging and predictive stability are available (404–409).

With all of the available tools for predicting drug stability, in the final analysis the actual long-term data under ambient conditions are required by the FDA.

Expiration Dating/Shelflife—The objective of stability testing is to determine for what time period and under what condition the product is satisfactory. Expiration dates had been used only for problem products with "limited" stability; those that were stable for longer, yet arbitrary periods, went undated. For New Drug Applications, the FDA has required that if no expiration date is proposed, its absence must be justified (see *Regulatory Considerations*). Interest and concern in the use of valid expiration dates have been shown by government, academia, and industry over the last several years, as evidenced by the frequent seminars held on the subject.

The first major conference was the "Seminar on Drug Stability as Affected by Environment and Containers,"

cosponsored by the FDA and the School of Pharmacy, University of Connecticut, in Washington, D.C., in 1967. Other major conferences were held in 1969 by the University Extension, University of Wisconsin, "The Dating of Pharmaceuticals," and in 1972 by the School of Pharmacy and Pharmacal Sciences, Purdue University, "Implementation of Product Expiration Dating Systems in the Pharmaceutical Industry."

Many manufacturers had voluntarily instituted expiration dates for some or all of their products by 1969 (5), even though there was no official requirement. Federal regulations have required expiration dates for products liable to deterioration or for certain classes of drugs and have proposed expiration dates for all drug items (see *Regulatory Considerations*). In addition, the USP (410) had required that:

> *"In the absence of a specific requirement in the individual monograph for a dosage form, the label shall bear an expiration date assigned for the particular formulation and package of the article. This date identifies the time during which the article may be expected to meet the requirements of the Pharmacopeial monograph provided it is kept under the prescribed storage conditions. The expiration date limits the time during which the product may be dispensed or used."*

The same requirement appears in the latest edition of the NF (411).

The preamble to the proposed CGMP (412) justifies the requirement for expiration dating for all drug products as follows:

> *"Consumers of pharmaceuticals have a vital interest in having those products maintain the identity, strength, quality, and purity essential to render them safe and effective for use. The stability of a drug product during the period of time between its manufacture and its delivery to the patient can have a major influence on such identity, strength, quality, and purity. Many factors affect drug product stability. These include the stability of the active ingredients, the interaction of active and inactive ingredients, the manufacturing process, the storage conditions, the dosage form, the container closure system, the conditions under which the drug product is shipped, stored, and handled by wholesalers and retailers, and the length of time between initial manufacture and final use. Although some of these factors are not within the direct control of the manufacturer, the use of expiration dating by the manufacturer will encourage the removal of outdated or aged stocks."*

It is further stated that: "The appropriate expiration date for each drug product must be determined by suitable stability studies."

Johnson (5) indicated that expiration date intervals reflect the length of time required for: (*a*) the least stable active component to degrade to about 90% of the label claim, (*b*) some aspect of pharmaceutical elegance to become unacceptable, or (*c*) a maximum of 5 years.

Many definitions and uses of the terms normally associated with stability testing and expiration dating have been evident in the literature. Carstensen and Nelson (413) proposed a nomenclature for the various phrases (shelflife, outdate, expiration, and label date) to make scientific and technological dialog more precise in this area.

The USP (414) directs dispensing pharmacists to rotate stocks based on age and states that the expiration date is guaranteed by the manufacturer only if the product is stored in the original container at the recommended conditions.

REGULATORY CONSIDERATIONS

The multitude of recently promulgated governmental regulations concerning the stability of pharmaceutical preparations emphasizes the importance of this subject. These regulations include specific requirements for stability testing on which expiration dating is to be based. The most noteworthy of these regulations are discussed here.

IND/NDA—The Federal Food, Drug, and Cosmetic Act and its amendments require that a manufacturer demonstrate the safety and efficacy of a new drug prior to introducing it into interstate commerce. This requirement is more clearly defined in the Notice of Claimed Investigational Exemption for a New Drug (IND) (415) and the New Drug Application (NDA) (416).

IND's require "a statement of the methods, facilities, and controls used for the manufacturing, processing, and packing of the new drug to establish and maintain appropriate standards of identity, strength, quality, and purity as needed for safety and to give significance to clinical investigations made with the drug." Based on this regulation, available stability data on the new drug substance and dosage forms concerned with a particular IND are required by the FDA.

The requirements for stability information under the regulations for NDA's are more specific and detailed than those for IND's. They require:

> *"a complete description of, and data derived from studies of the stability of the drug, including information showing the suitability of the analytical methods used. Describe any additional stability studies underway or contemplated. Stability data should be submitted for any new-drug substance, for the finished dosage form of the drug in the container in which it is to be marketed, including any proposed multiple-dose container, and if it is to be put into solution at the time of dispensing, for the solution prepared as directed. State the expiration date(s) that will be used on the label to preserve the identity, strength, quality, and purity of the drug until it is used. (If no expiration date is proposed, the applicant must justify its absence.)"*

Under the regulations for Antibiotic Drugs (417), an expiration date is required for the product label of any antibiotic drug. These regulations are detailed in the Antibiotic Application, FD Form 1675 (1/71):

> *"h) A complete description of, and data derived from stability studies of the potency and physical characteristics of the drug, including information showing the suitability of the analytical methods used. Describe any additional stability studies underway or contemplated. Stability data should be submitted for any new antibiotic, for the finished dosage form of the drug in the container including a multiple-dose container in which it is to be marketed, and if it is to be put into solution at the time of dispensing, for the solution prepared as directed.*
>
> *i) The expiration date needed to preserve the identity, strength, quality, and purity of the drug until it is used."*

Guidelines (418) were published to assist drug sponsors and applicants in developing information required by the FDA for IND's and NDA's. These guidelines detail the many factors involved in the stability evaluation of a drug, indicating what is necessary for a stability profile and what is required to satisfy the regulations. Further clarification of the Federal requirements for stability of new drugs was provided by Silk (419) of the FDA.

GMP Requirements—Good Manufacturing Practice

in Manufacture, Processing, Packing, or Holding of Drugs was first promulgated in 1963. In 1969, revisions of these regulations were proposed (420); in 1971, a final revised version of Current Good Manufacturing Practice (421) was implemented. Section 133.13—Stability provided for the assurance of the stability of the finished drug product, requiring that it be:

"a) Determined by reliable, meaningful, and specific test methods.
b) Determined on products in the same container—closure systems in which they are marketed.
c) Determined on any dry drug product that is to be reconstituted at the time of dispensing (as directed in its labeling), as well as, on the reconstituted product.
d) Recorded and maintained in such a manner that the stability data may be utilized in establishing product expiration dates."

Section 133.14—Expiration dating provided:

"assurance that drug products liable to deterioration meet appropriate standards of identity, strength, quality, and purity at the time of use, the label of all such drugs shall have suitable expiration dates which relate to stability tests performed on the product.
a) Expiration dates appearing on the drug labeling shall be justified by readily available data from stability studies such as described in Section 133.13.
b) Expiration dates shall be related to appropriate storage conditions stated on the labeling wherever the expiration date appears.
c) When the drug is marketed in the dry state for use in preparing a liquid product, the labeling shall bear expiration information for the reconstituted product, as well as, expiration date for the dry product."

Revisions of these regulations have been proposed (422) to expand the current regulations for stability testing (Section 211.166) by requiring that a written testing program designed to assess the stability characteristics of drug products and used to establish storage conditions and expiration dates be followed. Additional proposed requirements include:

"1) Statistical criteria, including sample size and test intervals, for each attribute examined to assure statistically valid estimates of stability;
2) Storage conditions for samples tested."

The proposal also requires:

"an adequate number of batches of each drug product shall be tested to determine an appropriate expiration date and a record of such dates shall be maintained. Accelerated studies, combined with basic stability information on the components, drug products, and container–closure system, may be used to support tentative expiration dates provided adequate shelf life studies are not available and are being conducted. Where data from accelerated studies are used to project a tentative expiration date that is beyond a date supported by actual shelf life studies, there must be stability studies conducted, including drug product testing at appropriate intervals, until the tentative expiration date is verified or the appropriate expiration date determined."

The regulation proposed for expiration dating (Section 211.137) requires that the date be statistically valid and that it be applied to all drug products, not only to products liable to deterioration. Thus revision requiring dates for all products is in line with the current compendial policy (410, 411): "in the absence of a specific requirement in the individual monograph for a dosage form, that the label bear an expiration date assigned for the particular formulation and package of the article."

PPA: Child Resistant Closures—To emphasize the concern for the stability of pharmaceutical products in alternate packaging, the rules and regulations for Special Packaging (423) pursuant to Section 3 of the Poison Prevention Packaging Act of 1970 required that for "immediately effective supplemental applications" to qualify child resistant containers, the container and closure composition and the torque of the container be consistent with those provided for in the approved new drug application. Stability commitments to test the stability of initially marketed batches of the drug and to report the results to the FDA quarterly the 1st year, semiannually the 2nd year, and annually thereafter through the expiration date of the article were required. The law also required a commitment to withdraw from the market any batch falling outside the approved specifications for the drug. In cases where the container composition and the composition of the closure component in contact with the drug varied from those in the approved new drug application, appropriate submissions for approval had to be made to the new drug application.

In place of data showing the package to be a satisfactory barrier to moisture and gas transmission, stability data obtained at conditions of exaggerated temperatures and humidity for 3 months could be submitted. This provision again emphasizes the importance of actual stability determinations on the product in the container–closure combination.

It is obvious that great importance is placed on any change in packaging components and its possible effect on drug product stability.

Formulation Changes—Significant formulation changes are normally accompanied by appropriate stability evaluation to determine any untoward effect on the product. However, minor formulation changes, required by government action, have stipulated the need for stability studies. Examples of these changes are: termination of provisional listing and certification of amaranth (FD&C Red No. 2) (424), elimination of chloroform as an ingredient of human drug and cosmetic products (425), and termination of provisional listing of carbon black (426).

Each regulation required essentially the same information and/or commitment, namely that manufacturers of new drugs who either deleted or replaced the delisted component submit data to establish the stability of the revised formulation. If the data were too limited to support a conclusion that the drug would retain its declared potency for a reasonable marketing period, then an alternate three-part commitment was required:

1. Commit to test the stability of marketed batches at reasonable intervals.
2. Submit data as they become available.
3. Recall from the market any batch found to fall outside the approved specifications for the drug.

The formulation change required to conform to these regulations could involve a simple deletion of the item or a replacement of the component; however, the regulations apply equally to all changes. The item may have been an intimate part of the product composition or used only on the surface as an ingredient of printing inks. The regulations do not consider the extent of the formulation change.

GLP Requirements—Recently, guidelines (427) for

good laboratory practices in nonclinical studies of pharmaceutical compounds were adopted by the Pharmaceutical Manufacturers Association, and regulations for such guidelines were proposed by the FDA (428). The FDA-proposed regulations indicate that: "identity, strength, quality, and purity of each batch of a test or control substance should be determined and documented."

The FDA proposal also requires that the stability of the test substance will, where possible, be established by the testing facility under circumstances of the intended study. Where it is not feasible to determine accurately the stability of the test substances prior to study initiation, periodic reanalysis is indicated. Since nonclinical work is basic to the investigation and evaluation of possible medicinal agents, the integrity of the test substance is critical for proper evaluation of test results.

Computerization/Records/Reports—The increasing regulatory requirements outlined above generate additional stability studies which, in turn, produce voluminous data. Since most data accumulated on dosage forms are eventually submitted to a regulatory agency as part of a submission for a new drug application, for a supplemental application, to fulfill regulatory commitments, or in periodic reports, a means of collating and reporting these data is essential. The obvious course of action when multitudes of numbers are concerned is computerization.

Many manufacturers currently utilize computers from the initial stages of stability determination for statistical evaluation of kinetic data to the preparation of reports on dosage forms for submission to the FDA. Computers allow for the design of more complex models, which would be impractical and tedious by manual calculations. (See *Predictive* under *Marketed Product Stability.*)

One of the first approaches to the use of a computer system for processing stability data was a system that aids in the planning, interpretation, and submission of stability data (5). It was noted that data processing is in a dynamic state, and system efficiencies change with new equipment and programs. Blanco *et al.* (429) reported on a computerized stability program permitting maximal use of development and analytical manpower. This system includes capabilities to schedule, to flag exceptional behavior, to interpret data, and to prepare reports for filing with government authorities. The implementation of a computer program involving nearly 400 products was described by Newman (430); other applications of computer uses in stability programs have been reported (431, 432).

With appropriate design, a computer program for dosage form stability evaluation can provide for the control of sample storage at given conditions, control inventory, schedule test intervals, sample at prescribed intervals, delineate test requirements for each sample, review data, highlight untoward results, provide numerous administrative lists and search capability, interpret data, and prepare reports. As automation in the laboratory enables the analyst to perform more determinations, computerization allows for the efficient handling of complex administrative functions and the coordination of voluminous amounts of data by technical personnel which would otherwise require more personnel and increase the potential for human error. Human error is not eliminated by computerization; but with appropriate edit programs and design, it can be minimized.

Several programs specifically for pharmaceutical stability are commercially available from within the pharmaceutical industry and from consultants.

Failure of Drug Product to Meet Required Specifications—A stable product can be legally defined as a product that meets the required specifications for the indicated shelflife. A drug product that does not meet this definition creates significant problems for both the consumer and the manufacturer.

The FDA may initiate action, under the Food and Drug Act, to cause removal of a product from the market. Or, as noted previously, there is provision for the manufacturer to recall batches voluntarily from the marketplace that do not meet established specifications. With instability, there is a potential for the product to be rendered unsafe and ineffective through formation of toxic degradation products or loss of activity. If the instability does not pose a health hazard but, nevertheless, causes the material to be outside established specifications, then a recall results in the possible unavailability of medication to the consumer which would otherwise be adequate. Secondary consequences of an economic nature are incurred due to the expense of a recall and the intangible expense associated with damage to the manufacturer's reputation. The legal, economic, moral, and safety problems that could be incurred by instability are obvious and should further emphasize the need for the manufacturer to provide for a sound program that will ensure that the product is satisfactory throughout its useful life.

A modern, effective stability program, therefore, must encompass basic mechanistic studies on drug degradation, followed by appropriate preformulation studies and a thorough evaluation of the product–container combination to establish a valid expiration date and storage requirements to maintain the integrity of the drug.

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