Modification of Ethylene Acrylic Acid Film for Antimicrobial Activity

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ABSTRACT: Benzoyl chloride is commonly used as a food preservative to control microbial contamination by reducing the growth rate and maximum growth population and extending the lag period of the target microorganisms. Benzoyl chloride was successfully incorporated into a matrix of an ethylene acrylic acid polymer. The reaction of benzoyl chloride with ethylene acrylic acid was confirmed by Fourier transform infrared spectroscopy. The antimicrobial activity of modified ionomer films was studied through the monitoring of the growth of *Penicillium* sp. and *Aspergillus* sp. on the modified films. An untreated film did not show any inhibition of microbial growth. The inhibition activity was least in an ionomer film treated with acid and benzoyl chloride, and this was followed by a film treated with alkali and benzoyl chloride. The maximum inhibition was observed in a film treated just with benzoyl chloride. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 100: 3063–3068, 2006

Key words: ethylene acrylic acid; antimicrobial activity; benzoyl chloride

INTRODUCTION

Foods, being perishable products, have very short shelf life. The packaging of perishable products is quite different from the packaging of other products. Earlier food packaging materials were used to provide only barrier and protective functions. However, various kinds of active substances can now be incorporated into packaging materials to improve their functionality. Such active packaging technologies are designed to extend the shelf life of foods while maintaining their nutritional quality and safety. Active packaging technologies involve interactions between the food, the packaging material, and the internal gaseous atmosphere.[1] The extra functions they provide include oxygen scavenging, antimicrobial activity, moisture scavenging, and ethanol emitting.

When antimicrobial agents are incorporated into a polymer, they retard or prevent microbial growth. This application could be used for foods effectively, not only in film forms but also as containers and utensils. Sachet systems have been used to control the gas composition inside a package; for example, an ethanol-vapor-generating sachet can inhibit mold growth on bakery products.[2] An oxygen-scavenging system absorbs oxygen gas in the package and prevents the growth of aerobic microorganisms, especially mold, as well as the oxidation of food components. Antimicrobial packaging materials have to extend the lag period and reduce the growth rate of microorganisms to prolong shelf life and maintain food safety. They have to reduce the microbial growth of nonsterile foods or maintain the stability of pasteurized foods without postcontamination. If the packaging materials have self-sterilizing ability because of their own antimicrobial activity, they may eliminate the chemical sterilization of packages with peroxide and simplify the aseptic packaging process.[3] Table I reviews some typical applications of antimicrobial packaging systems.

Antimicrobial substances

A chemical preservative can be incorporated into a packaging material to add antimicrobial activity to it. For example, preservative-releasing films provide antimicrobial activity by releasing the preservative at a controlled rate. Oxygen absorbents also reduce headspace oxygen and partially protect food against aerobic spoilage such as mold growth.[35] Most of the common antimicrobial chemicals for food products are some organic acids and their salts, sulfites, nitrites, antibiotics, and alcohols, as shown in Table I. For example, sorbic acid and its potassium salts have been studied as preservatives for the packaging of cheese products. These were mixed into a wax layer for natural cheese,[36–40] a wet wax coating on packaging paper,[10,11] and an edible protein coating on intermediate-moisture foods.[41] However, the release rate and mi-

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TABLE I
Application of Antimicrobial Food Packaging

Antimicrobial agent	Packaging material	Food
Potassium sorbate	LDPE	Cheese[4]
	LDPE	Culture media[5]
	MC/palmitic acid	Culture media[6]
	MC/HPMC/fatty acid	Culture media[7]
	MC/chitosan	Culture media[8]
	Starch/glycerol	Chicken breast[9]
Calcium sorbate	CMC/paper	Bread[10,11]
Propionic acid	Chitosan	Water[12]
Acetic acid	Chitosan	Water[12]
Benzoic acid	PE-*co*-PE	Culture media[13]
Sodium benzoate	MC/chitosan	Culture media[8]
Sorbic acid anhydride	PE	Culture media[14, 15]
Benzoicacid anhydride	PE	Fish fillet[16]
Benomyl	Ionomer	Culture media[17]
Imazalil	LDPE	Bell pepper[18]
	LDPE	Cheese[15]
Nisin (peptide)	Silicon coating	Culture media[19]
	SPI, corn zein films	Culture media[20]
	PVOH, nylon, cellulose acetate, SPI film, corn zein films	Culture media[21]
		Culture media[20]
Glucose oxidase	Alginate	Fish[22]
Alcohol oxidase[23]	—	—
Ethanol	Silica gel sachet	Culture media[24]
	Silicon oxide sachet (Ethicap)	Bakery[25]
Hinokithiol	Cyclodextrin/plastic (Seiwa)[26]	—
Reduced iron complex	Sachet (Ageless)	Bread[27]
BHT	HDPE	Breakfast cereal[28]
CO_2	Calcium hydride	Coffee[29]
	Sachet	Fruit/vegetable[30]
SO	Sodium metabisulfite	Grape[26]
UV radiation	Nylons	Culture media[31,32]
Silver zeolite	LDPE	Culture media[33]
Grapefruit seed extract	LDPE	Lettuce, soybean sprouts[34]

LDPE = low-density polyethylene; MC = methyl cellulose; HPMC = hydroxypropyl methyl cellulose; CMC = carboxyl methyl cellulose; PE = polyethylene; MA = methacrylic acid; SPI = soy protein isolate; PVOH = poly(vinyl alcohol) BHT = butylated hydroxy toluene; HDPE = high-density polyethylene.

gration profile of antimicrobial agents in these applications were not specifically controlled.

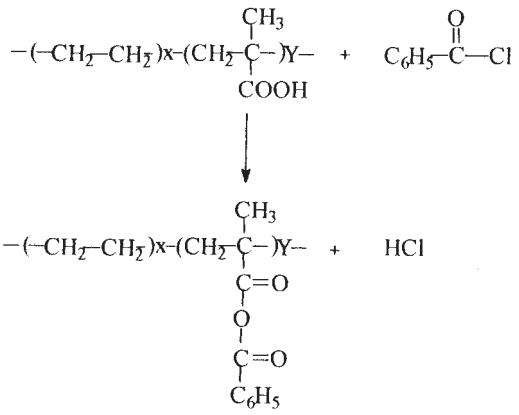
Other attempts at incorporating chemicals into plastics for use as antimicrobial packaging films involved antimycotics and antibiotics. Imazalil was used as the active substance and was chemically coupled to plastic films to delay the growth of molds. A shrink-wrapping film of imazalil in low-density polyethylene for use on peppers[42] and cheddar cheese[43] and imazalil-bound ionomer films[44] had antifungal properties and controlled the contamination of cheese and peppers.

Biodegradable polymers are currently being studied as edible coatings or film materials.[45] Padgett et al.[20] demonstrated the antimicrobial activity of lysozyme and nisin in soy protein isolate films and corn zein films. The use of edible films or coatings and the incorporation of food preservatives and natural antimicrobial agents may become popular areas of packaging research.

Factors causing food spoilage come mainly from microbial contamination on the food surface.[46–48] To inhibit microbial growth on the food surface, many direct techniques, such as dipping, dusting, and spraying have been developed to apply preservatives (antimicrobial agents) to the food surface.[49–51] However, these methods are laborious and inconvenient.

Ionomer films have been used as food packaging materials because of their unique properties, including a high degree of transparency, strength, resilience, stiffness, toughness, and inertness to organic solvents and oils. Halek and Garg[44] successfully demonstrated the incorporation of a fungicide (benomyl) onto the carboxylic groups of ionomer films. However, beno-

myl is not a legal food preservative and is not allowed to be used in foods. The applications of an antimicrobial ionomer in food packaging, particularly when coupled with legal food preservatives, are rarely found in the literature and need more research. Benzoic acid and its salts are among the most commonly used food preservatives. The incorporation of benzoic acid with an ionomer as an antimicrobial packaging film might provide several advantages over the methods mentioned previously. Thus, the preparation of modified ionomer films formed by the treatment of an ionomer film with benzoyl chloride films is examined in terms of the release of benzoic acid from the film.



Scheme 1

EXPERIMENTAL

Preparation of the films

Extruded ionomer film with an average thickness of 37–50 μm, procured from DuPont (Mumbai, India), were treated with 1*M* HCl and 1*M* NaOH solutions separately for 1 day at room temperature (25–30°C) with constant stirring. The treated ionomer films with 1*M* HCl and 1*M* NaOH were rinsed with distilled water to remove the excess of HCl and NaOH from the surface of the films and were further dried in a vacuum oven at 55°C and 28 in. of vacuum for 2 h. Both treated films along with a control ionomer film were further treated with a 0.1*M* benzoyl chloride solution in *n*-hexane for 1 h and dried in a vacuum of 28 in. at 55°C for 2 h.

Preparation of the media

Commercially available potato dextrose agar (PDA) was used for testing the antimycotic effect of the films. Media were prepared according to the manufacturer's instructions and autoclaved at 121°C for 15 min. Two flasks containing 100 mL of the prepared media were tempered, and 1 mL of a 10% tartaric acid solution was added to each flask for a pH adjustment to 3.5 ± 0.2. Spore suspensions of *Aspergillus* sp. and *Penicillium* sp. were prepared in saline solutions.

Preparation of the petri plates

PDA media were poured into each plate and spread evenly. The plates were kept open to dry the surface of the PDA media. A spore suspension (1 mL) was poured onto the surface of each plate and spread evenly with a surface-sterilized glass hockey stick. The ionomer film was kept in the center of the plate and pressed from all sides to remove the air bubbles under the surface of the film. The experiment was done in four replicates. The various combinations used in duplicate for microbial testing were as follows:

1. Untreated ionomer film (control).
2. Acid-treated, benzoyl chloride modified ionomer film.
3. Alkali-treated, benzoyl chloride modified ionomer film.
4. Benzoyl chloride modified ionomer film.

The samples were observed for fungal growth/inhibition zones for a maximum period of 15 days. In the case of the control, the plate was discarded after the 3rd day because of heavy fungal growth.

RESULTS AND DISCUSSION

The anhydride linkages in the modified ionomer films were formed with benzoyl chloride with the carboxylic group of the ionomer films according to the expected reactions shown in Scheme 1. Sandler and Kaor[52] proposed similar reactions.

Confirmation by IR spectroscopy

The reaction of the ionomer films with benzoyl chloride in different acid/alkali-treated films showed the formation of anhydride linkages in modified ionomer films according to the proposed reaction and was further confirmed by Fourier transform infrared (FTIR) spectra [Fig. 1(a–d)]. The formation of extra new peaks at 1741 and 1809 cm^{-1} as distinct vibration stretching confirmed the formation of anhydrate from P(CO—O—CO), and a stretching peak at 1013 cm^{-1} confirmed the formation of —C—O—C—. These peaks, coupled with the carbonyl vibration at 1709 cm^{-1}, proved the formation of anhydride bonds in the films.

Antimicrobial activity of the benzoyl chloride modified ionomer films

The antimicrobial activity of the modified ionomer films was determine through the monitoring of the

growth of *Penicillium* sp. and *Aspergillus* sp. for 15 days on the surface of the films, as shown in Figures 2(a–d) and 3(a–d), respectively. The growth of both molds was inhibited by all the modified ionomer films. Benzoic acid is a well-documented antimicrobial agent;[53] it was not necessary to test the inhibitory effects of the ionomer films over a wide range of microorganisms. The inhibition of the two mold species was enough to serve a demonstrative purpose. The very high level of contamination was designed to compare the antimicrobial ability of the modified ionomer.

The inhibition zone for *Penicillium* sp. in a Petri dish with a 60-mm diameter was observed to be maximum on film samples modified with benzoyl chloride film

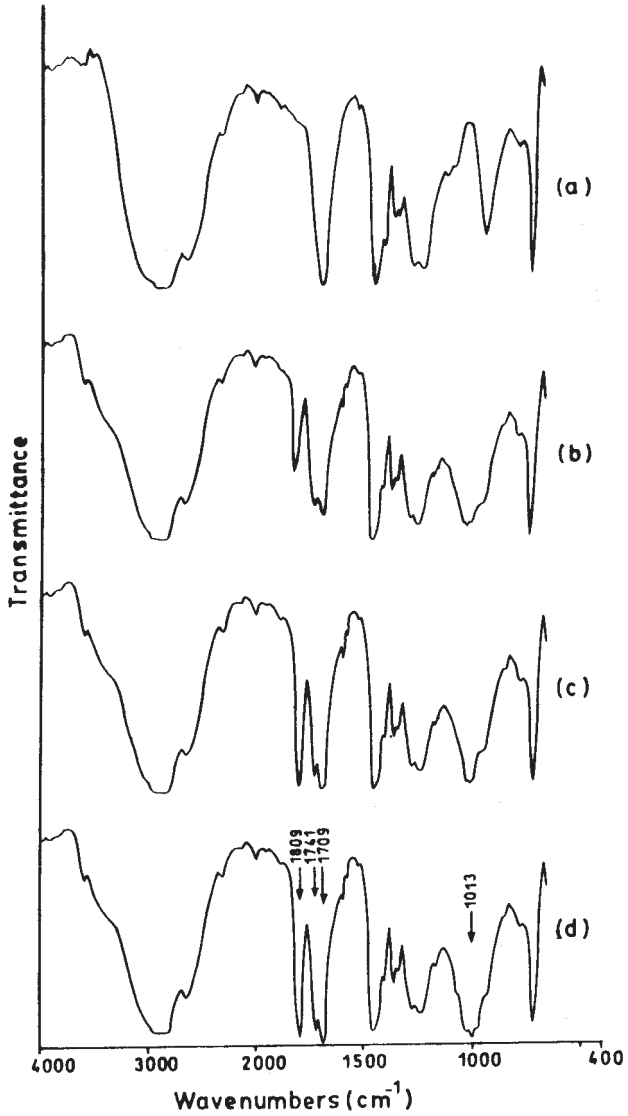


Figure 1 FTIR spectra of the ionomer films: (a) untreated ionomer film (control); (b) acid-treated, benzoyl chloride modified ionomer film; (c) alkali-treated, benzoyl chloride modified ionomer film; and (d) benzoyl chloride modified ionomer film.

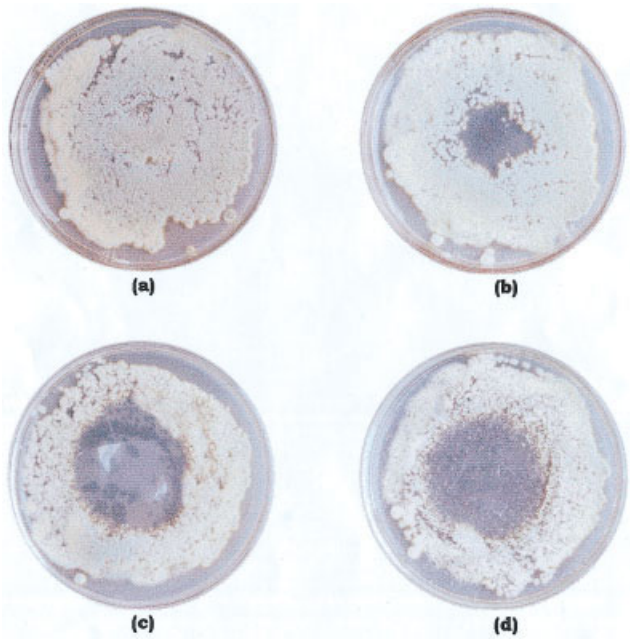


Figure 2 Inhibition zones of *Penicillium* sp. on the ionomer films: (a) untreated ionomer film (control); (b) acid-treated, benzoyl chloride modified ionomer film; (c) alkali-treated, benzoyl chloride modified ionomer film; and (d) benzoyl chloride modified ionomer film. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

[Fig. 2(d); diameter of the inhibition zone = 26.5 ± 1 mm], followed by alkali-treated, benzoyl chloride modified film [Fig. 2(c); diameter of the inhibition zone = 24.5 ± 1 mm] and acid-treated, benzoyl chloride modified film [Fig. 2(b); diameter of the inhibition zone = 15.5 ± 1 mm]. Similar results were observed for *Aspergillus* sp. The inhibition zone for *Aspergillus* sp. was observed to be maximum on film samples modified with benzoyl chloride [Fig. 3(d); diameter of the inhibition zone = 50.5 ± 10 mm], followed by alkali-treated, benzoyl chloride modified film 3[Fig. 3(c); diameter of the inhibition zone = 17.5 ± 2.5 mm] and acid-treated, benzoyl chloride modified film [Fig. 3(b); diameter of the inhibition zone = 14 ± 2 mm]. The maximum inhibition of both molds by the modified film treated with benzoyl chloride clearly indicated that the release of benzoyl chloride was maximum in the benzoyl chloride modified film [Figs. 2(d) and 3(d)]. It is well known that zones of inhibition occur only when an antimicrobial agent is released. In this case, the agent present in the modified film was only benzoyl chloride. The acid- and alkali-treated films controlled the rate of release of benzoyl chloride from the films, resulting in a reduced inhibition zone. Depending on the requirement of the inhibition quantity, suitable modified films can be used by the selection of a benzoyl chloride modified film for maximum inhibition, followed by an alkali-treated, benzoyl chlo-

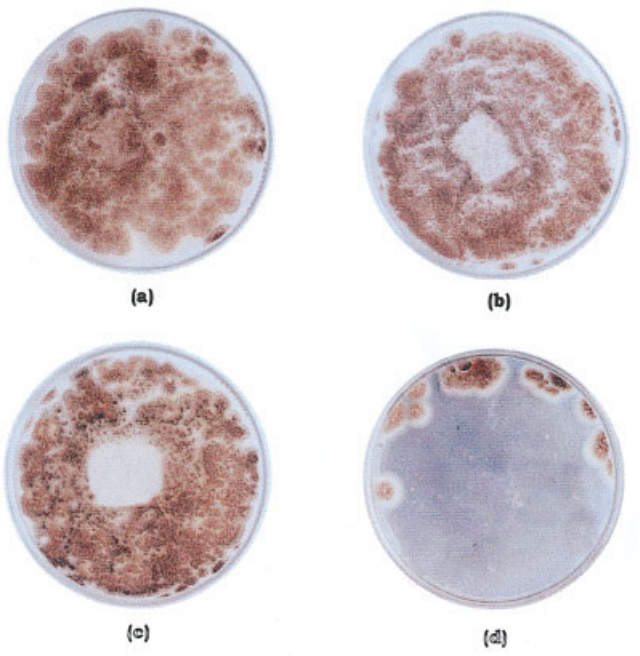


Figure 3 Inhibition zones of *Aspergillus* sp. on the ionomer films: (a) untreated ionomer film (control); (b) acid-treated, benzoyl chloride modified ionomer film; (c) alkali-treated, benzoyl chloride modified ionomer film; and (d) benzoyl chloride modified ionomer film. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

ride modified film for moderate inhibition and an acid-treated, benzoyl chloride modified film for low inhibition of microorganisms.

CONCLUSIONS

Most packaged perishable food products are heat-sterilized, and microbial contamination can occur on the surface or a damaged area of the food through a package defect or restoration after opening. An antimicrobial substance, such as benzoyl chloride, incorporated into packaging materials can control microbial contamination on the surface of food by inhibiting microbial growth and extending the lag period of the target microorganism. Benzoyl chloride modified ionomer films were successfully prepared, and the reaction was confirmed by FTIR spectra. All the modified films showed positive effects on the inhibition of microbial growth. The maximum inhibition was observed with films treated with benzoyl chloride, followed by films treated with alkali and benzoyl chloride and with acid and benzoyl chloride.

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