

Chitosan-Lipid Films: Microstructure and Surface Energy

Dominic W. S. Wong,* Francois A. Gastineau,† Kay S. Gregorski, Sandra J. Tillin, and Attila E. Pavlath

Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 800 Buchanan Street, Albany, California 94710

Composite films of chitosan-laurate were shown to have low water permeability, while films containing other fatty acids or esters were not effective in this respect. Electron microscopy revealed the microstructure of these films to be very different, in terms of density, pore formation, channeling, surface contour, and packing pattern. The surface energy of the films was estimated, and the dispersion force component (γ_s^d) and the polar component (γ_s^p) were calculated. The chitosan-laurate film exhibited higher surface energy, but no variation on either surface, while all other films tested differed in both surface energy components depending on the surface orientation. In general, these physical attributes can be correlated quite well with the permeability characteristics of the particular film. It is postulated that the unique properties of the chitosan-laurate film could suggest the importance of the morphological arrangement of the lipid within the chitosan matrix.

Chitosan is a linear polyamine obtained by the alkaline deacetylation of chitin which is an *N*-acetylglucosamine-glucosamine polymer. The unique positively charged chitosan in acidic solution has found potential uses in agriculture as a carrier for the controlled release of pesticides and/or fertilizers (Anon., 1987), in the food industry as an adsorbent for waste and water treatment (Knorr, 1991), and in the area of nutrition as a lipid binder (Furda, 1980). In the pharmaceutical area, chitosan can be used as an antimicrobial agent and also as encapsulation material for drug ingredients (Bodmeier et al., 1989; Vorlop and Klein, 1981).

The film-forming qualities of chitosan have been studied (Averbach, 1978) and found to be dependent on the homogeneity of the bulk material, the degree of acetylation, and acid degradation during dissolution. Chitin and chitosan films have been used to coat a variety of natural and synthetic fibers producing sheath-core bicomponent fibers (Allan et al., 1989). Chitosan has also been used as a hemostatic agent in films for sealing vascular grafts (Fradet et al., 1985). A composite film derived from chitosan and fine cellulose fiber has been developed having good wet strength, gas barrier, and biodegradability (Hosokawa et al., 1990).

Chitosan, like many carbohydrate polymer films, tends to exhibit selective permeability to gases but lack resistance to water transmission. Therefore, the incorporation of fatty materials to provide hydrophobicity and increase film resistance to water transmission is being investigated in the present study. Basically, the product is a composite film in which the fatty acid molecules are distributed in the chitosan matrix. The effects of lipid type on the permeability characteristics of these films are evaluated and explained in terms of their microstructures and structure-function correlation.

MATERIALS AND METHODS

Materials. Commercial grade chitosan (1.0% insolubles, >70% deacetylation) was obtained from Protan, Inc. (Commack, NY). Myvacet 5-07 (acetylated monoglycerides) and Myverol

P-06 (propylene glycol monoesters) were provided by Eastman Chemical Products, Inc. (Kingsport, TN). All other chemicals and reagents were of analytical grade.

Film Preparation. A 1% chitosan solution was prepared by adding 1.0 g of chitosan powder in 100 mL of 1% formic acid aqueous solution by vigorously stirring the chitosan suspension. The chitosan solution was filtered through a coarse sintered glass filter. A 50-mL volume of the chitosan solution was then mixed with various amounts of fatty acids in a high-speed blender for 10 min. For fatty acids of 12 carbons and longer chain length, the mixture was heated above the melting point of the respective lipid before blending and casting. Films were cast on a poly(methyl methacrylate) (Lucite or Plexiglas) plate, and the dried films had a thickness of 25.4–38.0 μm .

Permeability Measurements. Water permeabilities were determined using the ASTM (1987) procedure. A controlled atmosphere glovebox (Labconco Corp., Kansas City, MO) equipped with a humidity indicator and an analytical balance was used. The permeability of carbon dioxide gas through the films was measured by using a gas permeation cell fabricated of stainless steel (Gilbert and Pegaz, 1969; Karel et al., 1968). The test film was clamped between the stainless steel sections, forming an upper and a lower chamber. Both chambers were purged with helium before the gas line in the upper chamber was switched open for carbon dioxide to flow through. The gas in the lower chamber was analyzed by gas chromatography at regular time intervals. A Hewlett-Packard 5990 (Palo Alto, CA) equipped with a thermal conductivity detector and a packed column (183 cm \times 0.32 cm ss) of Chromosorb 107 was used. Column temperature was maintained at 60 °C and flow rate at 30 mL/min. Oxygen transmission rates were monitored by a Mocon Ox-Tran 100A with Datalogger DL200 (Mocon Modern Control, Inc., Minneapolis, MN). Oxygen molecules permeating through the fixed film in the diffusion cell were detected by an oxygen-specific coulometer detector.

Scanning Electron Microscopy. The chitosan film was mounted on an aluminum plancette and coated with a 100–200-Å thickness of gold-palladium in a Polaron (Hatfield, PA) SEM coating system. The specimen was scanned using a Hitachi SEM S-530 electron microscope equipped with a Varian 801 (Mountain View, CA) view station. Images were recorded on Polaroid film type 52. Samples were photographed at tilt angles of 30–60° to the electron beam for views in the cross section.

Contact Angle Measurement. Contact angle measurements were performed with glycerol, ethylene glycol, water, and methylene iodide using a goniometer (Rame-Hart, Inc., Mountain Lakes, NJ). A small drop of liquid was deposited on the film.

* Present address: Coex Packaging Technical Center, Cryovac, Via Trento 7, 20017 Passirana di Rho (Milano), Italy.

Table I. Water Vapor Permeability and Gas Transmission of Chitosan-Lipid Films

film type	permeability, (g mm)/ (m ² day mmHg)	gas transmission rates, cm ³ /(m ² day atm)	
		CO ₂	O ₂
chitosan	0.414 (0.020) ^a	48.60 (5.83)	11.02 (0.34)
-lauric acid	0.203 (0.027)	176.74 (7.68)	25.06 (1.10)
-palmitic acid	0.693 (0.003)	3.12 (0.11)	1.39 (0.09)
-octanoic acid	0.549 (0.020)		
-butyric acid	0.307 (0.112)		
-methyl laurate	0.957 (0.022)		
-myvacet	0.628 (0.002)	13.48 (0.24)	0.48 (0.08)
-myverol	0.740 (0.003)		

^a Number in parentheses is the standard deviation.

The angle was measured through the liquid where the tangent to the drop meets the surface. For each film type, at least 10 measurements were made and the average was taken. A plot of $\cos \theta$ vs the liquid surface tension, γ_l , yielded a straight line, the slope of which was used for the calculation of the surface energy according to the modified Young equation, $\gamma_l(1 + \cos \theta) = 2(\gamma_s^d \gamma_l^d)^{1/2} + 2(\gamma_s^p \gamma_l^p)^{1/2}$, where γ_l is the surface energy of the liquid used for θ measurement, γ_s^d is the dispersion force of the surface energy of the substrate (film), and γ_s^p is the hydrogen bonding or polar component of the surface energy (Ko et al., 1981; Owens and Wendt, 1969). Contact angle measurements were made on both sides of the cast films, i.e., on the film side exposed to air and on the film side exposed to the poly(methyl methacrylate) surface.

RESULTS AND DISCUSSION

The effect of incorporating fatty acids to chitosan films on the permeability is presented in Table I. The degree of water permeability did not follow the increasing chain length and hydrophobicity of the acid. The incorporation of lauric acid decreased the permeability by 49.0% as compared with chitosan film [0.203 vs 0.414 (g mm)/(m² day mmHg)], while other fatty acids did not show the same effect.

To investigate whether the carboxyl function of the fatty acids was required for the characteristic permeability, a number of fatty acid esters were used for constructing chitosan films. Substitution of methyl laurate for lauric acid resulted in an increase in water permeability. Both acetylated monoglyceride and propylene glycol stearate showed permeability values 3.0–3.6 times higher than that of lauric acid.

The effect of fatty materials on the gas transmission rates is in contrast to that on the water permeability. The carbon dioxide transmission rate increased in the order lauric acid > myvacet > palmitic acid. In the case of oxygen transmission, the rates also followed this same pattern.

In an attempt to find an explanation for the unique behavior of the chitosan-laurate film distinguishable from all of the other chitosan-lipid films tested, we have examined the microstructures of both the surfaces and cross sections of the various chitosan-lipid films. Morphological differences among the various chitosan-lipid films are evident from a comparison of the photomicrographs. Variations in their microstructures include density, pore formation, channeling, and packing pattern, attributes which may be correlated to the permeability characteristics of the films.

Chitosan Film. Pure chitosan films are compact, and the film surface has a smooth contour without pores or cracks (Figure 1A). Since chitosan polymers are cationic and the resulting films are strongly hydrophilic, water molecules can interact with the matrix and increase the permeation rate (Pascat, 1986). The hydrophilic nature

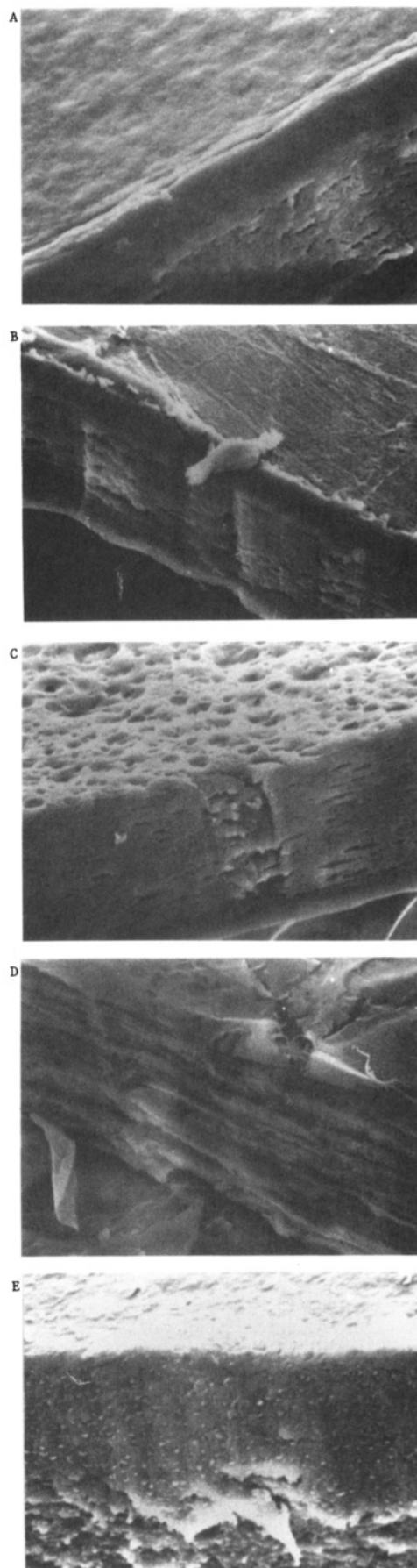


Figure 1. Electron microscopy photomicrograph of chitosan-fatty acid films (2:1 w/w): (A) pure chitosan film; (B) chitosan-butyrate; (C) chitosan-octanoate; (D) chitosan-laurate; (E) chitosan-palmitate.

of the film therefore accounts for the water permeability value obtained in the present experiment (Table I). In contrast to the results on water permeability, the chitosan film formed a very effective gas barrier as suggested in the literature (Muzzarelli, 1977). This particular property is probably related to the dense structure of the film. Transmission of gases through packed polymers is the lowest when compared with that in water or air with differences of 3 orders of magnitude (Mannapperuma and Singh, 1990). In general, films constructed from polysaccharides and their derivatives have only minimal moisture barrier properties but are highly effective in controlling diffusion of various gases (Banker et al., 1966).

Chitosan-Butyrate Film. The chitosan-butyrate film retained most of the characteristics exhibited by the straight chitosan film (Figure 1B). The film matrix was compact and dense, although there seemed to be a slight difference in the surface contour. Apparently, the short-chain butyric acid raised the hydrophobicity of the film enough to reduce the water permeability from 0.414 to 0.307 (g mm)/(day m² mmHg).

Chitosan-Octanoate Film. The chitosan-octanoate film showed a more loose and pliable texture as shown in Figure 1C. The surface of the film was covered with craterlike pits, and a small degree of channeling might exist in the film matrix. It is speculated that the loose structure observed in this film type may well be caused by the octanoic acid chains disrupting the ordered structure of the chitosan polymers. Chitin, which is similar to cellulose in its chemical structure, is known to exist in well-defined crystalline polymorphic forms, and chitosan membranes have shown X-ray diffraction lines indicating ordered structures (Mike and Blackwell, 1978). Although the long carbon chains of octanoic acid could affect the hydrophilic/hydrophobic property of the film, the physical factors had a dominant influence on the water vapor permeability through the film. Consequently, the film exhibited a slightly higher water permeability than that shown by the chitosan-butyrate film.

Chitosan-Laurate Film. When lauric acid was incorporated into the chitosan film, the microstructure was changed dramatically. The surface view suggested a sheet-like structure, while the cross section revealed the sheets stacked in layers (Figure 1D). The lauric acid seemed to be incorporated uniformly in the matrix. There was no obvious migration of the fatty acid to the air-film surface as noted by other authors using methylcellulose derivatives as the base component (Greener and Fennema, 1989; Vojdani and Torres, 1989, 1990). The arrangement of stacking layers of chitosan-lipid sheets means that to penetrate the film, water molecules have to pass through an extended network of channels lined with hydrophobic molecules. In the case of gases, the diffusion rate is not affected as much by hydrophobicity of the film but depends mostly on the medium. The permeability of gases in air and in water is much higher than in polymers. For oxygen, the permeability is 20.3×10^{-6} m²/s in air as compared to 0.072×10^{-9} m²/s in water and 0.038×10^{-12} m²/s in poly(vinyl chloride). A similar pattern of diffusion values for carbon dioxide has been noted (Mannapperuma and Singh, 1990).

Chitosan-Palmitate Film. The results obtained from the chitosan-laurate film led us to the assumption that palmitic and stearic acid with longer carbon chains could yield an even better film. To our surprise, the films had a poor resistance to water. The microstructure of the film showed palmitic acid forming discrete particles throughout the entire chitosan matrix (Figure 1E). Therefore, the

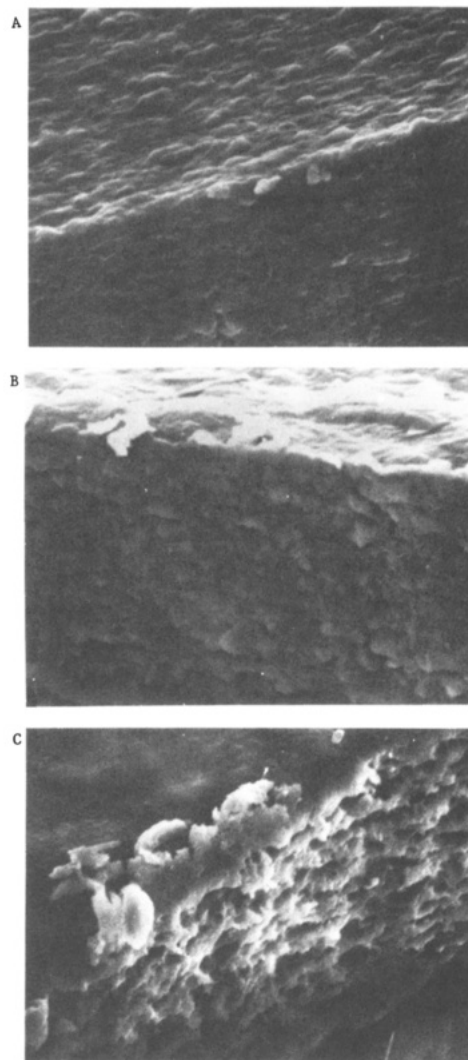
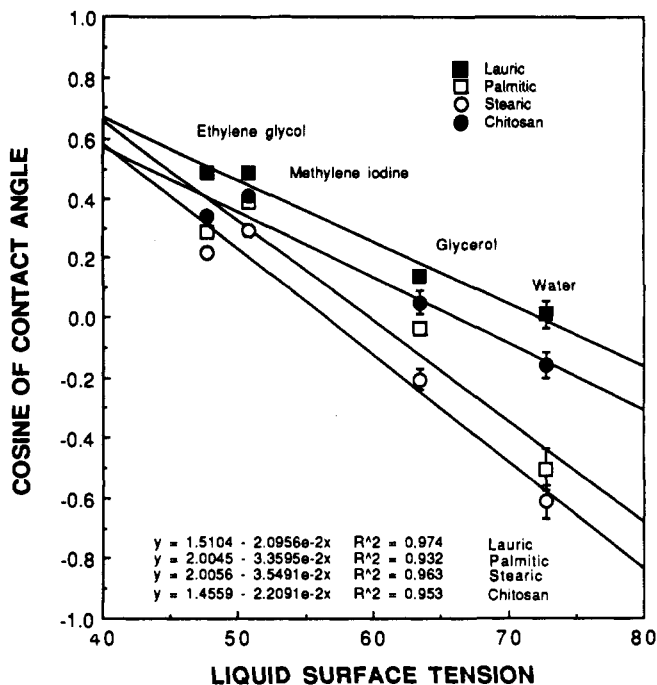


Figure 2. Electron microscopy photomicrograph of chitosan-fatty ester films (2:1 w/w): (A) chitosan-myvacet; (B) chitosan-myverol; (C) chitosan-methyl laurate.

hydrophobic areas corresponding to the fat globules are localized in the film, resulting in an ineffective barrier to water. By the same argument, the comparatively dense structure means the gas diffusion rates could be low. This conclusion is borne out by the findings presented in Table I.

Chitosan-Fatty Ester Film. Fatty esters are usually good emulsifiers that stabilize emulsions in a heterogeneous system. The three types of films constructed with the addition of the fatty esters, acetylated monoglyceride, propylene glycol monostearate, and methyl laurate, exhibited a loose and spongelike structure. In the chitosan-myvacet film, the surface and subsurface are spongelike (Figure 2A). The chitosan-myverol film showed a very similar structure in cross section, but the myverol formed solid platelets on the surface (Figure 2B). The chitosan-methyl laurate film has a surface similar to that observed in the straight chitosan film. The cross section revealed a highly spongelike structure (Figure 2C). Because of the porous structures, these ester-containing films exhibited poor resistance to water.

Surface Energy. The contact angles of various liquids on the films are presented by plotting $\cos \theta$ vs liquid surface tension (γ_l) in Figure 3. Fowkes (1964), in a theoretical study of attractive forces at interfaces, postulated that the total liquid surface energy comprises two additive terms: a dispersion force (γ_l^d) and a hydrogen-bonding or



Film	γ_s^d	γ_s^p	γ_s^{tot}
Lauric	20.2	8.7	28.9 (29.6)
Palmitic	15.6	8.7	24.3 (31.9)
Stearate	13.2	8.0	21.2 (26.6)
Chitosan only	17.0	7.7	24.8 (35.6)

number in parenthesis = γ_s^{tot} of plexiglass side of the film.

Figure 3. Plot of cosine contact angle for a number of polar liquids on films. Each datum represents an average of 20 measurements. Vertical bars are standard deviations. The slope was calculated from the regression line.

polar component (γ_1^p). Thus, with nonpolar liquids, such as aliphatic alkanes, the γ_1^p term is negligible and $\gamma_1^{tot} = \gamma_1^d$. Young's equation can be modified to be applied to liquid-solid interactions as follows (Ko et al., 1981; Ruckenstein and Gourisankar, 1986): $\gamma_1(\cos \theta + 1) = 2(\gamma_s^d)^{1/2}(\gamma_1^d)^{1/2}$. For polar liquids, such as those used in this experiment, the γ_1^p and γ_s^p terms can also be expressed in geometric terms. Thus, the equation becomes $\gamma_1(\cos \theta + 1) = 2(\gamma_s^d \gamma_1^d)^{1/2} + 2(\gamma_s^p \gamma_1^p)^{1/2}$. Using two pairs of x, y points from the regression line, γ_s^d and γ_s^p can then be calculated by solving two simultaneous equations (Figure 3). Calculations based on the above equation indicate that the chitosan-laurate films had a relatively higher value of $\gamma_s^d = 20.2$ and $\gamma_s^p = 8.7$ and $\gamma_s^{tot} = 28.9$ dyn/cm. The higher chain fatty acids, in general, decreased the total surface energy of the chitosan film. It is speculated that the decrease in the γ^p component might be due to shielding of polar residues of the chitosan molecules by the nonpolar acids which do not fit in with the structure of chitosan. The γ^d component of the surface energy also decreased because the longer acids formed discrete hydrophobic regions that did not interact. In contrast, in the case of the chitosan-laurate film, the lauric acid fits in with the chitosan and did not form a shielding effect, while the γ^d component was increased, accounting for the higher total surface energy as compared with that of the pure chitosan film. It is also important to note that the chitosan-laurate film showed very similar γ_s^{tot} values for

both sides of the polymer film [i.e., the side exposed to air and the side exposed to the poly(methyl methacrylate)], while other film types differed with respect to the surfaces tested. The results correlate well with the microstructural aspects of the films in that the lauric acid is believed to be incorporated evenly throughout the film, and hence a constant surface energy value is obtained for both sides of the film. These results further suggest that the uniformity of the distribution of the lipid molecules might be a crucial factor in the determination of the effectiveness of the water barrier.

Correlation of Characteristic Properties. The permeability characteristics generally correlate quite well with the microstructures as well as the physical characteristics of the films studied. However, there is still no adequate explanation for the dramatic difference in the morphology between the lauric acid and the palmitic acid molecules in the chitosan matrix. It is known that long-chain fatty acids exist in two or three polymorphic forms, each giving a different spacing in X-ray diffraction (D'Souza et al., 1990). These modifications in crystal forms depend on the following conditions: the purity of the acid, the solvent used, the temperature, and the cooling rate. The stable β form is usually obtained in the crystallization of even-carbon-number fatty acids, but fatty acids crystallized from strongly polar solvents yield the α forms. Palmitic and stearic acids have been shown to exist in a mixture of α and γ forms when crystallized from nonpolar solvents (Malkin, 1952). It is tempting to speculate that the lauric acid exists in a crystal form in conformational alignment with the chitosan polymer, while palmitic and stearic acids do not fit in with the chitosan structure. Interactions analogous to this type have been shown in the complex formation between amylose and saturated fatty acids (Karkalas and Raphaelides, 1986) and also monoglycerides (Batres and White, 1986; Carlson et al., 1979). The amylose chain coils up to form a helix with the lipid molecule fitting inside the core.

The nature of the crystalline structure of these chitosan films could be studied using thermogravimetry, differential scanning calorimetry, or X-ray diffraction. These studies are currently in progress in this laboratory. Preliminary data indicate that the chitosan-laurate film has a crystallinity pattern distinguishable from that of the chitosan-palmitate and other films. The results obtained seem to support the hypothesis that the morphological arrangement of the fatty acid chains with respect to the chitosan polymers affects the microstructure and the permeability of the film.

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LITERATURE CITED

- Anon. Chitin and its derivatives. *Bioprocess. Technol.* 1987, 9 (7), 4.
- Allan, G. G.; Carroll, J. P.; Hirabayashi, Y. Chitosan-coated films. In *Chitin and Chitosan, Sources, Chemistry, Biochemistry, Physical Properties and Applications*; Skjak-break, G., Anthonsen, T., Sandford, P., Eds.; Elsevier Applied Science: London, 1989; pp 765-776.
- ASTM. *Standard Test Methods for Water Vapor Transmission of Materials*; American Society for Testing and Materials: Philadelphia, 1987; Vol. 15, pp 748-755, Method E96-80.
- Averbach, B. L. Film-forming capability of chitosan. In *Proceedings of the First International Conference on Chitin/Chitosan*; Muzzarelli, R. A. A., Pariser, E. R., Eds.; MIT: Cambridge, MA, 1978; pp 199-209.

- Banker, G. S.; Gore, A. Y.; Swarbrick, J. Water vapor transmission properties of free polymer films. *J. Pharm. Pharmacol.* **1966**, *18*, 457.
- Batres, L. V.; White, P. J. Interaction of amylopectin with monoglycerides in model systems. *J. Am. Oil Chem. Soc.* **1986**, *63*, 1537-1540.
- Bodmeier, R.; Oh, K.-H.; Pramari, Y. Preparation and evaluation of drug-containing chitosan beads. *Drug Dev. Ind. Pharm.* **1989**, *15*, 1475-1494.
- Carlson, T. L.-G.; Larsson, K.; Dinh-Nguyen; Krog, N. A study of the amylose-monglyceride complex by raman spectroscopy. *Stärke* **1979**, *31*, 222-224.
- D'Souza, V.; deMan, J. M.; deMan, L. Short spacings and polymorphic forms of natural and commercial solid fats: A review. *J. Am. Oil Chem. Soc.* **1990**, *67*, 835-843.
- Fowkes, F. M. Attractive forces at interfaces. *Ind. Eng. Chem.* **1964**, *56*, 40-52.
- Fradet, G.; Brister, S.; Mulder, D. S.; Lough, J.; Averbach, B. L. Evaluation of chitosan as a new hemostatic agent: in vitro and in vivo experiments. In *Chitin in Nature and Technology*; Muzzarelli, R., Jeuniaux, C., Gooday, G. W., Eds.; Plenum Press: New York, 1985; pp 443-451.
- Furda, I. Nonabsorbable lipid binder. U.S. Patent 4,223,023, Sept 16, 1980.
- Gilbert, S. G.; Pegaz, D. Find new way to measure gas permeability. *Packag. Eng.* **1969**, 66-69.
- Greener, I. K.; Fennema, O. Evaluation of edible, bilayer films for use as moisture barriers for food. *J. Food Sci.* **1989**, *54*, 1400-1406.
- Hosokawa, J.; Nishiyama, M.; Yoshihara, K.; Kubo, T. Biodegradable film derived from chitosan and homogenized cellulose. *Ind. Eng. Chem. Res.* **1990**, *29*, 800-805.
- Karel, M.; Issenberg, P.; Ronsivalli, L.; Jurin, V. Application of gas chromatography to the measurement of gas permeability of packaging materials. *Food Technol.* **1963**, *17*, 91-94.
- Karkalas, J.; Raphaelides, S. Quantitative aspects of amylase-lipid interactions. *Carbohydr. Res.* **1986**, *157*, 215-234.
- Knorr, D. Recovery and utilization of chitin and chitosan in food processing waste management. *Food Technol.* **1991**, *45*, 114-122.
- Ko, Y. C.; Ratner, B. D.; Hoffman, A. S. Characterization of hydrophilic-hydrophobic polymeric surfaces by contact angle measurements. *J. Colloid Interface Sci.* **1981**, *82*, 25-37.
- Malkin, T. The molecular structure and polymorphism of fatty acids and their derivatives. In *Progress in the Chemistry of Fats and Other Lipids*; Holman, R. T., Lunberg, W. O., Eds.; Academic Press: New York, 1952; pp 1-15.
- Mannapperuma, J. D.; Singh, R. P. Modeling of gas exchange in polymeric packages of fresh fruits and vegetables. Presented at the 1990 IFT Annual Meeting, Anaheim, CA, June 16-20, 1990; Abstract 646.
- Mike, R.; Blackwell, J. The structure of α -chitin. *J. Mol. Biol.* **1978**, *120*, 167-181.
- Muzzarelli, R. A. *Chitin*; Pergamon Press: Oxford, U.K., 1977; p 71.
- Owens, D. K.; Wendt, R. C. Estimation of the surface free energy of polymers. *J. Appl. Polym. Sci.* **1969**, *13*, 1741-1747.
- Pascat, B. Study of some factors affecting permeability. In *Food Packaging and Preservation: Theory and Practice*; Mathlouthi, M., Ed.; Elsevier Applied Science: London, 1986; p 7.
- Ruckenstein, E.; Gourisankar, S. Y. Surface restructuring of polymeric solids and its effect on the stability of the polymer-water interface. *J. Colloid Interface Sci.* **1986**, *109*, 557-566.
- Vojdani, F.; Torres, A. Potassium sorbate permeability of methylcellulose and hydroxypropyl methylcellulose multi-layer films. *J. Food Process. Preserv.* **1989**, *13*, 417-430.
- Vojdani, F.; Torres, J. A. Potassium sorbate permeability of methylcellulose and hydroxypropyl methylcellulose coatings: Effect of fatty acids. *J. Food Sci.* **1990**, *55*, 841-846.
- Vorlop, K.-D.; Klein, J. Formation of spherical chitosan biocatalysts by ionotropic gelation. *Biotechnol. Lett.* **1981**, *3*, 9-14.

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Registry No. Chitosan laurate, 139168-36-8; chitosan stearate, 139168-37-9; chitosan palmitate, 139168-38-0; chitosan octanoate, 139198-66-6; chitosan butyrate, 139168-39-1; chitosan methyl laurate, 139168-40-4.