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Chitosan in Nanostructured Thin Films

Felipe J. Pavinatto,^{*,†} Luciano Caseli,[‡] and Osvaldo N. Oliveira, Jr.[†]

Instituto de Física de São Carlos, Universidade de São Paulo, São Carlos, SP, Brasil, and Departamento de Ciências Exatas e da Terra, Universidade Federal de São Paulo, Diadema, SP, Brasil

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This review paper brings an overview of the use of chitosans in nanostructured films produced with the Langmuir–Blodgett (LB) or the electrostatic layer-by-layer (LbL) techniques, with emphasis on their possible applications. From a survey in the literature one may identify three main types of study with chitosan in nanostructured films. First, the interaction between chitosans and phospholipid Langmuir monolayers has been investigated for probing the mechanisms of chitosan action in their biological applications, with the monolayers serving as cell membrane models. In the second type, chitosan serves as a matrix for immobilization of biomolecules in LB as well as in LbL films, for which chitosan is suitable to help preserve the bioactivity of such biomolecules for long periods of time even in dry, solid films. An important application of these chitosan-containing films is in sensing and biosensing. The third type of study involves exploiting the mechanical and biocompatibility properties of chitosan in producing films with enhanced properties, for example, for tissue engineering. It is emphasized that chitosans have been proven excellent building blocks to produce films with controlled molecular architecture, allowing for synergy between distinct materials. We also discuss the prospects of the field, following a critical review of the latest developments in nanostructured chitosan films.

1. Introduction

The increasing importance of materials from renewable sources has put chitosans in the spotlight, especially due to their biological properties, which have been exploited in many applications.^{1,2} Chitosan is a biopolymer found as structural material in some fungi, but it is mainly obtained from the deacetylation of chitin extracted from shells of crustacean and mollusks such as shrimps, crabs, and squids. Chitin is produced in the amount of one hundred billion tons per year in nature, but its practical use is severely limited by its insolubility, in contrast to its derivative chitosan.^{3,4} Because the deacetylation of chitin hardly proceeds completely in normal heterogeneous reactions, chitosans are actually random copolymers with acetylated and deacetylated 2-deoxy-D-glucopyranose units joined by β (1 \rightarrow 4) links. The structures of chitin and chitosan are given in Figure 1.

The most distinguishing chitosan properties are its biodegradability and biocompatibility, which makes it a green polymer. Being biocompatible in this context means that chitosan can interact with biomolecules without degrading them. Also important is that chitosan is the only positively charged, naturally occurring polysaccharide,⁵ for chitosan may then interact strongly with negatively charged entities, including many lipids and proteins. This is advantageous in several of the applications envisaged, such as in transfection, where stable complexes between chitosan and DNA fragments can be formed.^{6–8} With regard to LbL films, the cationic nature of chitosan is exploited in distinct architectures with polyanions, as will be extensively discussed in this review.

Among the various uses of chitosans, of special relevance have been the biologically related applications, for chitosan was found able to promote tissue regeneration, which is used in tissue

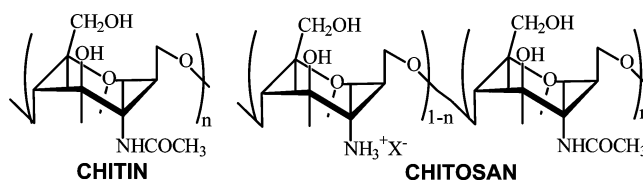


Figure 1. Chemical structure of chitin and chitosan (in its protonated form).

engineering, wound healing and implants.^{9,10} Chitosan formulations are exploited in drug delivery, where chitosan in various forms, for example, nanoparticles, microparticles, and fibers, is the vehicle for delivering drugs.² Chitosan is also used as an antimicrobial agent^{11,12} to avoid bacterial growth or kill microorganisms, in actions explained in terms of disruption of the cell membrane and penetration of chitosan with disturbance of the cell functions. Another characteristic of chitosan is its suitability as a matrix for immobilizing enzymes,^{13,14} where the enzyme activity is preserved in a solid film probably because chitosan retains a considerable amount of bound water molecules. Examples of such nanostructured films containing enzymes will be detailed in section 3.2.1.1.

This paper is organized as follows. After an introduction to nanostructured films in section 2, section 3 brings a survey of Langmuir monolayers, LB and LbL films made with chitosans. In addition to grouping the contributions of the literature into three main classes, a critical review is provided for some of the work presented in the literature. For some of the papers identified in the survey as relevant, we restricted ourselves to a succinct description in order to be as comprehensive as possible in covering the subject without turning the review excessively long. The main challenges and opportunities for the near future in this field are discussed in section 4.

2. Nanostructured Films

Nanostructured films can be built in a way that parameters such as thickness, composition, morphology, surface density,

* To whom correspondence should be addressed. E-mail: pavinatto@ifsc.usp.br.

[†] Universidade de São Paulo.

[‡] Universidade Federal de São Paulo.

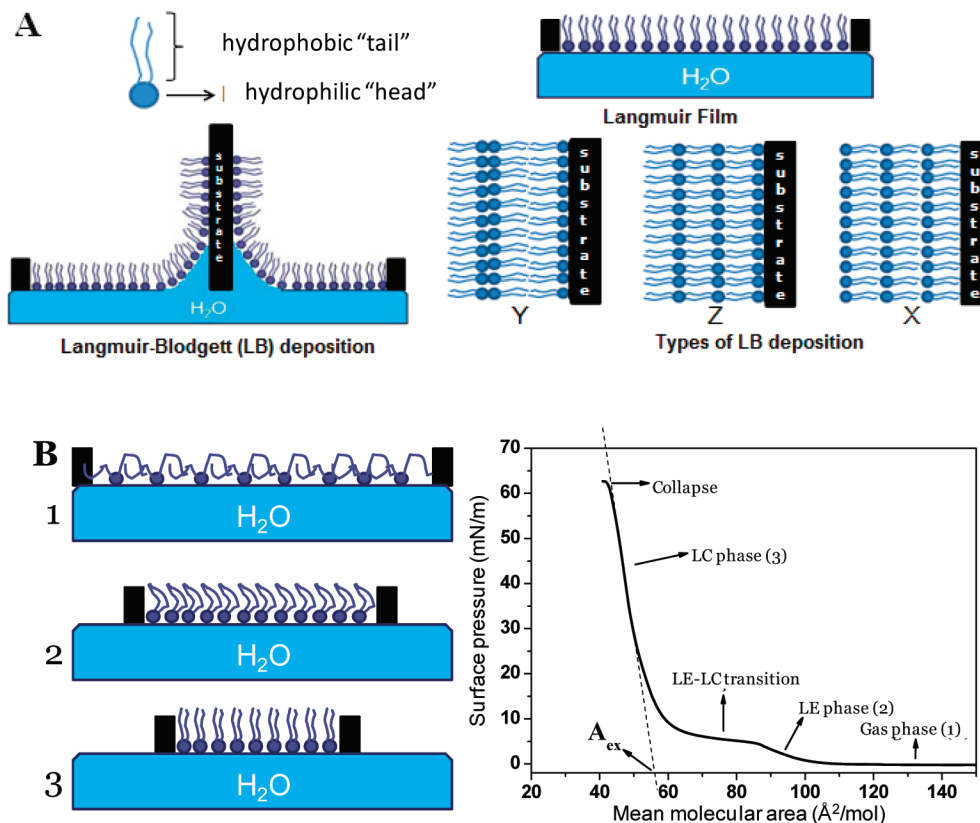


Figure 2. (A) Schematic architecture of Langmuir and LB films for an ideal amphiphilic molecule; the types of LB films that can be formed are also shown. (B) Illustration of the relative order of the molecules at the air–water interface and its correspondence with the different phases of the π – A isotherm for the phospholipid dipalmitoyl phosphatidyl choline (DPPC). A_{ex} indicates the extrapolated area of the condensed phase, which roughly matches the cross section area occupied by each molecule. LE: liquid-expanded, LC: liquid-condensed.

and roughness are accurately controlled. In many cases, these properties are manipulated at the molecular level, with intermolecular interactions investigated in detail. The approaches employed to produce such films include the Langmuir, Langmuir–Blodgett, and the layer-by-layer techniques.

A Langmuir monolayer is formed on liquid surfaces, usually at the air–water interface, when amphiphilic, insoluble molecules are spread through a solution of organic, volatile solvents. After solvent evaporation, the hydrophobic moieties of the compound spread are directed toward the air, while the hydrophilic ones are anchored to the aqueous subphase. These films can attain several degrees of packing depending on the molecular surface density, usually determined by measuring the surface tension. The decrease in surface tension caused by the presence of the interfacial film, from γ_0 for the pure aqueous (or pure water) subphase to the tension γ in the presence of the monolayer, is referred to as surface pressure (π ; where $\pi = \gamma_0 - \gamma$). A Langmuir film may then be characterized by measuring π as a function of the average area per molecule (A), which is varied by action of lateral barriers, thus yielding π – A isotherms. The surface compressional modulus (C_s^{-1} ; calculated from π – A isotherms with the equation: $C_s^{-1} = -A \cdot d\pi/dA$) is used to determine the physical states of the monolayer. Analogous to a 3D system, one may define gaseous, liquid-expanded, liquid-condensed, and solid phases for Langmuir monolayers, even including first- and second-order transitions in some cases. The breakdown of the monolayer structure, denoted by a decrease or stabilization of π at high surface pressures, is called collapse, being attributed to the formation of multilayers or vesicles as the monolayer is compressed beyond its limits in terms of molecular packing.

In addition to the surface pressure isotherms, other methods have been used to characterize Langmuir films, including surface potential measurements, Brewster angle microscopy, fluorescence microscopy, and optical spectroscopy.^{15–17} Though the study and fabrication of Langmuir films is normally aimed at optimizing conditions for the deposition of Langmuir–Blodgett (LB) films (see below), a Langmuir monolayer is itself useful for investigation of intermolecular interactions, especially in mimicking biointerfaces, such as half of cell membranes, digestive droplets, and biological tissues.

The pioneering work on liquid monolayers by Irving Langmuir in 1917¹⁸ was complemented by Katharine B. Blodgett in 1934,¹⁹ who transferred the films from the liquid surface onto solid supports. This was performed by dipping or removing a solid support from the aqueous subphase that intercepted vertically the monolayer, in a process that can be repeated to produce a multilayer film. The transfer of the monolayer takes place at a constant surface pressure, which needs to be sufficiently high, with an equally high C_s^{-1} , for restoring the initial value of π that tends to decrease as molecules are removed from the interface to be transferred. The films on the solid support are the so-called Langmuir–Blodgett (LB) films, for which many potential applications have been suggested.

The architecture of Langmuir and LB films is depicted in Figure 2A, with different phases in the π – A isotherms and the idealized molecule organization shown in Figure 2B.

LbL films are formed with the spontaneous adsorption of charged molecules in an aqueous solution, in alternating layers of oppositely charged molecules.²⁰ Adsorption is ionic and does not involve covalent bonding. In addition to the ionic interaction, secondary forces, including H-bonding, may be responsible for

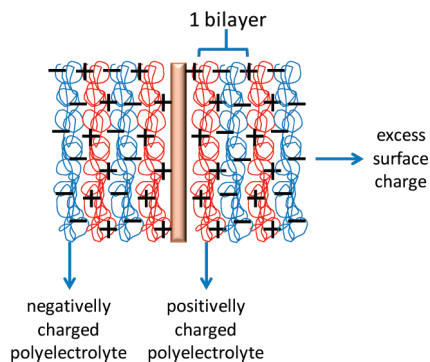


Figure 3. Typical structure of an LbL film made from polyelectrolytes.

the adsorption of LbL films,^{21,22} and therefore, a large number of molecules can be used in film formation. These include polyelectrolytes,²³ functionalized nanoparticles²⁴ and nanotubes,²⁵ phthalocyanines,²⁶ and biomolecules.²⁷ An equally wide variety of applications have been suggested for the LbL films, such as sensors,²⁸ membranes,²⁹ nanocapsules,³⁰ liposomes,³¹ and new materials for tissue engineering.³² The typical structure for LbL films is shown in Figure 3.

3. Chitosan-Containing Nanostructured Thin Films

3.1. Chitosan-Containing Langmuir and LB Films. Because chitosan is not soluble in organic solvents, its processing as Langmuir and LB films is feasible only if chemical modifications are performed in its structure. Reports have appeared in the literature where chitosan derivatives were employed, as discussed in subsection 3.1.1. Besides chemical modifications, another strategy for the incorporation of water-soluble biomacromolecules at the air–water interface is their adsorption from the water subphase, which is the topic in subsection 3.1.2.

3.1.1. Langmuir and LB Films of Chemically Modified Chitosans. To our knowledge, the first report of a Langmuir film made of derivatives of chitosan appeared in 1993,³³ with chitosan incorporating long alkyl chains attached to the primary OH and amine groups. These chitosan derivatives were soluble in chloroform and formed stable, condensed monolayers. Also, LB films up to 20 layers could be deposited, whose layer thickness was about 2 nm, consistent with the calculated size of the alkyl chain plus the size of the polymer backbone. The aim of their work was to apply the films in drug delivery systems and chiral devices.

Miyashita and co-workers described the synthesis and processing as LB films of derivatives of chitosan pentamers.^{34,35} A series of *N,N*-dialkyl derivatives were produced through the reductive amination using ethyl, butyl, heptyl, or lauryl aldehyde, and sodium borohydride. The low molecular weight and reduced interchain hydrogen bond, promoted by the insertion of the lateral alkyl groups, made the products soluble in chloroform, and thus, Langmuir films could be spread on an aqueous subphase.³⁴ The area per molecule, extrapolated to zero surface pressure for the condensed phase, was consistent with the size of the glucopyranose ring of chitosan, around 50–60 Å².³⁶ The film stability decreased when the pH of the subphase lowered, due to the higher water solubility of the chitosan derivative. Y-type LB films with up to 60 layers could be transferred onto quartz substrates. The films were smooth and had 1.74 nm per layer, according to X-ray diffraction (XRD) data.

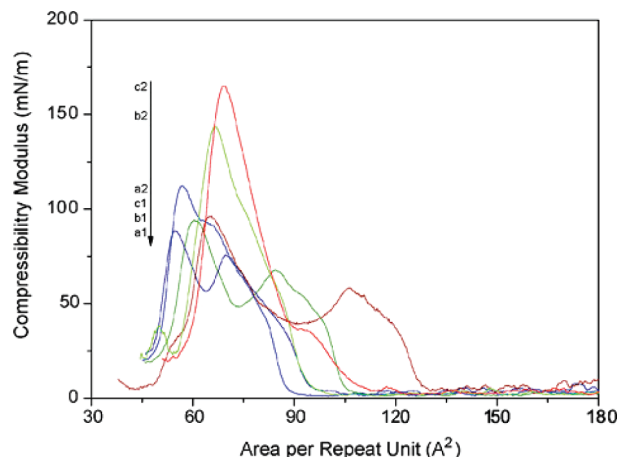


Figure 4. Compressibility modulus calculated from π - A isotherms of *N,N*-dialkyl chitosans with different side groups and chain length (see text). Reprinted with permission from ref 38. Copyright 2007 Elsevier.

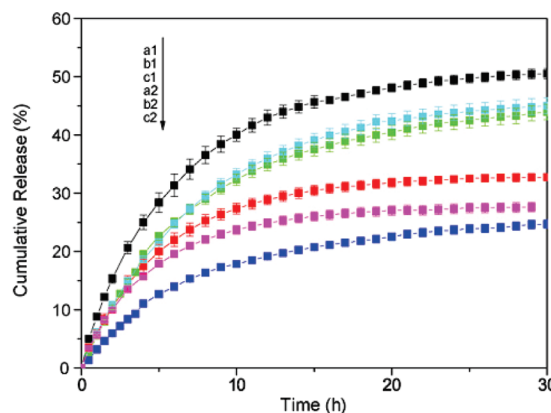


Figure 5. Drug release kinetics for self-assembled vesicles formed from the same *N,N*-dialkyl chitosans used in the experiments of Figure 4. Reprinted with permission from ref 38. Copyright 2007 Elsevier.

The same group extended the work to other amphiphilic chitosans, namely, *N,N*-dialkyl-chitosans of molecular weight ranging from 3 to 10 kDa of various chain lengths.^{37,38} The Langmuir films exhibited similar behavior to the pentamers, with condensed, stable films. The main goal was to produce vesicles from the modified polymers, which were used in drug release trials. As a general trend, the rate of drug release was slower for vesicles made of high molecular weight chitosans and longer alkyl groups as side chains. The efficiency of drug release of the vesicles could also be related to the profile of the surface pressure isotherms. In general, the higher the compressibility modulus of the Langmuir film (C_s^{-1} , calculated from the π - A isotherms) the slower was the release rate from the vesicles of vitamin B₁₂ (model drug). This relationship is shown in Figures 4 and 5, where the maximum compressibility modulus increased for the products according to the order $a_1 < b_1 < c_1 < a_2 < b_2 < c_2$ (Figure 4), and the inverse trend (decreasing in the same order of samples) is observed for the release rate (Figure 5). As a vesicle is formed by one or multiple bilayers of lipids, which have a similar structure to a Langmuir monolayer, the correspondence of Figures 4 and 5 is an interesting example of how vesicle properties can be predicted upon studying Langmuir monolayers. This correspondence is expected because monolayers with high C_s^{-1} are more rigid, and therefore, vesicles made with such membranes should be a more difficult barrier for the drug to pass. Chitosan samples represented with the subindex “1” were produced from a 3 kDa starting material,

while those with the subindex "2" were obtained with a 10 kDa chitosan. The letters a, b, and c indicate the substitution with octyl, decyl, and dodecyl groups, respectively.

Wu Y. and co-workers synthesized amphiphilic chitosans with an alkyl group combined with a cinnamoyl chromophore to facilitate the optical characterization of the LB films.³⁹ Through amine protection with a methanesulfonic counterion combined with a two-step synthesis, a chitosan could be modified with octanoyl moieties at both hydroxyl groups and cinnamoyl units at the amine group. This derivative was named octanoylchitosan cinnamate, for which the Langmuir film displayed a low collapse pressure of 25 mN/m and a limiting area of 100 Å². This large area, twice the value expected for the glucopyranose ring, was attributed to the hindering of packing imposed by the cinnamate side groups, which have restricted mobility. LB films of this derivative were deposited onto hydrophobic quartz plates at 10 mN/m. Because the transfer ratios during the downstroke were only 0.6, while they were close to 1.0 in the upstrokes, the films were considered the XY-type, as they possess an intermediate structure between X- and Y-type LB films depicted in Figure 2A. The main feature of the deposited LB films was the chirality arising from the chitosan backbone helices, which was preserved in the compressed monolayer. Indeed, in contrast to drop-cast films or solutions, the circular dichroism signal pointed to a uniaxial orientation of the polymer chains.

Mixed films were obtained with cholesterol and another chloroform-soluble derivative of chitosan, namely, *O,O*-dipalmitoyl chitosan.⁴⁰ This derivative formed nonmonomolecular Langmuir films whose surface pressure isotherms depended on the volume of solution spread. When mixed with cholesterol, the isotherms had a well-defined collapse pressure, but the miscibility was not ideal for any proportion of cholesterol. The neat films of the chitosan derivative could be transferred from the air–water interface to gold plate substrates, and the LB film homogeneity was confirmed by Fourier-transformed infrared spectroscopy (FTIR). The *O,O*-substituted chitosan could be cross-linked with glutaraldehyde, thus, forming a polymer gel employed in removing cholesterol from THF–water solutions. Tong et al.⁴⁰ showed that 20 mg/mL of the chitosan derivative gel in solution could recover up to 33% of the cholesterol initially dissolved (initial concentration of 1.5 mmol L⁻¹). This binding to cholesterol was not observed for unmodified chitosan or chitin samples, being ascribed to dispersive interactions between the alkylic palmitoyl chains and the sterol. The authors then suggested that this chitosan derivative may be used in sequestering cholesterol from foods.

3.1.2. Langmuir and LB Films Formed by Chitosan Adsorption from the Subphase. Many water-soluble biomacromolecules exhibit a surface excess, thus, forming Gibbs monolayers at the air–water interface. (A Gibbs monolayer at the air/water interface is formed by a soluble material that comes from the bulk of the subphase.) For instance, the hydrophobic and hydrophilic amino acid residues in polypeptide structures confer surface activity to proteins. However, Gibbs monolayers are not normally formed with chitosan owing to its very limited surface activity that depends on the molecular weight, pH, and degree of acetylation (DA). To overcome the poor surface activity, one can form a Gibbs or a Langmuir monolayer at the air–water interface of a compound that promotes the chitosan interaction. The film may be made with polymers or typical amphiphilic molecules, but in most cases, biologically relevant molecules are used, including phospholipids and cholesterol, as they not only promote chitosan adsorption, but also serve as biomembrane models.

3.1.2.1. Adsorption of Chitosan to a Bare Air–Water Interface. Chitosan in acidic aqueous solutions is supposed to remain soluble and does not present any surface activity, analogous to typical polyelectrolytes. Schulz et al.⁴¹ studied the emulsification properties of chitosan and stated that chitosan was not surface active for the air/water interface, but had non-negligible activity for oil–water interfaces from which its emulsifier properties arose. The lack of surface activity of chitosan in diluted solutions was also discussed by Babak and co-workers,⁴² while studying complexes of chitosan and carboxymethyl-chitin with the surfactants sodium dodecyl sulfate (SDS) and tetradecyltrimethylammonium bromide (TDAB). The surfactant efficiency, that is, the ability to induce a decrease in surface tension, was higher for the complex than for the pure surfactants in solution at the same concentration, which was attributed to the formation of ionic pairs at the interface. This improved surface activity was also observed when alkylated chitosan and the surfactants (SDS and TDAB) were in the solution. But polysoaps with a degree of substitutions equivalent to the proportion of surfactant in the complexes (with alkyl chains of the same size) were less efficient at causing reduction in the surface tension. The surface activity of a chitosan sample with a molecular weight of 330 kDa and DA of 10% was almost nonexistent up to the concentration of 1.6 mg/mL.

The surface activity of alkylated chitosans was enhanced by increasing the size of the alkyl chains up to 12 carbons, forming diffusion-dependent Gibbs monolayers.^{43–49} These derivatives formed physical gels owing to intermolecular hydrophobic interactions or hydrogen bonding. The elastic properties of the Gibbs films were inferred from dynamic elasticity measurements. After the adsorption of these modified chitosans at the air–water interface, aggregation occurred via lateral hydrophobic interactions between covalently bound alkyl chains and the hydrophobic moieties (acetylamide groups) of chitosan.

Gargallo et al. used chitosan dissolved in the subphase to study its effect over a Langmuir monolayer of poly(maleic anhydride-*alt*-stearyl methacrylate) (P(MA-*alt*-StMA)).⁵⁰ The lift-off area in the surface pressure isotherm increased from 56 to 64 Å²/mol and the collapse pressure decreased from 52 to 31 mN/m with the addition of 3 mg/mL of chitosan in the acetic acid solution (0.3 mol/L) as the subphase. Such changes in the surface-pressure–area isotherms were attributed to the migration of chitosan from the bulk of the solution to the interface. In contrast to the reports of Babak et al. and Schulz et al.,^{48,49} Gargallo et al.⁵⁰ suggested that chitosan is itself surface active on the basis of surface tension measurements using the de Du Noüy method. Adsorption of chitosan from the solution led to an increase in surface pressure, which increased with the chitosan concentration until saturation that occurred at about 5 mg/mL. The maximum increase in pressure ($\Delta\pi$) was 11 mN/m for subphase temperatures from 10 to 35 °C, while for 45 °C it was 15 mN/m, as illustrated in Figure 6. Through thermodynamic calculations, the authors confirmed that the chitosan adsorption (molecular weight of 161 kDa and DA of 18%) was spontaneous, with an estimated $\Delta G_{\text{adsorption}}$ of -9.9 kJ/mol at 25 °C.

Qun et al.⁵¹ measured the surface tension (γ) of several chitosan samples and correlated the changes in γ with varying chitosan concentrations with the polymer conformation in solution. They showed that the surface tension of chitosan solutions in the range of concentrations between 0 and 4 mg/mL is the same surface tension of the pure water, and structural

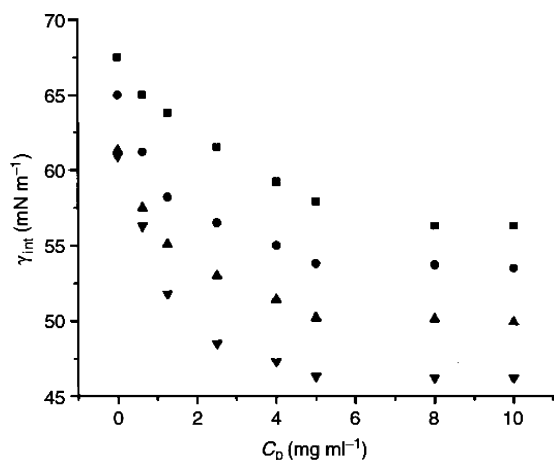


Figure 6. Decrease in surface tension vs concentration of chitosan in 0.3 M aqueous acetic acid solutions at four different temperatures: (■) 283 K; (●) 298 K; (▲) 308 K; (▼) 318 K. Reprinted with permission from ref 50. Copyright 2004 John Wiley and Sons.

parameters of the polymer do not have any effect over this property. However, for chitosan solutions with concentration above 4 mg/mL the surface tension was affected.

There has been considerable work with chitosans adsorbed on polymer films at the air/water interface. Mai-ngam⁵² synthesized a chitosan grafted-copolymer with poly[ethyleneoxide] (PEO) and hexyl side chains, where the starting material was a low molecular weight chitosan chloride ($M_w = 5$ kDa). This chitosan chloride was soluble in water, but the authors still claimed that it exhibited surface activity.

Pépic and co-workers⁵³ used chitosan as an additive in a triblock-copolymer ((EO)₁₀₀-(PO)₆₅-(EO)₁₀₀) solution, then affecting the surface activity and formation of aggregates of the copolymers in a way that depended on the ionic strength, pH, temperature, and polymer concentration. In general, the CMC (critical micellar concentration) and CAC (critical aggregation concentration) increased with the relative concentration of chitosan. The authors measured a very small surface activity for the bare chitosan. The polymer, with molecular weight of 150 kDa and DA of 15.5%, when employed in concentrations below 0.5 mg/mL, caused a decrease smaller than 2.5 mN/m in the surface tension of pure water or pure acetate buffer, pH 5.9. Only for chitosan solutions at 0.5 mg/mL in acetate buffer with pH of 6.4 a shift in γ of 6–7 mN/m was observed. But this can be attributed to the poor solubility of chitosan in pH values close to the pK_a of the amine groups.

The absence of surface activity for chitosan in very diluted solutions (concentrations below 0.5 mg/mL) was also stated by Stenger and coauthors,⁵⁴ who used chitosan to enhance the surface activity of pulmonary surfactant aggregates used as a drug against lung diseases. This increase is caused by the formation of ion pairs of chitosan-surfactant in solution.

It is concluded that discrepancies exist in the literature with regard to the surface activity of chitosan. Even though chitosan behaves as a polyelectrolyte at low pH, which makes it highly soluble in water, some authors reported the intrinsic tendency of chitosan to migrate to the surface. The reports on chitosan surface activity are summarized in Table 1. The reasons why these discrepancies appear may be that (i) chitosans can exhibit a variety of structures and compositions, depending on the molecular weight, DA, and degree of ionization of the amine group and that (ii) viscous effects for increasingly concentrated chitosans may affect the surface tension.

3.1.2.2. Adsorption of Chitosan to Preformed Langmuir and LB Films: Biomembrane Models. In many biological applications, chitosan interacts with interfaces such as cell membranes, human tissues, and digestive droplets.^{2,4,5} A suitable model to mimic cell membranes is a Langmuir monolayer or an LB film, as already demonstrated in theoretical and experimental studies.^{55–57} While liposomes and vesicles can better mimic the bilayer structure of a cell and allow the study of transport phenomena, Langmuir monolayers and LB films offer the advantages of easier control of film composition and state of packing. Furthermore, with Langmuir and LB films a number of surface specific techniques can be used to study the molecular level interactions.

Fang and Chan⁵⁸ studied the interaction of chitosan with DPPC bilayers formed onto silica wafers with the fusion of small unilamellar vesicles. Chitosan was capable of nucleating at the defects of the lipidic bilayer, forming 33 nm high aggregates after 1 h of dipping in a 0.025 mg/mL chitosan solution in a phosphate buffer. For a longer dipping time of 48 h, AFM images showed that the film had chitosan clusters with 1 μ m of lateral dimension and 56 nm in height. No adsorption of chitosan was noted on bare mica surfaces. The nucleation of chitosan clusters in the DPPC bilayer was correlated with the condensing effect that chitosan had on DPPC Langmuir monolayers when adsorbed from the subphase. The chitosan used had molecular weight of 113 kDa and DA of 12.5 and was dissolved in a subphase in the concentration of 0.008–0.024 mg/mL. The condensation of the DPPC monolayer increased with the chitosan concentration.⁵⁸

The interaction between chitosan and Langmuir monolayers of cholesterol was studied for a chitosan sample with $M_n = 108.7$ kDa and DA = 15%,⁵⁹ dissolved in an acidic subphase in the concentration range from 0.050 to 0.300 mg/mL. Chitosan induced the monolayer to expand, as inferred through surface pressure and surface potential isotherms and Brewster angle microscopy (BAM) images. The extent of expansion saturated at 0.100 mg/mL of chitosan in the subphase. The results were rationalized by assuming an interaction between specific groups of chitosan ($-\text{NH}_3^+$ and $-\text{OH}$) and cholesterol ($-\text{OH}$), mainly via hydrogen bonds. This hypothesis was also later considered by other authors.^{60,61}

Parra-Barraza et al.⁶⁰ used monolayers of stearic acid and cholesterol as membrane models to interact with four distinct chitosans: HMW: high molecular weight ($M_v = 267$ kDa and DA = 16%); MMW: medium molecular weight ($M_v = 102$ kDa and DA = 22%); chitosan chloride ($M_v = 3.5$ kDa and DA = 22%); and HYPM: hydrophobically modified chitosan = *N,N*-dodecyl chitosan (M_v not informed, DS not informed, and DA = 27%). For the same concentration in the subphase (0.100 mg/mL), all the chitosans caused expansion of cholesterol and stearic acid Langmuir monolayers. The effects were more pronounced for cholesterol films, with the largest expansion induced by HMW that caused a change of 15.9 $\text{\AA}^2/\text{mol}$ in the extrapolated area. HYPM was the only one to induce the appearance of a liquid-expanded phase in the isotherm of cholesterol, but all chitosans made the film more compressible, as measured through the surface compressional modulus (C_s^{-1}). Interestingly, the degree of expansion for cholesterol monolayers increased with the concentration of MMW in the subphase, with no saturation up to 0.300 mg/mL. In contrast, for stearic acid monolayers the magnitude of expansion was almost the same, regardless of the MMW concentration. For both amphiphilic materials, the effects caused by chitosan chloride were smaller than for the other chitosans, which was attributed to the low

Table 1. Summary of the Results on the Chitosan Surface Activity from the Literature

molecular weight (kDa)	DA (%)	concentration (mg/mL)	$\Delta\pi$ (mN/m)	solvent	ref
NA ^a	11	NI	negative	1% (v/v) AcOH ^b	48
330	10	lower than 1.6	0	acetate buffer 0.05 mol/L; pH 4.6	49
161	18	5 ^c	11	0.30 mol/L AcOH	50
5 samples from 80 to 1880	6 or 10	lower than 4	0	0.10 mol/L AcOH	51
1880	10	higher than 5	-13	0.10 mol/L AcOH	51
870	11	lower than 10	0	0.10 mol/L HCl	51
5	3.5	60 ^d	17	pure water	52
150	15.5	lower than 0.5	2.5	acetate buffer 0.05 M; pH 6.5	53
120	20	lower than 0.5	0	saline-HCO ₃ buffer ^e	54

^a NA: not available. ^b AcOH: acetic acid. ^c Concentration at which the highest $\Delta\pi$ of 11 mN/m was obtained (temperature 298 K). The surface activity exponentially increased from 0 to 5 mg/mL, and saturated above this concentration. ^d CMC measured for chitosan chloride. ^e Buffer composition: 150 mM NaCl + 2 mM CaCl₂ + 0.2 mM NaHCO₃ and pH adjusted to 5.5 with the addition of HCl and NaOH.

Table 2. Features from the π -A Isotherms of Cholesterol with the Different Chitosan Samples Added in the Subphase^a (Reproduced from Ref 60. Copyright 2005 American Chemical Society)

chitosan mixed with cholesterol	chitosan % (w/w)	A_0	A_{ex}	C_s^{-1}	A_m
cholesterol	0	44.0	39.6	417	41.6
HYPM	0.01	64.9	48.0	272	46.1
	0.02	69.2	55.8	207	51.0
	0.03	76.6	61.5	191	52.8
HMW	0.01	58.3	55.5	233	53.1
	0.02	60.6	56.9	187	52.7
	0.03	71.5	68.9	160	53.5
MMW	0.01	54.0	51.4	282	49.1
	0.02	59.8	56.7	202	50.7
	0.03	71.2	67.2	133	60.1
chitosan-chloride	0.01	49.5	47.5	255	43.7
	0.02	51.5	49.5	229	43.0
	0.03	51.5	49.8	254	43.4

^a A_{ex} = extrapolated area; A_0 = area of the "onset of condensation"; C_s^{-1} = maximum compressional modulus; A_m = area at which C_s^{-1} was attained.

molecular weight and high solubility of this compound. Some of the results mentioned are summarized in Table 2.

Langmuir-Blodgett films of cholesterol and stearic acid were deposited from Langmuir films formed over subphases containing different chitosans, and characterized by AFM. In contrast to the work of Fang and Chan,⁵⁸ MMW-chitosan could be deposited by dip coating over mica slides, forming a film with root-mean-square (rms) roughness of 12.3 and 45.6 nm high aggregates. When deposited together with the lipids, the chitosans appeared also as aggregates in the film, but in this case the size of such aggregates was smaller and depended on the molecular weight. In addition, it was inferred from the AFM images that the aggregate distribution on the surface was more homogeneous in films of cholesterol than in the stearic acid films. For the latter, a higher quantity of isolated domains of chitosan could be seen. It was then concluded that chitosan interacts more effectively with cholesterol than with stearic acid. The author also conducted theoretical calculations to show that the interaction between chitosan and the sterol occurs via $-\text{NH}_3^+$ and $-\text{OH}$ groups. For chitosan and deprotonated stearic acid, this interaction was rather dominated by the electrostatic binding between $-\text{NH}_3^+$ and $-\text{COO}^-$ groups.

The interaction between chitosan and Langmuir monolayers of cholesterol was also described by Wydro et al.⁶¹ Chitosan caused the cholesterol monolayer to expand, an effect that increased with chitosan concentration up to saturation. Interestingly, despite the use of a different chitosan sample ($M_w = 330$ kDa and DA = 30%), saturation occurred at 0.100 mg/mL, the same value reported by Pavinatto et al.⁵⁹ The monolayer C_s^{-1} was continuously reduced with the addition of chitosan up to a concentration of saturation of 0.200 mg/mL. This shows that the incorporation of chitosan in the film may saturate at 0.100

mg/mL, but the film compressibility is affected up to a higher chitosan concentration. The effects of chitosan over Langmuir monolayers of stearic acid (SA) and fatty acids with the same chain length but distinct degrees of saturation (oleic, linoleic and α -linoleic) were reported in the same paper. Chitosan also expanded these films up to a concentration for saturation between 0.050 (for SA) and 0.100 mg/mL (for linoleic and α -linoleic acids). The most remarkable feature was the more effective interaction for the disaturated and trisaturated acids linoleic and α -linoleic acids. Furthermore, the fact that C_s^{-1} decreased for SA and increased for all the other fatty acids also deserves attention. Based on their experiments the authors proposed that the interaction of chitosan with the studied materials was initiated by chitosan adsorption to the films, triggered by electrostatic and hydrogen bond interactions. Moreover, they speculated that in a second step chitosan penetrated into the film establishing hydrophobic interactions with the apolar region of the monolayers.

After the initial work with cholesterol,⁵⁹ our group in Brazil started using Langmuir monolayers of the phospholipids DPPC and dipalmitoyl phosphatidyl glycerol (DPPG), and a chitosan sample with M_n of 108.7 kDa and DA = 15%.⁶² Analogous to the films of cholesterol, chitosan expanded the phospholipid monolayers and a concentration of saturation of 0.200 mg/mL was observed. The surface compressional modulus calculated from the surface pressure-area isotherms decreased with chitosan in the subphase. However, the dilatational elasticity measured with the pendant drop technique showed a dramatic increase with the incorporation of chitosan, especially for the negatively charged DPPG. One may understand such different behavior because C_s^{-1} is taken from the isotherm obtained in equilibrium, while the elasticity from drop experiments is

measured dynamically, at a frequency that can prevent film accommodation. The most important conclusion from this first work arose from the combination of surface pressure and surface potential measurements. As the maximum signals at the end of the compression for both measurements were almost the same with or without chitosan in the subphase (for both lipids), a model was constructed for the interaction. It was supposed that chitosan, which presented surface activity induced by the interfacial film of phospholipid, could adsorb and penetrate in the film modulating its properties. However, for higher states of packing of the membrane, it was assumed that chitosan was expelled from the interface, being located at the subsurface of the monolayer (see ref 62 Figure 8 and its discussions).

In a subsequent work, the phospholipid dimyristoyl phosphatidic acid (DMPA) was used for taking advantage of the easiness in transferring multiple layers of DMPA onto solid supports as LB films.⁶³ In the latter paper and in all other subsequent works from our group, the chitosan sample used had a M_n of 113 kDa and DA = 22%. For Langmuir films of DMPA approximately the same trend of effects found for DPPC and DPPG was observed with the addition of chitosan to the subphase, that is, expansion of the film and decrease in the surface compressional modulus. However, in this case the dynamic surface elasticity also decreased with the addition of chitosan, which was attributed to the strong electrostatic binding between the materials. This hinders sudden conformation changes in phospholipid packing and chain orientation when the surface goes through a mechanical deformation. In addition, the higher surface potential signal observed for the compact film in the presence of chitosan led us to infer that chitosan was not expelled from the interface in this system.

With the help of LB film characterization, we confirmed the hypothesis that chitosan was not expelled. The transfer of about 150 ng of chitosan along with about 110 ng of DMPA for each layer in an 11-layer LB film was measured through QCM nanogravimetry. The presence of chitosan in the LB film was confirmed by FTIR measurements. The AFM images of chitosan-containing LB films displayed large patches with height between 50 and 150 nm, resembling the patches reported by Parra-Barraza et al. for LB films of cholesterol.⁶⁰ An increase in the film roughness (rms) from 0.26 to 25.8 nm was caused by the presence of 0.200 mg/mL of chitosan in the subphase of the precursor Langmuir film. Even when the large patches were excluded from the calculations, the rms value (3.16 nm) was 1 order of magnitude higher than that for the pure DMPA LB film. Therefore, chitosan was present both in large aggregates and as subsurface for the DMPA monolayer.

A more complex model for the membrane was studied that involved mixed films of cholesterol and phospholipids, and using additional, sophisticated surface characterization techniques, namely, PM-IRRAS (polarization modulated infrared reflection absorption spectroscopy) and SFG (sum-frequency generation spectroscopy).⁶⁴ The surface pressure and surface potential isotherms were not altered when the proportion of cholesterol and DMPA in the Langmuir film was varied, provided that the subphase contained chitosan. This unexpected result means that replacing cholesterol by DMPA molecules did not change the isotherms. The explanation was based on the driving force for the chitosan action, believed to arise from the electrostatic interaction between NH_3^+ groups from chitosan and DMPA. Because such interaction occurs in a restricted number of sites, not all DMPA molecules interact with chitosan. Those that remain surface inert can be replaced by cholesterol molecules without affecting the isotherms significantly because the cho-

lesterol molecules occupy the area left by DMPA. In spite of this inert role, Pavinatto et al. concluded that cholesterol modulated the chitosan penetration and its effect on the ionization of DMPA head groups.

The effects from chitosan on DMPA/cholesterol monolayers also included an increased order of the DMPA chains, which was inferred from the appearance of a peak at 2883 cm^{-1} in the PM-IRRAS spectrum. The chain alignment was preserved in deposited LB films, according to SFG measurements, where the order parameter of the mixed film increased with incorporation of chitosan.⁶⁴ Other effects from chitosan which could be identified with the PM-IRRAS measurements were the slight change in orientation of the phosphate groups in DMPA and the change in the extent of chitosan incorporation in the monolayer. The amount of chitosan at the interface increased up to a pressure of 15 mN/m, which points to penetration into the region of the alkyl tails of DMPA. From the observations above, the authors proposed a model for the interaction of chitosan with DMPA/cholesterol monolayers, which comprises two steps: (i) first, chitosan migrates from the subphase to the interface and interacts with the mixed monolayer, especially via electrostatic forces between its protonated amine groups and the negatively charged phosphate groups of DMPA; (ii) then chitosan penetrates and expands the monolayer, also causing the DMPA hydrophobic tail to be more oriented.

The initial studies and the available literature pointed to the electrostatic interactions between amine groups of chitosan and phosphate moieties in the phospholipid headgroups as the driving force for the interaction.⁶⁵ However, from a comparison with the effects of an analogous polyelectrolyte (polyallylamine: PAH), we could observe that the global effects from chitosan are also due to additional contributions from other intrinsic parameters of the polymer. Such parameters are believed to be mainly specific chain conformations adopted by chitosan in solution (e.g., rod-like, random-coil, helix, etc.) and synergistic effects from hydroxyl groups and from the polysaccharide backbone.⁶⁵

A promising, almost unexplored, application of chitosan was investigated in ref 66 in which chitosan was able to remove a protein, β -lactoglobulin (BLG), from negatively charged phospholipid monolayers. This was inspired in the work by Casal et al.⁶⁷ who showed that chitosan could sequester BLG from milk. In the experiments with Langmuir films, Caseli et al. showed that BLG could be incorporated into DMPA monolayers at the air–water interface, but BLG was removed when chitosan was injected after the saturation of BLG adsorption. This effect on the adsorption kinetics is illustrated in Figure 7, which features a final pressure that is about 2 mN/m smaller than the initial pressure, and indicates that some DMPA molecules were removed from the monolayer together with BLG. A model for this phenomenon is shown in Figure 8. This action of chitosan is specific for membranes made with negatively charged phospholipids and for the protein BLG. Such specificity indicated again that chitosan activity is associated with its protonated amino groups and interaction with negatively charged groups.

3.2. Chitosan-Containing LbL Films. Chitosan, being positively charged at low pHs, can be attached electrostatically to negatively charged compounds, including polymers, carbon nanotubes, and inorganic complexes. The electrostatic LbL approach to produce nanostructured films suggested by Decher et al.²² is based on the deposition of alternating layers of positively and negatively charged species. Usually the thickness per layer depends on the physical conditions of deposition and

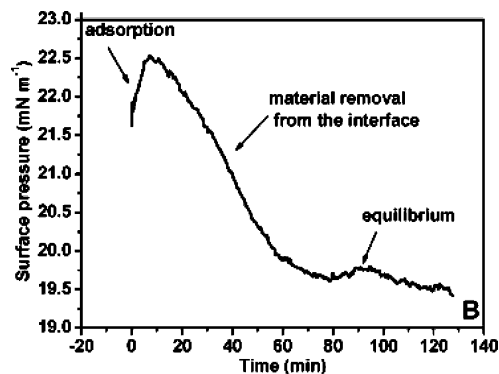


Figure 7. Kinetics of BLG adsorption onto a DMPA Langmuir monolayer in the presence of chitosan. Three events can be recognized: (i) BLG adsorption; (ii) chitosan incorporation and material removal from the interface; and (iii) stabilization. BLG and chitosan concentration were 0.25 mg/mL and 0.20 mg/mL, respectively. Reproduced from ref 66. Copyright 2008 American Chemical Society.

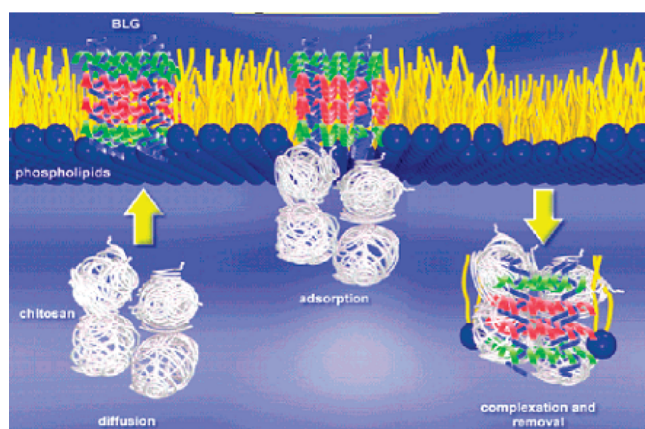


Figure 8. Model for the BLG removal from a DMPA Langmuir monolayer caused by chitosan. Reproduced from ref 66. Copyright 2008 American Chemical Society.

on the nature of the material. In many cases, monomolecular layers can be produced.

Chitosan has been deposited in LbL films for more than a decade,⁶⁸ serving as matrix to build devices with tailored properties for a variety of purposes, including sensing, drug and gene delivery, anticoagulant devices, osseous implant, and healing. Some of the applications of chitosan-containing LbL films are reported as follows.

3.2.1. Matrix for Active Elements in Sensors. Chitosan can be coimmobilized with several substances on solid supports for sensors, normally biomolecules with molecular recognition properties, including enzymes, antigens, and parts of nucleic acids. Electrochemical sensors can have their performance improved by enhancing the electron transport with incorporation of carbon nanotubes, phthalocyanines, and porphyrins, whose adsorption may be facilitated with a chitosan matrix.

3.2.1.1. Sensors with Enzymes. Chitosan has been the matrix for several enzymes using various techniques,¹³ with the enzyme activity being preserved,⁶⁹ probably owing to its biocompatibility. Sensors have been produced to identify wines, sugar, and fish and to detect organic compounds in waste waters, in addition to sophisticated biosensors for in situ measurements of environmental pollutants and metabolite control in artificial organs. Table 3 provides a list of enzymes immobilized in chitosan matrices. The most popular enzyme is glucose oxidase, while the most common method for analyte determination is

electrochemistry, with analysis of the electric current generated during the oxidation of glucose into hydrogen peroxide and gluconic acid.

Optical methods have also been employed, as in the determination of paraoxon,⁶⁹ an organophosphate compound that is an acetylcholinesterase inhibitor used as an active metabolite of the insecticide parathion. Proteins with heme groups attached, such as hemoglobin and myoglobin, are used due to the specificity of the heme chemical group, able to transport oxygen, and serve as a catalyst of hydrogen peroxide.

In sensors based on electrochemical measurements, using chitosan as a matrix may be disadvantageous because chitosan may hamper the flux of electrons between the analyte aqueous solution and the electrode. For instance, successive layers of chitosan/glucose oxidase may not necessarily increase the current signal because only the enzyme at the uppermost layer seems to be active.⁹² Therefore, optimized conditions must be sought for enhancing sensing performance.

3.2.1.2. Sensors with Antigens and Parts of Nucleic Acids.

There have also been cases where the molecular recognition capability of the biosensor involves interaction with other biomolecules. For instance, DNA has been adsorbed with chitosan for detecting oligonucleosides electrochemically.^{93,94} In another study,⁹⁵ *Escherichia coli* cells were encapsulated with LbL films of chitosan and a polyanion (alginate, hyaluronic acid, or a oligonucleoside). This system was then used as a biorecognition system, which is useful for gene therapy. Furthermore, chitosan intercalated with DNA was fabricated to enhance the voltammetric signal of uric acid, with high selectivity to ascorbic acid.⁹⁶ Antigen–antibody systems have been used for fabrication of immunosensors, with chitosan layers alternated with a negatively charged polysaccharide (alginate) being used to immobilize an antigen on the uppermost layer.⁹⁷ The combination of glassy carbon, chitosan, gold nanoparticles, and carcinoembryonic antibody produced a bioinspired system with enhanced electrochemical properties, producing therefore an immunosensor with high sensitivity, selectivity, and stability.⁹⁸

3.2.1.3. Sensors with Compounds that Enhance the Electric Signal. These sensing systems are based on the specificity of the enzyme by its catalytic substrate, but sensors have been constructed when the electrode is modified with chitosan-based LbL films to enhance the electric signal. In this case, chitosan-based LbL films can be seen as a new hybrid material protecting the electrode. One of the most popular strategies is the use of carbon nanotubes⁹⁹ combined with chitosan, used for increasing the oxidation current in detecting dopamine,¹⁰⁰ NADH (nicotinamide adenine dinucleotide),¹⁰¹ DNA (deoxyribonucleic acid) damaging (with DNA interaction with chitosan and carbon nanotubes layers),⁹⁴ glucose (with Prussian blue as mediator),¹⁰² and hydrogen peroxide.¹⁰³ In the latter case, chitosan and carbon nanotubes were immobilized in an LbL fashion on gold nanoparticles, with cytochrome C immobilized on the uppermost layer. This example shows how the fabrication of hybrid materials with chitosan, in a variety of architectures, has provided materials with tailored properties. Also, Fe₃O₄ nanoparticles were coimmobilized with chitosan to enhance the electrode conductivity,¹⁰⁴ analogous to the use of dyes to enhance the optical signal for ethanol sensing.¹⁰⁵ LbL films of metal-phthalocyanines alternated with chitosan have been used to detect dopamine with low interference, where the electrochemical properties were enhanced due to optimization of the electron transfer through the film.¹⁰⁶

3.2.1.4. Other Kinds of Sensors. Chitosan-alginate LbL films were deposited on a gold support and made to interact with

Table 3. Enzymes and Heme-Proteins Immobilized on Chitosan Matrix with the LbL Technique for Sensing

enzyme/protein	analyte	method of detection	ref
glucose oxidase	glucose	optical	70
urease + superoxide dismutase	urea	electrochemical	71
glucose oxidase	glucose	electrochemical	72
glucose oxidase	glucose	optical	73
pectinase	pectin	optical	74
hemoglobine	hydrogen peroxide	electrochemical	75
glucose oxidase	glucose	electrochemical	76
glucose oxidase	glucose	electrochemical	77
glucose oxidase	glucose	electrochemical	78
glucose oxidase	glucose	electrochemical	79
glucose oxidase	glucose	electrochemical	80
tyrosinase	neurotransmitter metabolite	electrochemical	81
polyphenol oxidase	catechol and phenol	electrochemical	82
organophosphorus hydrolase	paraoxon	optical	69
organophosphorus hydrolase	paraoxon	optical	83
horseradish peroxidase and myoglobin	hydrogen peroxide	electrochemical	84
myoglobin	hydrogen peroxide and trichloroacetic acid	electrochemical	85
myoglobin	hydrogen peroxide and trichloroacetic acid	electrochemical	86
hemoglobin	hydrogen peroxide	electrochemical	87
horseradish peroxidase	hydrogen peroxide	electrochemical	88
pectinase	polygalacturonic acid	optical	89
glucose oxidase	glucose	optical	90
horseradish peroxidase	hydrogen peroxide	optical	91

Pseudomonas aeruginosa cells, which were detected optically by the change in refractive index of substrate + film.¹⁰⁷ Chitosan simply deposited in a layer-film on silicate was used to remove heavy metals from water such as Cd(III), Cr(III), and Cr(IV),¹⁰⁸ due to its good sorption capacity, and to detect Cu(II) with impedance spectroscopy.¹⁰⁹ LbL films containing chitosan and synthetic polymers were used in taste sensors for beverages.^{110,111}

3.2.2. LbL Films with Chitosan for Membranes and for Improving Physical and Biological Properties of Materials. In most applications for sensing with LbL films, as discussed in the last subsection, chitosan was used merely as a matrix to help preserve the activity of a biomolecule, being therefore relatively inert. The mechanical and biocompatibility of chitosan are, however, exploited in membranes and other materials. Being the only positively charged polysaccharide in nature, it can be combined with negatively charged or neutral materials, especially those of biological interest, including heparin, cellulose, hyaluronan, and dextran sulfate, for various applications. These include adsorption of proteins,^{112–118} increase in mechanical resistance,^{119–123} cytotoxicity, and protection against wound infections, enhancement of electrical conductivity,^{110,111,124–126} changes in optical and electrochromic properties,^{127–134} and use of fluorescence probes.¹³⁵

For many nanocomposites made from chitosan, it is a challenge to obtain good mechanical properties because chitosan is sensitive to humidity. To obviate this limitation, researchers^{136–138} have described the reinforcement of chitosan by nanofillers in LbL films to increase their mechanical strength. For instance, Darder et al.¹³⁹ intercalated chitosan with Na-montmorillonite, providing robust nanocomposites used in bulk-modified electrodes with easy surface renewal, ruggedness, and long-term stability. Wang et al.¹⁴⁰ prepared chitosan/carbon nanotube composites with the solution evaporation method, in which the nanotubes were homogeneously dispersed throughout the chitosan matrix. The mechanical properties (tensile modulus and strength of the nanocomposites) were greatly improved with incorporation of nanotubes into the chitosan matrix. LbL films from deacetylated chitosan and eucalyptus wood cellulose nanowhiskers (CNWs) were formed with hydrogen bonding and

electrostatic interactions between the negatively charged sulfate groups on the whisker and the positively groups of chitosan.¹⁴¹ These biodegradable nanocomposites are also expected to have enhanced mechanical and thermal properties.

Of particular importance is the interaction of chitosan with hyaluronan. Both are biocompatible materials and have been used in membranes. Salomäki et al.¹²⁰ reported the enhanced viscoelastic properties of chitosan/hyaluronan LbL films, which displayed higher mechanical strength. Chitosan-alginate was used for other purposes, as for tissue engineering in attaching hepatic cells,³² where the biocompatibility of the polysaccharide-based adsorption matrix was explored. The resistance of chitosan against external factors is also useful, for example, in preventing enzyme degradation,^{141–146} coagulation,¹⁴⁷ and microorganism attack.^{87,148–150}

Biomaterials that use LbL films with chitosan are also important. For instance, chitosan coimmobilized with heparin in several layers have been used to tailor the properties of coronary stent in terms of anticoagulation for clinic use.¹⁴⁶ The chitosan/heparin system was reported as antiadhesive and bactericide, forming a biomacromolecular self-assembly that is safe and efficient in promoting the re-endothelialization and healing after stent implantation. The LbL technique was suggested to be easy to apply to the surface of drug healing stent systems. The combination of polysaccharide LbL films with cell adhesive peptides may enhance orthopedic implant properties, replacing Ti and its alloys, thus, avoiding bacterial infection.⁸⁷ Also worth mentioning is the combination of materials to seek synergy in their properties, as gold nanoparticles, poly(L-lactic acid), chitosan, and dextran sulfate.¹⁵⁰ The metal nanoparticles usually have bactericide properties that can be combined with polysaccharides to enhance their characteristics of hydrophobicity, hemocompatibility, cytocompatibility, and antibacterial activity against Methicilin-resistant *Staphylococcus aureus*.

Actuators have been produced with chitosan LbL films in surface acoustic impedance,¹⁵¹ robotic rapid systems,¹⁵² and electroactive paper actuators.¹⁵³ Chitosan coimmobilized with poly(vinyl-sulfonic acid) and H₂PtCl₆ on platinum plates can also be applied as a catalyst layer for proton exchange membrane in direct methanol fuel cells.¹⁵⁴

3.2.3. *Chitosan Vehicles for Drug Delivery and Other Applications.* LbL films with chitosan have been reported for protein and drug encapsulation for controlled release,^{102,155–166} including gene delivery¹⁶⁷ and encapsulation of lipid capsules and liposomes.¹⁶⁸ The encapsulation of these materials imparts a higher resistance and biocompatibility with more durable drug delivery systems. Chitosan/alginate structures, for instance, are able to encapsulate enzymes and drugs for release, with a decrease in the drug bioactivity, but with enhanced storage stability.⁷¹ Grech et al. produced beads with chitosan and hyaluronic acid for controlled release of gentamicin sulfate (antibiotic to treat bacterial infection and osteomyelitis), producing biodegradable microspheres (beads) that do not require surgical removal from the body.¹⁶⁹ In this case, the release depends on the permeability and on the breakdown of the multilayer structures. The drug release has also been prolonged when ibuprofen was encapsulated with chitosan/alginate microcapsules produced by the LbL technique, with reduction of the initial burst related to the drug delivery.¹⁶⁵

The covering of liposomes and lipid emulsion by chitosan-negative polysaccharide LbL films has been reported to improve liposome stability which may control the delivery of encapsulated proteins and drugs.^{168,170–173} For instance, lecithin and chitosan LbL films encapsulating lipid droplets (emulsion) have been used to prove that chitosan does not inhibit in vitro digestibility, which may have important implications for the possible chitosan activity as a fat reducer agent in food ingestion.¹⁷⁰ Lecithin and chitosan encapsulating oil droplets have also been used as food additive.^{174,175}

4. Concluding Remarks and Perspectives

The extensive use of chitosan in a variety of applications is reflected in a large number of papers published. By way of illustration, a search on the ISI Web of Science with the keyword “chitosan” leads to almost 17000 entries in May, 2010. Most of the applications are related to its biological properties, including antimicrobial activity, wound healing ability, and ability to encapsulate biomolecules for drug delivery. In spite of the prolific scientific production in the field, little is known about the molecular mechanisms responsible for the biological activity of chitosan. It is precisely within this context that the nanostructured films may be exploited, which was also the main motivation for us to produce this review.

Using Langmuir monolayers and LB films is essential to understanding some of the molecular-level mechanisms, especially those associated with the interaction with cell membranes. The recent findings with cell membrane models have allowed one to infer that electrostatic interactions may be the most important feature in the action, but they are not the only one. Further studies are now required to elucidate this point, which should include the following as major topics: (i) use of chitosan derivatives, especially those with surface-active properties that may lead to the formation of Langmuir monolayers on their own; (ii) combination of various biomolecules in Langmuir monolayers in order to achieve a more realistic cell membrane model, as has been done with mixtures of cholesterol and phospholipids⁶⁴ and with proteins;⁶⁶ and (iii) attempts to identify the sites of interaction between chitosan and the lipids. It is likely that sophisticated experimental methods will be required to study such interactions, and here emphasis should be placed on the already mentioned spectroscopic techniques PM-IRRAS and SFG. The ultimate understanding of the molecular-level interactions will also depend on the capability of researchers in

the field in theoretical modeling. The first attempts in this regard involved molecular dynamics simulations,¹⁷⁶ but the results are only modest in terms of possible interactions with membrane models or other biomolecules. The sheer size of chitosan molecules and the intricate possibilities of conformation prevent any accurate modeling. For this very reason we shall have to wait a considerable time for realistic simulations to be carried out with quantum mechanical methods.

Though limited in the quantification of the interactions, as mentioned above, molecular modeling and the investigation of molecular-level interactions with experimental techniques may help in predicting chitosan properties with practical implications. For example, it may be possible to determine the chemical modifications to be made in the chitosan for optimizing its bioactivity in specific cases, in addition to obtaining the ideal dosage in medical applications to reduce side effects.

Equally important for nanostructured films of chitosan are the applications exploiting the control of molecular architecture of these films. As discussed in this review, chitosan films have been used in various types of biosensors, particularly because chitosan has been proven to be excellent scaffolding material to preserve the activity of biomolecules. Two additional applications are for drug delivery in patches made with chitosan films and surface modification for cell growth aimed at tissue engineering. In conclusion, the abundant evidence for the action of chitosan in nanostructured films holds the promise for real applications to be made over the next few years.

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