



Polímeros: Ciência e Tecnologia

ISSN: 0104-1428

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Associação Brasileira de Polímeros
Brasil

Pavinatto, Felipe J.; dos Santos, David S.; Oliveira, Osvaldo N.
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Polímeros: Ciência e Tecnologia, vol. 15, núm. 2, 2005, pp. 91-94
Associação Brasileira de Polímeros
São Paulo, Brasil

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Interaction between Cholesterol and Chitosan in Langmuir Monolayers

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Abstract: Chitosan incorporated in the aqueous subphase is found to affect the Langmuir monolayers of cholesterol, causing the surface pressure and the surface potential isotherms to become more expanded. The mean molecular area per cholesterol molecule in the condensed monolayer increases from 53 \AA^2 in the absence of chitosan to 61 \AA^2 for a concentration of 0.100 mg/mL of chitosan in the subphase. If additional chitosan is incorporated in the subphase, no change is noted, which points to saturation in the effects from chitosan. The interaction between chitosan and cholesterol probably occurs via hydrogen bonding. The monolayer expansion is also manifested in the monolayer morphology, as indicated by Brewster angle microscopy measurements, where larger cholesterol domains are visualized when chitosan is present in the subphase.

Keywords: Cholesterol, chitosan, Langmuir monolayers.

Introduction

Cholesterol is present in practically all cells of mammals, being essential for the functioning of some glandules and interacting with many compounds such as the lipids from the membrane and other cell components. When in excess, cholesterol may cause a number of health problems, and this has prompted researchers to search for ways of reducing it in the human body. Recently, chitosans have been suggested to be efficient in obesity treatments^[1], probably because they can interact with cholesterol. Chitosan is a biocompatible polysaccharide derived from chitin extracted from the exoskeleton of crustaceans and arthropods^[2], which has been employed in a number of applications, mainly in cosmetics^[3]. With its biodegradability, chitosan can also be used in drug delivery systems^[4]. It is clear, therefore, that studies of molecular-level interactions between chitosan and cholesterol are relevant. One possible way to investigate these interactions is to employ Langmuir monolayers that serve as simplified model for the cell membrane^[5]. In most cases, phospholipid monolayers are used^[6-8], but a number of studies exist with cholesterol, which also forms stable monolayers^[9,10] and interact with phospholipids^[6,11-13]. With this approach, information can be obtained on a number of issues, including the drug location in the membrane and the cooperative response of the membrane to drugs^[8] and peptides^[9].

In this work we obtained Langmuir monolayers of cholesterol either on a pure water subphase or on a subphase containing dissolved chitosan. When chitosan is present, the system considered is a two-phase system with an aqueous subphase covered with an amphiphilic material (cholesterol with hydrophilic and hydrophobic groups) and interacting with a third component (chitosan, soluble in

acidic medium). The monolayers were characterized with surface pressure, surface potential and Brewster angle microscopy (BAM) measurements. This study is a first step in the attempt to elucidate the activity of chitosan, which can also throw light into the interaction mechanisms between chitosan and cholesterol. It is remarked that Langmuir-Blodgett (LB) films of an amphiphilic chitosan were reported by Li et al.^[14], but to our knowledge there is no investigation of the effects from chitosan in the subphase on Langmuir monolayers.

Experimental

The cholesterol samples used in the experiments was 3β -hydroxy-5-cholestene (Aldrich), employed as received. Chitosan was obtained from desacetylation of chitin extracted from shells of shrimps^[15] and the degree of acetylation was determined as 15% by nuclear magnetic resonance, according to the method of reference^[16]. The chemical structures of these materials are shown in Figure 1.

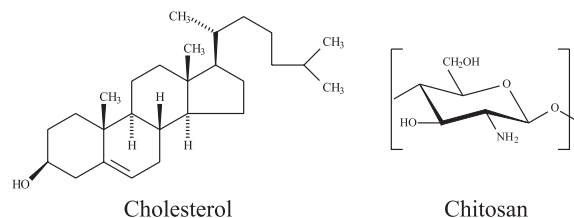


Figure 1. Cholesterol and chitosan structures

The Langmuir films were fabricated with a KSV 5000 Langmuir trough, equipped with a surface pressure sensor (Wilhelmy method) and a Kelvin probe to measure surface potential, housed in a class 10,000 clean room. Aliquots of a

chloroform (Mallinckrodt) cholesterol solution, 0.450 mg/mL, were spread on an aqueous subphase with an acetate buffer, pH 3.5. The chitosan samples were dissolved in the same buffer solutions using concentrations between 0.050 mg/mL and 0.300 mg/mL. Brewster angle microscopy (BAM) images were obtained with a BAM2 system from Nanofilm Technology (Germany), according to the experimental procedures described in reference^[17].

Results and Discussion

Langmuir films from pure cholesterol display surface pressure isotherms as shown in Figure 2A.

They are stable films, with a well-defined phase transition from the gaseous to the liquid condensed phase at 53 \AA^2 per molecule. The collapse pressure is approximately 35 mN/m, when an area of ca. $49 \text{ \AA}^2/\text{mol}$ is reached. When added to the subphase, chitosan has strong interaction with the cholesterol monolayer, and the isotherms become more expanded (Figures 2B to 2F) with the extrapolated area to zero pressure increasing. While the extrapolated area was 54 \AA^2 per molecule for pure cholesterol, it increased to 59 \AA^2 and 65 \AA^2 for 0.050 mg/mL and 0.100 mg/mL chitosan concentrations in the subphase respectively, i.e. an increase between 9% and 20% in area for these concentrations. The expansion saturates when a concentration of 0.100 mg/mL of chitosan is employed. Further addition of chitosan in the subphase has no significant effect on the monolayer properties, with the maximum area per molecule being approximately 65 \AA^2 , as shown in isotherms 2E and 2 F. Figure 3 shows the increase in mean molecular area with the chitosan concentration, where a clear saturation is observed for 0.100 mg/mL of chitosan in the subphase.

In addition, the surface pressure isotherms become less steep if chitosan is contained in the subphase. Chitosan affects the cholesterol monolayers, either by incorporating into the

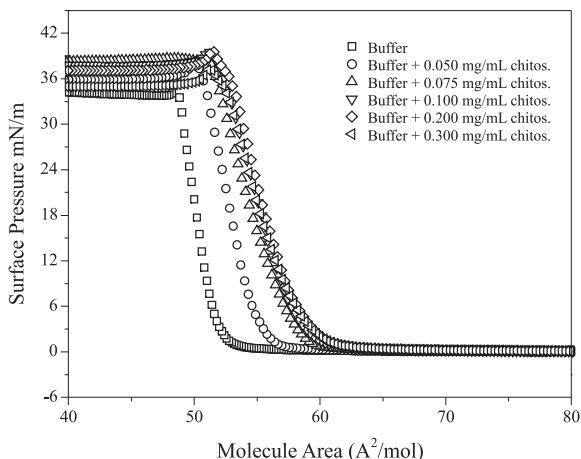


Figure 2. Surface pressure isotherms for cholesterol monolayers spread on the following subphases: (○A) buffer pH 3.5 ; (□B), (△C), (▽D), (◇E) and (◁F) buffer pH 3.5 + 0.050 mg/mL, 0.075 mg/mL, 0.1 mg/mL, 0.2 mg/mL and 0.3 mg/mL of chitosan respectively.

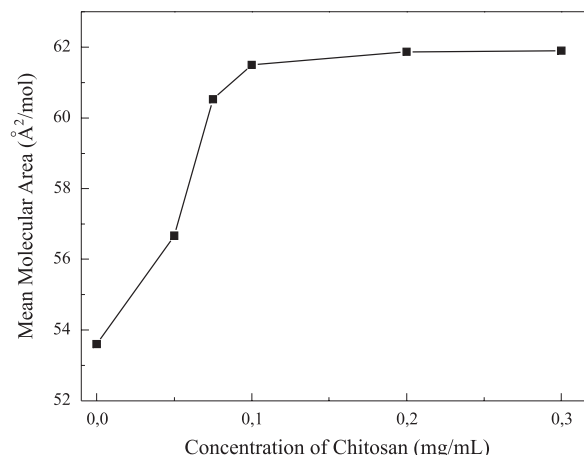


Figure 3. Mean area per molecule (extrapolated to zero pressure) vs. concentration of chitosan in the subphase

monolayer itself or by affecting the cholesterol conformation. In subsidiary experiments (results not shown) we observed that the cholesterol monolayers with chitosan in the subphase are stable for all chitosan concentrations employed in this study. For example, there is little change in area per molecule (less than 1%) during several hours when the monolayer is kept at a fixed pressure of 18 mN/m.

With regard to the surface potential isotherms, as expected they are more expanded for cholesterol monolayers spread on chitosan-containing subphases (Figure 4).

The maximum surface potential also increases slightly with the chitosan concentration. Note that the surface potential is already non-zero at the largest area per molecule plotted. In order to verify if a zero could be achieved – which means absence of large domains before compression starts – further experiments will be carried out with the compression starting at larger areas per cholesterol molecule. For aliphatic^[18] and aromatic compounds^[19] with well-defined headgroups, the measured surface potential can be related to the molecular dipole moments

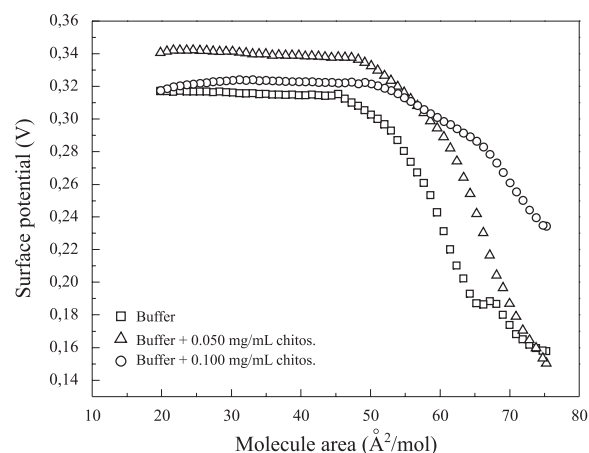


Figure 4. Surface potential isotherms for cholesterol monolayers spread on the following subphases: (□A) buffer pH 3.5 ; (△B) buffer pH 3.5 + 0.050 mg/mL of chitosan and (○C) buffer pH 3.5 + 0.100 mg/mL of chitosan.

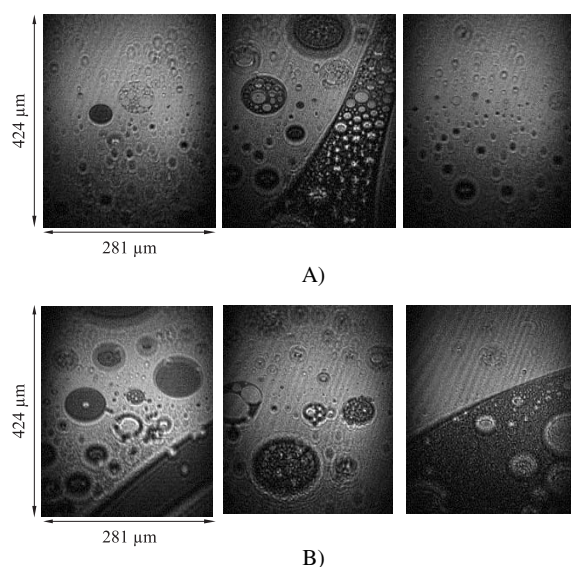


Figure 5. BAM images for cholesterol monolayers on the gaseous phase spread on (A) pH 3.5 buffer and (B) pH 3.5 buffer + 0.050 mg/mL of chitosan

using the Demchak-Fort model^[20], which depicts the Langmuir monolayer as a 3-layer capacitor with dipole moments contributing at the air/monolayer and monolayer/water interfaces, in addition to reorientation of water molecules in the subphase due to the presence of the monolayer. For the cholesterol monolayers studied here, however, this quantitative treatment cannot be performed, as the precise orientation of the molecules at the interface is not known. It can be inferred, nevertheless, that chitosan does not alter the contribution to surface potential from the cholesterol dipole moments significantly, as demonstrated by the small changes in surface potential in Figure 4 upon addition of chitosan in the subphase.

The interaction between chitosan and cholesterol is also manifested in the morphology of the monolayers, as displayed in the images acquired with Brewster angle microscopy (BAM). Figure 5 shows images obtained for cholesterol monolayers spread on the pH 3.5 buffer and another spread on a subphase containing chitosan.

Even though a number of features seen in the images may be related to experimental artifacts with interference fringes in several places, owing to the fluidity of the monolayer, it is clear that with chitosan in the subphase of the cholesterol domains become much larger, pointing to a considerable interaction between chitosan and cholesterol. It may be speculated that such interactions may occur via hydrogen bonding between hydroxyl groups present both in cholesterol and chitosan. The interaction between hydroxyl groups from cholesterol and the amine groups from chitosan is less important because at pH 3.5 almost all the amine groups are protonated and not available to establish hydrogen bonds. A quantification of the effects is not possible with the experiments carried out here because even though the degree of desacetylation of chitosan was 75 and the exact concentration of cholesterol are known, we cannot estimate how much of the chitosan comes to the monolayer interface to participate in the interaction. Experiments to

establish the kinetics of the adsorption of chitosan are being carried out to determine such an amount.

Conclusions

The interaction between chitosan and cholesterol has been probed by spreading cholesterol monolayers on subphases containing chitosan. The latter was found to expand the cholesterol monolayer considerably and also affected the monolayer morphology as seen in BAM micrographs. There is a concentration of approximately 0.100 mg/mL at which the effect from chitosan is saturated. The interaction probably involves H-bonding between the hydroxyl groups of cholesterol and hydroxyl and amine groups from chitosan. One should also mention that with the results presented here we cannot establish whether the interaction between chitosan and cholesterol is specific or not.

Acknowledgments

This work was supported by CNPQ, CAPES, FAPESP, IMMP/MCT and PRP-USP.

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