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Langmuir films containing ibuprofen and phospholipids

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ABSTRACT

This study shows the incorporation of ibuprofen, an anti-inflammatory drug, in Langmuir monolayers as cell membrane models. Significant effects were observed for dipalmitoyl phosphatidyl choline (DPPC) monolayers with relevant changes in the elasticity of the monolayer. Dipalmitoyl phosphatidyl glycerol (DPPG) monolayers were affected by small concentrations of ibuprofen, from 1 to 5 mol%. For both types of monolayer, ibuprofen could penetrate into the hydrophobic part of the monolayer, which was confirmed with polarization-modulated infrared reflection–absorption spectroscopy (PM-IRRAS). Brewster angle microscopy (BAM) images showed that ibuprofen prevents the formation of large domains of DPPC. The pharmacological action should occur primarily with penetration of ibuprofen via electrically neutral phospholipid headgroups of the membrane.

1. Introduction

The cell membrane is a complex system essential for cellular functions because it mediates interactions between the cell and its external environment. The differentiation and function, for example, depend on the composition of lipids in the membrane, which is the reason why delivery of drugs varies for different cell types [1–3]. A drug administered orally or injected intravenously seldom arrives in a specific target in the body in the appropriate concentrations to cause the expected therapeutic effect. This is easily explained by the obstacles of various kinds (anatomical, chemical and biological) that must be overcome before the drug reaches the target organ or tissue [4,5]. Ibuprofen is a non-steroidal anti-inflammatory drug, with low solubility in water (maximum solubility of 0.011 g/L or 53 μM at 25 °C) [6], which displays prolonged side effects. The inconvenience in the use of ibuprofen is mostly associated with gastrointestinal complications, since 15 to 30% of patients using this drug for a long time have gastrointestinal ulcers and bleeding, in addition to renal dysfunction [7].

Interactions of drugs with cell components involve changes in the organization of the biological membrane. Therefore, it is essential to characterize the interaction between drugs and membranes, particularly obtaining molecular-level information. Currently, this is normally done with membrane model systems, such as Langmuir monolayers [8–10] and liposomes [11]. The incorporation of substances in a Langmuir film has varying effects depending on the substance location, its electrical charge, and the method for incorporation. Though lipid monolayers are much less complex than the real biological membranes, they have been useful in modeling the interactions [12,13].

This paper deals with the effects from ibuprofen interacting with Langmuir monolayers with a zwitterionic phospholipid (DPPC) and an anionic (DPPG) phospholipid. The monolayers were characterized using surface pressure, surface potential, Brewster angle microscopy (BAM), and polarization-modulated infrared reflection absorption spectroscopy (PM-IRRAS) measurements. The main objective was to identify how ibuprofen affects the molecular packing, in addition to studying the dependence on the ibuprofen concentration and type of lipid, so that biological implications can be drawn as to the mode of action for the drug and its possible incorporation in drug delivery systems.

2. Materials and Methods

Dipalmitoyl phosphatidyl glycerol (DPPG) sodium salt and dipalmitoyl phosphatidyl choline (DPPC) were purchased from Avanti Polar Lipids, and analytical grade chloroform and methanol were purchased from Merck. Ibuprofen [α-methyl-4-(2-methylpropyl) benzeneacetic acid] of the highest purity available was acquired from Sigma. Figure 1 shows the chemical structure of DPPC, DPPG, and ibuprofen.

Two methodologies were employed to study interaction between phospholipids and ibuprofen. The first was co-spreading both components from the same solution, which is suitable to verify whether the drug can interact with the phospholipid and be incorporated in liposomes as a carrier for ibuprofen delivery [14]. DPPC and ibuprofen were dissolved in pure chloroform, and a

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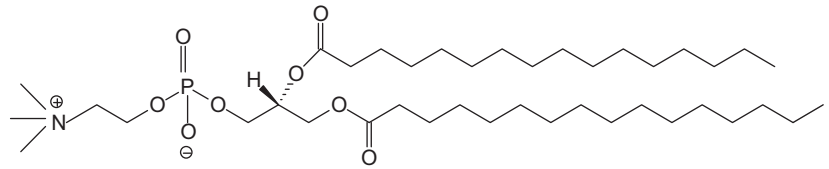
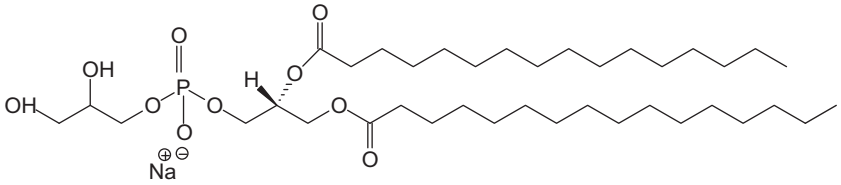
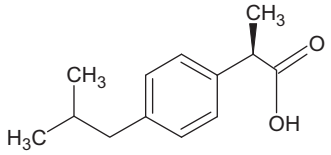


DPPC



DPPG



Ibuprofen

Figure 1. Chemical structures of dipalmitoyl phosphatidyl choline (DPPC), dipalmitoyl phosphatidyl glycerol (DPPG), and Ibuprofen.

chloroform:methanol (9:1 v:v) mixture was employed to dissolve solutions containing DPPG. Initially, 40μL of solutions containing pure phospholipids or phospholipids/ibuprofen mixtures in a range from 0.1 to 5.0% in mol of ibuprofen were spread on the surface to obtain a Langmuir monolayer. Milli-Q water at pH of 5.6 and temperature of 21 ± 1 °C was used as subphase. After spreading, the solvent was allowed to evaporate for 10 min. In the second methodology, the ability of ibuprofen at incorporating into the phospholipid monolayers was evaluated with the drug in the subphase at the following concentrations: 12.5, 25.0 or 50.0 μM. The surface activity of the drug was also assessed without spreading the phospholipids by compressing the barrier to observe any increase in surface pressure. All experiments were repeated several times to ensure reproducibility of the isotherms.

Surface pressure and surface potential measurements were carried out with a Langmuir minitrough from KSV Instruments which total area is 23,625.00 mm^2 in a class 10,000 clean room. The surface pressure π was determined using the Wilhelmy plate method and the surface potential ΔV was measured using the vibrating plate method (frequency 300 Hz) using a KSV Kelvin probe with both reference and vibrating plate electrodes made of platinum, and the probe located at approximately 1–2 mm above the water surface. Film compression using two symmetrically movable barriers was carried out at a constant barrier speed of 10 mm min^{-1}. The system is computer-controlled, which allows the simultaneous recording of surface-pressure (π-A) and surface-potential (ΔV-A) isotherms.

The film morphology was studied with a Brewster angle microscope (BAM), Model BAM2Plus System from Nanofilm Technologies (NFT - Germany), mounted on the trough apparatus. The BAM principle is based on the fact that a *p*-polarized light beam impinging on the water surface at the Brewster angle is not reflected. Therefore, no light reaches a camera placed in the direction of the reflected beam. The Brewster angle is determined by the refractive index of the two media that form the interface, for example, water and air, for a clean water surface [15]. If a Langmuir film is formed, this new interface changes with the refractive index being slightly modified, producing reflection of the light toward the camera. An image of the interfacial film structure is formed by contrast between regions without film (dark regions – without reflection) and spots where the water surface is covered with film molecules (bright regions – reflection).

Polarization-modulated infrared reflection absorption spectroscopy (PM-IRRAS) was performed using a KSV PMI550 instrument (KSV, Finland) at a resolution of 8 cm^{-1} with Langmuir monolayers obtained by spreading mixtures of phospholipids and ibuprofen 5% in mol on the aqueous subphase. The experimental setup used was similar to that described by Pavinatto et al. [16]. The Langmuir trough was mounted for the light beam reach the monolayer at a fixed incidence angle of 80°. As the incoming light was continuously modulated between s- and p-polarization at a high frequency, the spectra could be measured for the two polarizations simultaneously. The two channels processing the detected signal give the differential reflectivity spectrum $\Delta R=(Rp - Rs)/(Rp + Rs)$, where Rp and Rs are respectively the polarized reflectivities for parallel and perpendicular directions to the plane of incidence. The absorption of the parallel polarized light beam comes mainly from vertically oriented dipoles, while those oriented horizontally give rise to absorption of the perpendicularly polarized beam. The difference of the two spectra thus provides information on oriented vibrational dipoles, which is generally surface specific as the molecules in the subphase have random orientation. Since the spectra were measured simultaneously and the IR spectrum was divided by the corresponding spectrum of the subphase, the effect of water vapor was reduced. In the angle used in this work, positive bands indicate a transition moment preferentially in the surface plane, whereas negative bands indicate preferential orientation perpendicular to the surface.

3. Results and Discussion

The main objective in studies of pharmaceutical drugs interacting with model membranes is to obtain molecular-level

information on the possible action that could be correlated with their physiological activity. The two most important modes of action are drug penetration into the membrane, altering the packing of the lipid bilayer and creating pores [17,18], and changes in the membrane elasticity induced by the drug [19]. Obviously, the mere coupling of the drug onto the membrane surface may also have an effect, but this would probably be not as important as the other two modes of action. Among the various factors affecting the interaction with the drug is the charged state of the membrane, which is the reason why in several studies use is made of neutral and anionic phospholipids. In most cases, interaction is stronger with charged lipids [20]. In this analysis, we only considered situations where the pharmacological action depends directly on the membrane and not on the receptor, because a Langmuir monolayer is probably not a good model to simulate the action, unless the receptor is included in the experiments.

When co-spread with DPPG monolayers, ibuprofen induced significant changes in the surface pressure isotherms at concentrations below 1 mol%. Figure 2 shows the isotherms at various concentrations, where a non-monotonic behavior is noted as the ibuprofen concentration changed. For the expansion induced by the drug was considerable for 0.1 mol%, but decreased for 0.2% and then increased again higher concentrations. This could be related to dynamic phenomena such as drug dissolution/incorporation and its aggregation/disaggregation that is characteristic of metastable systems. It should be stressed that the isotherm for neat DPPG is consistent with the literature [21,22]. With regard to the monolayer elasticity, we used the surface pressure-area isotherms to calculate the compressional modulus ($C_S^{-1} = -A(\partial\pi/\partial A)$), also referred to as equilibrium surface elasticity. The physical states of monolayers are classified on the basis of Cs^{-1}, as follows: 12.5–50 mN/m, liquid-expanded; 50–100 mN/m, liquid; 100–250 mN/m, liquid-condensed;>250 mN/m, solid [23]. A decrease in compressibility modulus was observed only at high surface pressures, as indicated in the inset of Figure 2. As we shall discuss later on, in the pressure region relevant for cell membranes (< 32 mN/m), there is practically no effect upon incorporating ibuprofen. At higher ibuprofen concentrations, between 1 and 4 mol% (results not shown), the changes in the isotherms were within the dispersion of the data, which was 0.1 mN m^{-1}. It seems that either self-aggregation or dissolution of the drug may have taken place, thus resulting in no change in the area per DPPG molecule in the isotherms. The changes were significant only for 5 mol% for which there was an increase in the compressibility modulus from 400 mN/m for DPPG to 700 mN/m for ibuprofen co-spread at higher pressures. The concentrations chosen for the experiments were based on our prior experience and values used in the literature.

Another interesting feature in the isotherms of Figure 2 is the lowering of the collapse pressure caused by ibuprofen, which may be attributed to penetration into the hydrophobic part of the DPPG monolayer, decreasing the stability of the monolayer, anticipating the 2D-3D process. Furthermore, considerable effects could be observed with very small ibuprofen concentrations. This points to the presence of cooperative effects, analogously to what has been reported for other drugs, such as dipyridamole [24] and phenotiazine derivatives [25].

The incorporation of ibuprofen also affected the monolayer surface potential, as shown in Figure 3. The surface potential increases with ibuprofen co-spread, with a non-monotonic dependence on concentration that results from the metastability of the monolayer. The inset depicts the change in the apparent dipole moment calculated with Equation (1) as the ibuprofen concentration was varied. Note that at large areas per molecule the surface potential of a neat DPPG monolayer is negative owing to the contribution from the electrical double-layer formed by counter-ions in solution with the negative charge of the DPPG headgroups [26,27]. At high pressures the hydrophobic tails are aligned and the surface potential becomes positive due to the positive contribution from the molecular dipole moments.

$$\Delta V = \frac{\mu}{A\epsilon_0} + \Psi \tag{1}$$

where ΔV is the surface potential, μ is the apparent dipole moment, A is the area per molecule, ε_0 is the vacuum permittivity [28], and Ψ is the double-layer contribution.

Since ibuprofen is a weak acid with pKa 4.6 [29], its molecules are expected to be partially ionized in the monolayer. The degree of dissociation depends on the pH at the interface, which is lower than the bulk pH (ca. 6.0) due to the negative double-layer potential [30]. If a comparison is made with the data for stearic acid, whose pKa lies between 5 and 5.6 in the bulk [31], the degree of ionization on a pure water surface will be a few percent. (There has been some controversy over the values adopted for the pKa of carboxylic acids. See Ref. [32] for a discussion on the topic).

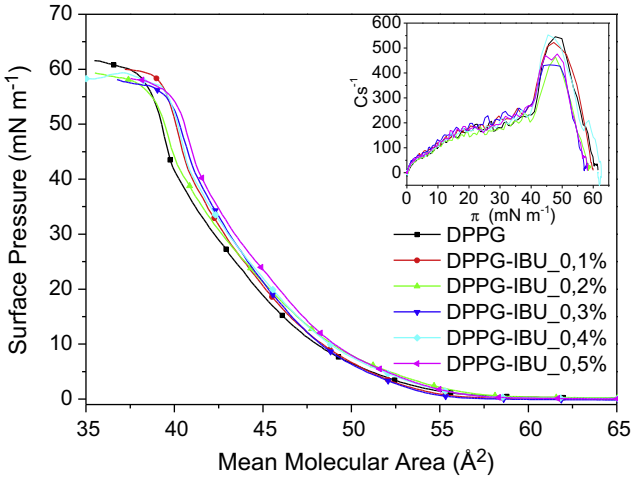


Figure 2. Surface pressure isotherms of DPPG-Ibuprofen monolayers at various drug concentrations. Each isotherm represents the media from at least 3 measurements, which error obtained was 0.1 mN m^{-1}. Inset: Compressibility modulus Cs^{-1} vs. surface pressure for DPPG monolayers.

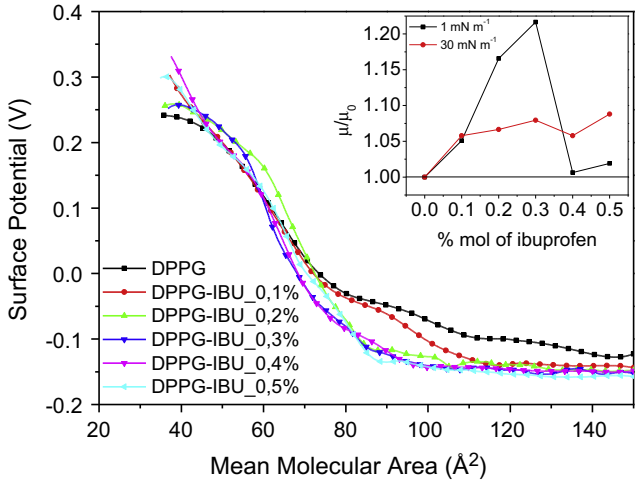


Figure 3. Surface potential isotherms of DPPG-Ibuprofen monolayers at various drug concentrations. Inset: Ratio between the apparent dipole moment of DPPG-Ibuprofen (μ) and pure DPPG (μ_o) vs. ibuprofen concentration at different surface pressures.

The negatively charged ibuprofen could in principle cause a decrease in surface potential, but this decrease cannot be very large because the ibuprofen concentration was very low. For these reasons, the contribution of the ibuprofen dipole moments should be discarded. Therefore, the change in surface potential may be ascribed to alterations in the molecular packing of the DPPG molecules, with an ensuing increase in the normal component of their dipole moments. A change in packing is consistent with the penetration of ibuprofen molecules deep into the hydrophobic part of the DPPG monolayer, inferred from the surface pressure isotherms. The changes in surface potential caused by such small amounts of ibuprofen can only be explained by effects owing to the interaction between the drug molecules that affect cooperatively the orientation of DPPG dipoles. By way of illustration, the presence of 0.5 % ibuprofen caused μ to increase by approximately 8% compared to the neat DPPG monolayer.

The non-monotonic dependence for the ibuprofen concentration in both surface pressure and surface potential isotherms for ibuprofen co-spread with DPPG monolayers was confirmed in the experiments where neat DPPG monolayers were formed on ibuprofen-containing aqueous subphases (results not shown). The incorporation of ibuprofen induced small expansions in the surface pressure isotherms, but the collapse pressure was little affected, which indicates the maintenance of the monolayer stability, probably because of a less extent of drug penetration owing to ionic-dipole repulsions. In addition, the compressibility modulus was only affected at high surface pressures, similarly to the data for the co-spread DPPG-ibuprofen monolayers. These data are therefore omitted. The only significant change was in the surface potential isotherms, as the surface potential increased with the incorporation of ibuprofen. It seems that upon adsorbing from the subphase the ibuprofen molecules are unable to affect the packing of DPPG to the same extent as in the co-spread monolayers. Therefore, not only the collapse pressure is barely altered but the change in surface potential was entirely different. Because the surface potential depends on the surface charge and orientation of the molecular dipole moments, it is a much more sensitive monolayer property than the surface pressure. Though with the present data we cannot identify the changes in molecular orientation responsible for the increase in DPPG monolayer surface potential upon adsorbing ibuprofen, it is clear that ibuprofen molecules affect the monolayer in different ways depending on whether they are co-spread or adsorbed from solution. We shall return to this point later on while discussing the biological implications of our findings.

In contrast to DPPG, monolayers of DPPC were barely affected by co-spreading small amounts of ibuprofen. The changes in surface pressure and surface potential isotherms for concentrations up to 0.5 mol% were within the dispersion of the data (results not shown). However, again differently from DPPG, higher ibuprofen concentrations did affect the isotherms, as shown in Figure 4a and b for surface pressure and surface potential, respectively. It is noted that the isotherms for neat DPPC agree well with the literature [33]. In addition to a considerable expansion of the isotherm at large areas per DPPC molecule, the liquid-expanded (LE) to liquid-condensed (LC) phase transition was affected significantly. This was also reflected in a large change in the compressibility modulus at surface pressures relevant for cell membranes, as can be seen in the inset of Figure 4a. The lowering in the modulus means that ibuprofen made the monolayers much more compressible. Because the monolayer expansion was kept even at high surface pressures, as shown in Figure 4a, the ibuprofen molecules appear to be inserted in the hydrophobic part of the DPPC monolayer, without being expelled upon compressing the monolayer.

The surface potential isotherms in Figure 4b also point to monolayer expansion induced by ibuprofen, an effect that increased with the concentration. Also, the ratio of apparent dipole moments, μ/μ_o, decreased monotonically with the ibuprofen concentration. Over the years, distinct models have been used to relate the measured surface potentials in Langmuir monolayers and the molecular dipole moments [34–37]. Applying such models requires the knowledge of the normal component of the dipole moments and therefore the orientation of the monolayer-forming molecules. For phospholipids interacting with ibuprofen it is not possible to obtain such precise knowledge, and therefore no quantitative treatment can be made of the surface potential data. Nevertheless, the decrease in the apparent dipole moment induced by ibuprofen for the DPPC monolayers, as it had not occurred for DPPG, indicates that ibuprofen does affect the packing of phospholipid molecules. Therefore, the monotonic expansion of the DPPC monolayer with ibuprofen analyzed by both, surface pressure and surface potential isotherms, may be related to the neutral nature of the lipid, that minimize electrostatic repulsions between lipid and drug, avoiding dynamic process involving drug aggregating, disaggregating and dissolution during monolayer compression. Also, the surface pressure of collapse increased with drug incorporation, showing enhancement of monolayer stability, with compression being possible until the surface tension almost reaches zero.

While discussing the data for DPPG, we stated that the methodology used to incorporate ibuprofen could indeed affect the interaction. It seemed that interaction was less strong – at least in terms of penetration into the DPPG monolayer – when ibuprofen was adsorbed from the subphase, rather than being co-spread. The same applies to DPPC. In subsidiary experiments with neat DPPC monolayers spread on ibuprofen-containing subphases (results not

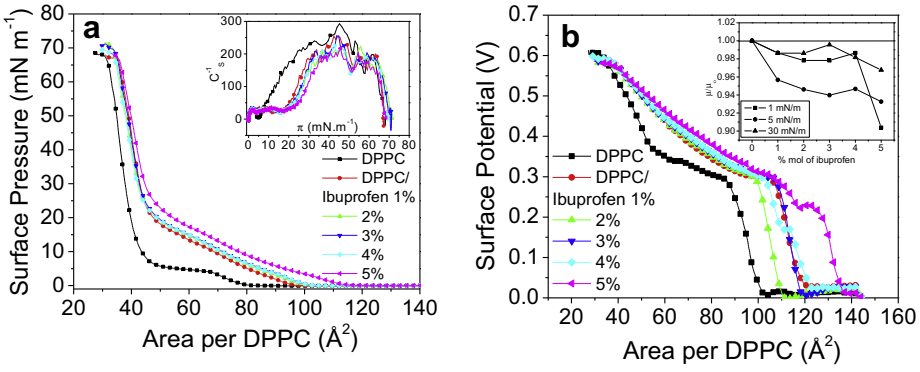


Figure 4. Surface pressure (a) and surface potential isotherms (b) for mixed DPPC/ibuprofen monolayers in the range of 1–5 mol% of drug concentration. Insets: (a) Compressibility modulus Cs^{-1} vs. surface pressure. (b) Ratio between the dipole moment of DPPC-Ibuprofen (μ) and pure DPPC (μ_o) vs. ibuprofen concentration at different surface pressures.

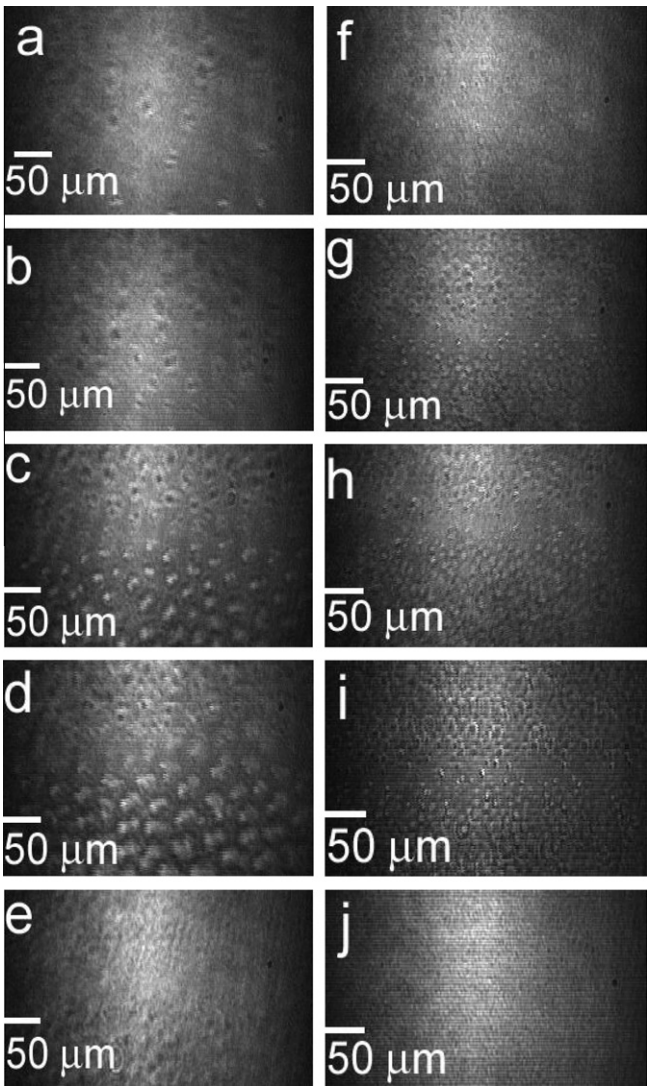


Figure 5. Brewster angle micrographs of pure DPPC monolayers (left) and DPPC/ibuprofen 5 mol% (right).

shown), we found that ibuprofen induced expansion in the surface pressure isotherms, but the shape of the LE-LC phase transition was not altered. Moreover, the compressibility modulus was affected to a much lower extent by ibuprofen, in comparison to the large changes in the co-spread DPPC/ibuprofen monolayers. The difference in behavior for the ibuprofen incorporation from the subphase was the dependence on the ibuprofen concentration, as the expansion did not increase monotonically with the concentration, similarly to the results reported in [38].

We investigated the effects from co-spread ibuprofen on the morphology of DPPC monolayers using BAM, whose images are shown in Figure 5 for a mixed DPPC-Ibuprofen (5% in mol) monolayer. In the gas phase, a neat DPPC monolayer displayed small, scattered domains (a), consistent with the literature [39]. As the monolayer was compressed, the domains increased in size, and in the LE-LC phase transition, at 5 $mN.m^{-1}$, large, irregularly shaped domains appeared (b, c). In particular, phase transition domains are observed in Figure 5d. A continuous increase in domain density with a solid, almost uniform monolayer is observed at 40 $mN.m^{-1}$ (e). When ibuprofen was co-spread, the change in morphology upon monolayer compression was similar to that for a neat DPPC monolayer, as can be seen in Figure 5 f through (j). The major difference lies in the smaller size of the domains, which appeared in a larger number compared to neat DPPC. Moreover, even during compression the domains remained small. Analogously to the surface pressure isotherms, the BAM images indicated that the LE-LC phase transition was affected by ibuprofen. With the compression the domains were joined to form an almost uniform monolayer (i, j), but the domains still visualized were still small. The large effect in the domain size is consistent with the changes in the surface pressure isotherms for DPPC. No distinctive structures were observable within the resolution of BAM during the compression of DPPG and DPPG-Ibuprofen films so these images have not been presented.

The large effects caused by ibuprofen when co-spread with DPPC are in contrast to the results by Jabłonowska et al. [40], who only observed slight changes in the surface pressure isotherms for DPPC-ibuprofen mixtures with mole fraction lower than 36%. Furthermore, larger effects were observed for DPPC monolayers spread on ibuprofen-containing subphases, again in contrast to our results. Motivated by this discrepancy we repeated our experiments and the reproducibility of the data was good. Therefore, it was not possible to identify the reason for the differences. One possible way to solve the discrepancy above is to try and confirm that ibuprofen interacts with the phospholipids using spectroscopic techniques. As we shall show below, upon employing PM-IRRAS we ensured that significant effects exist for ibuprofen co-spread with DPPC and DPPG.

The incorporation of ibuprofen affects the PM-IRRAS spectrum of a DPPC monolayer, as shown in Figure 6a. Of particular relevance was the change in the profile of the band assigned to the antisymmetric stretching vibration of the methylene group, [$\nu_a(CH_2)$], around 2920 cm^{-1}, which was narrower in the presence of ibuprofen. This is significant because for phospholipids, the CH_2 stretching mode between 2800 and 3000 cm^{-1} is useful as the frequency and width of the methylene bands are sensitive to the conformation of phospholipid acyl chains [41]. Therefore, the changes observed in the spectra are consistent with the penetration of ibuprofen into the hydrophobic tails inferred from the surface pressure isotherms.

Figure 6b presents the C = O stretching band at 1740 cm^{-1} for DPPC monolayer at 30 mN m^{-1} which is according to literature [42–44]. However, crystals of ibuprofen show an intense C = O stretching band at 1721 cm^{-1} [45]. The PM-IRRAS spectrum of the DPPC and ibuprofen 5% in mol mixture at 30 mN m^{-1} shows a shift to higher wavenumber (1743 cm^{-1}) and two shoulders are attached to this band (Figure 6b). The fact that the spectrum of the mixture is not a simple superposition of the spectra of DPPC and ibuprofen reveals that the lipid carbonyl groups are in a different environment when the molecules are mixed. The interaction between ibuprofen and DPPC molecules also appears to have affected H-bonding with water molecules in the neighborhood. This conclusion was based on the observed shifts to higher wavenumbers of the antisymmetric (from 1222 to 1229 cm^{-1}) and symmetric (from 1083 to 1087 cm^{-1}) P = O stretching vibrations, as shown in Figure 6c and d, respectively. Arrondo and coworkers investigated the 1000–1300 cm^{-1} region of IR spectrum of DPPC and other phosphate containing molecules [46]. Two bands have been assigned to various vibrational modes of DPPC phosphate group, with maximum wavenumbers at 1086 and 1222 cm^{-1}. They also observed shifts to higher wavenumbers with hydration of DPPC. It is likely that changes of H-bonding between the phosphate groups and water molecules are due to film disordering caused by ibuprofen.

Figure 7 shows the PM-IRRAS spectra for monolayers of neat DPPG and DPPG/ibuprofen, in which the expected bands for the acyl chains of DPPG appear at 2924, 2900 and 2851 cm^{-1}, assigned respectively to $\nu_a(CH_2)$, $\nu_s(CH_3)$ and $\nu_s(CH_2)$, as shown in Figure 7a. These bands are according to literature [47]. Similarly to DPPC, a

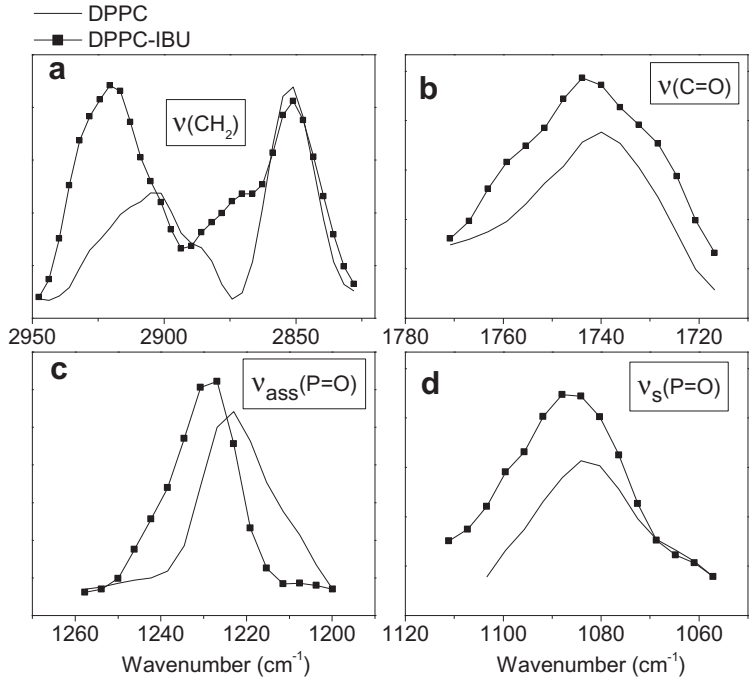


Figure 6. PM-IRRAS spectra for DPPC and DPPC/ibuprofen monolayers, with 5 mol% of ibuprofen, at 30 mNm^{-1}.

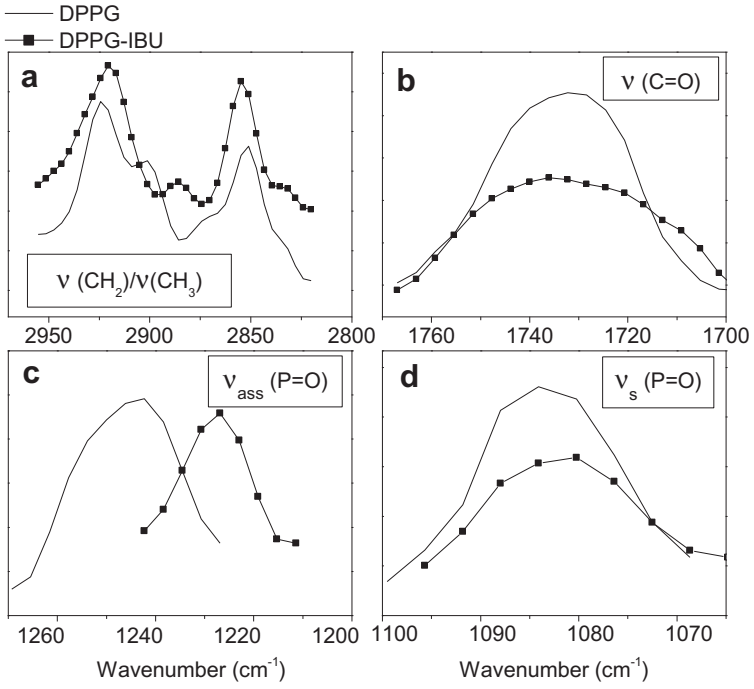


Figure 7. PM-IRRAS for DPPG and DPPG/ibuprofen monolayers, with 5 mol% of ibuprofen, at 30 mN m^{-1}.

large change was observed in the acyl chains, including a shift from 2924 to 2920 cm^{-1} for ν_a (CH_2) when ibuprofen was introduced. Moreover, for DPPG the intensity of the CH_3 symmetric band decreased in the presence of ibuprofen indicating the disorder of hydrophobic tails of the phospholipid. Likewise, ibuprofen decreased the order ratio [$\nu_s(CH_3/\nu_s(CH_2)$] from 0.78 to 0.19 for DPPG-drug mixed monolayers, which shows direct influence of ibuprofen over the alignment of DPPG. Again, this is consistent with the penetration of ibuprofen in the hydrophobic tails region of the DPPG monolayer, as indicated by the surface pressure isotherms. Figure 7b shows the stretching vibration band from the carbonyl group which appears at 1731 cm^{-1}. For DPPG monolayers containing ibuprofen this band is broader with a small shoulder at 1712 cm^{-1} due to the presence of ibuprofen. For the antisymmetric P = O stretching band a considerable shift, from 1243 to 1226 cm^{-1}, was observed in Figure 7c. According to Hübner and coworkers the shift of this band to lower wavenumbers points to hydration and a larger number of H-bonds with water molecules at the air–water

interface [48–49]. The symmetric P = O stretching band for DPPG monolayers appears at 1083 cm^{-1} (Figure 7d), characteristic of hydrated phosphate groups in lipids [50]. The slight shift to a lower wavenumber (1080 cm^{-1}) suggests changes in hydrogen bonding of the phosphate group.

3.1. Biological Implications

In the analysis of possible implications for the pharmacological action of ibuprofen, we shall start with the results from the adsorption of ibuprofen from the subphase, which is the methodology closer to the real systems. Obviously, one has to bear in mind that we can only establish possible correlations, because the membrane model represented by Langmuir monolayers is very simplified. The results discussed here pointed to incorporation of ibuprofen penetrating into the hydrophobic part of the Langmuir monolayers, but with only small changes in the elasticity properties of the membrane. The effects were somewhat larger for the neutral DPPC than for the anionic DPPG, for larger ibuprofen concentrations, because ibuprofen molecules are slightly negatively charged. The penetration does indicate that hydrophobic interactions are also relevant, in addition to the electrostatic interactions. Furthermore, the effects did not increase monotonically with the ibuprofen concentration for DPPG, which means that there is partition of ibuprofen molecules with the subphase and the formation of dynamic aggregates in the subphase during compression that could not adsorb on the monolayer.

In the experiments in which ibuprofen and DPPG or DPPC were co-spread, the most important pharmacological activity would be based on changes in the elasticity of the membrane. The effects, however, would be much stronger for DPPC, for which large monolayer expansions and lowering of the compressibility modulus could be observed. The changes in elasticity could be responsible for the modification in monolayer morphology, according to BAM experiments, where a predominance of small domains was noted throughout the monolayer compression. The formation of smaller domains associated to the increase in collapse pressure indicates the enhancement of monolayer stability due to ibuprofen incorporation in DPPC monolayers. This difference with DPPG must be attributed to electrostatic interactions. Possible effects from DPPG, however, cannot be ruled out because the Langmuir monolayers were affected by small amounts of ibuprofen. It is likely that the packing of cell membrane regions with charged phospholipids would be mediated by ibuprofen through an effect where basically the membrane is expanded without having its elasticity affected.

The results for co-spread monolayers also serve to infer implications for drug-delivery systems based on liposomes, since the drug would have to be trapped into phospholipid bilayers. From our findings, it is clear that larger ibuprofen loading can be achieved with DPPC, whose release may be controlled with the considerable changes in elasticity. Finally, we should mention that the ibuprofen concentrations employed in this study are much lower than those administered to patients, which highlights the importance of the interaction with the phospholipids and the bioavailability of the drug if incorporated into liposomes. Indeed, the incorporation of ibuprofen into DPPC and DPPG liposomes has been demonstrated, with slow release in layer-by-layer (LbL) films [14].

4. Conclusions

This paper described the interaction of an anti-inflammatory agent, ibuprofen, with model membrane systems consisting of DPPC and DPPG monolayers. Ibuprofen expanded the monolayers in both methods of incorporation, either from the subphase or when co-spread with the phospholipids. From the surface pressure isotherms, we could infer that ibuprofen penetrated into the hydrophobic part of the monolayer, with stronger effects being observed for DPPC. Penetration was confirmed with PM-IRRAS spectroscopy. This unusual stronger interaction with an electrically neutral phospholipid, rather than with the anionic DPPG, can be ascribed to the electrostatic interactions since ibuprofen molecules should be at least partially ionized at the pH of the experiments. One important feature was the large change in monolayer elasticity for DPPC when ibuprofen was co-spread at the air/water interface. Moreover, the dependence on the concentration of ibuprofen also varied with the phospholipid. Significant effects were only observed in DPPC when the ibuprofen concentration exceeded 1 mol%. In contrast, for DPPG the ibuprofen effects appeared only at small concentrations, typical of cooperative effects. The larger changes induced in the DPPC monolayers were also confirmed by studying the film morphology with BAM images.

With regard to the biological implications, the results presented here point to a physiological action for ibuprofen with preferential interaction with electrically neutral regions of the membrane, with the lipid packing being affected by the penetration of the drug into the hydrophobic chain regions. When ibuprofen was co-spread with the phospholipids, the physiological action could result from a large change in the compressibility modulus, particularly at surface pressures prevailing in a cell membrane. It was also clear from the overall results that ibuprofen can be incorporated into lipid structures, as already shown for liposomes in a previous study [14], which is relevant for further use in drug delivery.

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