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The influence of headgroup structure and fatty acyl chain saturation of phospholipids on monolayer behavior: a comparative rheological study

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Abstract

This paper compares six phospholipidic monolayers at the water/chloroform interface by performing dilational rheological measurements with a drop tensiometer apparatus. The chosen lipids differ both in their headgroup structure and fatty acyl chain saturation or symmetry. The study concentrated on monolayers formed with DPPC, DPPE, DOPC, DOPE, POPC and POPE. Using a generalized Maxwell rheological model, transposed at the interface, the intimate intermolecular interactions between amphiphilic molecules are studied on and off the monolayer plane. The equilibrium and nonequilibrium phenomena are analyzed and, respectively, correlated with monolayer cohesion and with monolayer/sub-surface interactions. The purpose of this work is to gain further insights into the influences (as slight as they are) of the weak changes in phospholipid structure and on the behavior of the monolayers. The results, widely described, provide further details on nuances existing between very similar molecules, and likewise, on the synergies created between the different effects.
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Keywords: DPPE; DPPC; DOPE; DOPC; POPE; POPC; Interfacial rheology; Dilational rheology

1. Introduction

Research focused on the self-assembly of phospholipids has proved to be of crucial importance in the study of many biological processes as well as in other medical, pharmaceutical and industrial applications. Overall, these phenomena are governed by the thermodynamic and physicochemical parameters of the bilayers, reflected by the cohesion of the layer related to in-plane order and disorder, by the hydration state of the headgroups, and even by the relative out-of-plane interactions

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of the amphiphile sub-surface. Furthermore, some models of lipid bilayers have been shown to be successful for fitting natural systems, and thus disclosing intimate interactions between phospholipidic amphiphiles within the membrane. A number of studies have been focused on the phase behavior of the binary lipid/water system and on the particular study of the L_α liquid crystalline phases, since amphiphilic mobility is comparable to that of biological membranes (Janiak et al., 1979). Besides, much research has studied simple lipid monolayers at the air/water interface, the so-called Langmuir monolayers, for their features of being *fully hydrated systems*, which resides on a semi-infinite reservoir of water much closer to the conditions of biomembranes than of partially dehydrated systems (Möhwald, 1993; Lui et al., 1994; Evans, 1995; Romao and Gonçalves da silva, 2004; Garidel and Blume, 2005; Dyck et al., 2005; Korchowiec et al., 2006). The usual experimental methods for studying such monolayers in great detail include optical techniques such as fluorescence microscopy, fluorescence spectroscopy and ellipsometry, etc., and X-ray or neutron scattering (Möhwald, 1993). Phospholipid monolayers spreading at the oil/water interface have also been widely studied (Brooks and Pethica, 1964, 1965), the surface pressure (π) being considerably higher with regard to the air/water interface. The cohesive interactions between lipid chains are reduced and the model of a biological membrane becomes more apparent.

In this study we focused, at the oil/water interface, on six different pure (i.e. one-component) phospholipid monolayers, made with molecules differing in their headgroup structures (PE and PC), in acyl chain saturation (DP and DO) and even in acyl chain symmetry (PO). Explicitly we used: DPPC, DPPE, DOPC, DOPE, POPC and POPE. The purpose was to gain further insights into the influence of weak structural variants of the amphiphile structure on the monolayer features and in-plane and out-of-plane intermolecular interactions as enumerated above. The monolayers were formed by the gradual adsorption of the surfactant at the chloroform/water interface of the pendant water drop, in a drop tensiometer apparatus. As a mechanical approach for studying such characteristics of lipid monolayers, we performed comparative dilational rheological experiments (Kretzschmar et al., 1996; Li et al., 1996a,b, 1999; Miller et al., 1998; Wüstneck et al., 2002a,b; Roberts et al., 2005) analyzed with a rheological model, based on the volumic generalized Maxwell model transposed at the interface (Saulnier et al., 2001).

2. Materials and methods

2.1. Materials

Dipalmitoylphosphatidylethanolamine (DPPE), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylethanolamine (DOPE), dioleoylphosphatidylcholine (DOPC), 2-oleoyl-1-palmitoylphosphatidylethanolamine (POPE) and 2-oleoyl-1-palmitoylphosphatidylcholine (POPC) obtained from Sigma were used. These lipids were dissolved in chloroform/methanol (3:1), HPLC grade, purchased from Prolabo (Fontenay-sous-Bois, France). This first dissolution is performed to obtain a phospholipid concentration of ∼6 mM, and a second dilution in pure chloroform that gives a concentration of ∼0.02 mM. Ultrapure® water was obtained with the MilliQ filtration system (Millipore, Saint-Quentin-en-Yvelines, France). Moreover, considering the low solubility of chloroform in water, they were previously mixed and saturated for about 1 week. The lipid concentration was chosen to correspond to the critical micelle concentration in the organic phase (which is mostly chloroform; for both instances, see Li et al., 1996a), forming an inverse micellar suspension. This method insured both the complete saturation of the water/organic interface, and the stable features of the forming monolayer in the bulk phases (an important detail for the rheological study).

2.2. Methods

2.2.1. Drop tensiometer

Rheological measurements were performed with a drop tensiometer device (Tracker Teclis, Longessaigne, France). The (instantaneous) axisymmetric shape of a drop, pendant or rising, is analyzed at constant temperature to provide the surface tension value. In our case, the drop is a pendant drop of water in the organic phase, which contains the phospholipids. The temperature was maintained at $(21 \pm 2)\,^\circ C$, and the measurements were performed three times. Each rheological experiment was performed at equilibrium, after the complete saturation of the water/chloroform interface. The first stage, prior to rheological experiments, is the formation of the monolayer by the gradual diffusion of the amphiphiles at the interface until complete saturation is achieved. Throughout the adsorption kinetics, the drop area is maintained constant in order to create a direct link between molecule adsorption and the surface tension; saturation is reached when the tension stabilizes. The kinetics appears relatively fast, these being consistent with the literature for similar systems at the water/chloroform interface (Li et

al., 1996a), typically less than 100 s for all the studied amphiphiles. At least 5 min were allowed after surface tension stabilization in order to strictly respect the same conditions of film maturation (or aging), between the different experiments.

The interfacial rheology study begins when a weak sinusoidal area fluctuation is imposed on the drop (typically with an amplitude of around 10% of the area value). Then, the response in terms of surface pressure $\pi = \gamma_0 - \gamma$ is followed up. This experiment was performed for a wide range of circular frequencies (as allowed within the experimental conditions) typically $0.05 < \omega < 7 \, \text{rad s}^{-1}$. The relationship between the imposed (sinusoidal) deformation, and the (sinusoidal) response of the surface tension, is expressed as the following transfer function (Eq. (1)), which may be split up into a real *conservative* elastic term, $G'(\omega)$, and an imaginary *dissipative* term, $G''(\omega)$

$$\bar{G}(j\omega) = \bar{A}\frac{d\pi}{d\bar{A}} = G'(\omega) + jG''(\omega) \tag{1}$$

where $\bar{A}$ represents the expression of the complex interfacial area. The interfacial rheological model provides physical characteristics of the film by fitting, as presented below, both experimental curves, $G'(\omega)$ and $\eta(\omega) = G''(\omega)/\omega$ as a function of ω, $\eta(\omega)$ representing the *interfacial viscosity* (Langevin, 2000; Miller et al., 2000).

2.2.2. Interfacial rheological model: a tool to explore the intermolecular interactions

The model chosen to deal with the transposition of the volumetric rheological phenomena, an interfacial approach, is based on a generalized Maxwell model, presented in Fig. 1. It is built as a linear combination of elastic and viscous behavior, described by Hooke's and Newton's laws, respectively, and therefore is only applicable for weak perturbations (less than 10%) of the surface pressure. In this paper we applied it to phospholipid monolayers in the case of sinusoidal perturbations of the drop area, but similar results would have been provided if the study had been transposed to ramp-type interface deformations (Saulnier et al., 2001).
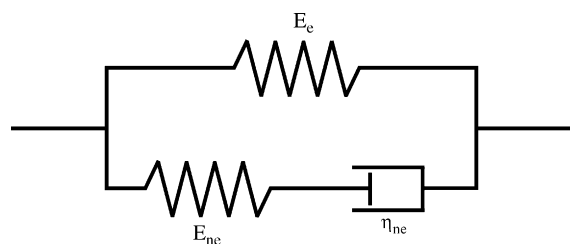

Fig. 1. Schema of the rheological model of the monolayer.

There are two parallel branches, the first one presenting a perfect elastic solid, where E_e is the elastic compliance, and the second one showing a viscoelastic Maxwell liquid, E_{ne} being the elastic component which reflects the dissipation in its own branch. The associated viscous term η_{ne} is a constant and provides the link between E_{ne} and the relaxation time τ according to Newton's law $E_{ne} = \eta_{ne}/\tau$. The idea was to consider that the viscoelasticity response (after the dilational perturbation) is exclusively due to the interaction of the monolayer with the sub-surfaces and not due to lateral diffusion or any reorganization within the layer itself. The interfacial film was formed within the *inter-phase* zone, and the *sub-surfaces* we defined are the thin liquid frontiers existing between this inter-phase region and the bulk phase. Hence the pure elastic branch is associated with monolayer cohesion whereas the viscoelastic one only concerns the affinities of the layer for the aqueous and organic phases. In this way we can consider the phenomenological links that exist between E_e, E_{ne}, τ, and the relative strength of the intermolecular interactions within the monolayer and with the sub-surfaces. Fig. 2 schematizes amphiphiles at the interface, and the four main types of interactions: (1) Acyl chain/organic sub-surface; (2) headgroup/aqueous sub-surface; (3) chain/chain; and (4) head/head.

The pure elasticity of the monolayer, E_e, therefore accounts for the cohesion and lateral interactions between amphiphiles, from their hydrophilic as much as from their lipophilic nature, presented with the interactions (3) and (4) in Fig. 2. On the other hand, E_{ne} is an elasticity term that reflects the energetic dissipation of the layer. This dissipative behavior is induced by the weak displacements of the surfactants to or from the sub-surface as a result of the compression or expansion, respectively, of the monolayer (Panaiotov et al., 1996;
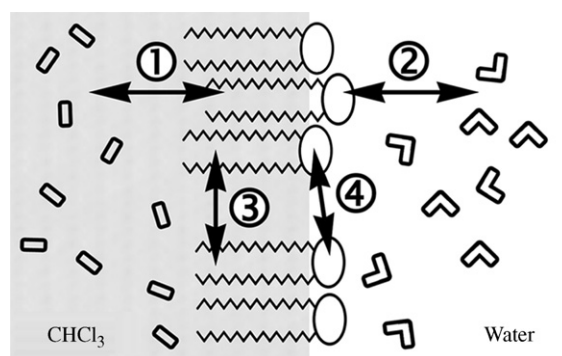

Fig. 2. Schematic representation of the phospholipidic interfacial film at the chloroform/water interface. Potential intermolecular interactions between headgroups, apolar tails, aqueous and organic sub-surfaces.

Boury et al., 1995a,b; Doisy et al., 1996), and therefore induced by the molecular amphiphile/sub-surface interactions ((1) and (2) in Fig. 2). The relaxation reflects the gradual reestablishment of equilibrium following the dilational perturbations that are modeled here as out-of-plane molecular movements between the interfacial film and the sub-surface; the relaxation time τ simply reflects the kinetics of these nonequilibrium phenomena linked to the dissipative terms.

It is noteworthy that this model built with one viscoelastic branch, involving that single relaxation time τ, governs the phenomena. Nevertheless, the *one-relaxation-time*-models have been proved to fit well the simple cases of one-component monolayers, from phospholipids or nonionic surfactants, to bigger molecules, polymers or even proteins (Malzert et al., 2002). The works of Saulnier et al. (2001) with their relaxation study of DPPC at the water/dichloromethane interface, showed particularly well the coherence of this *one-time*-model with our systems. However in our case, the fact that the relaxation behavior of the surface tension (after a weak perturbation) fitted well with one relaxation time, implies that the amphiphile displacements to the sub-surfaces are made preferentially in only one of the sub-surfaces. This consideration is one of the important assumptions established in this section, and it will be of fundamental importance in the analysis of the results.

The expressions of $G'(\omega)$ and $\eta(\omega)$ are provided as a function of E_e, E_{ne}, τ, and ω (Eqs. (2) and (3)).

$$G'(\omega) = E_e + E_{ne}\frac{\omega^2\tau^2}{1+\omega^2\tau^2} \qquad (2)$$

$$\eta(\omega) = \frac{G''(\omega)}{\omega} = E_{ne}\frac{\tau}{1+\omega^2\tau^2} \qquad (3)$$

The parameters are reached with the following interpolations, Eqs. (4)–(6).

$$\lim_{\omega\to 0} G'(\omega) = E_e \qquad (4)$$

$$\lim_{\omega\to\infty} G'(\omega) = E_e + E_{ne} \qquad (5)$$

$$\lim_{\omega\to 0} \eta(\omega) = \eta_{ne} = \tau\, E_{ne} \qquad (6)$$

3. Results and discussion

3.1. Influence of PE and PC headgroups on monolayer properties

Interfacial rheology experiments showed fundamental differences between PE and PC monolayers, and this was so whatever the extent of acyl chain saturation. Figs. 3–5 present the experimental values of E_e,
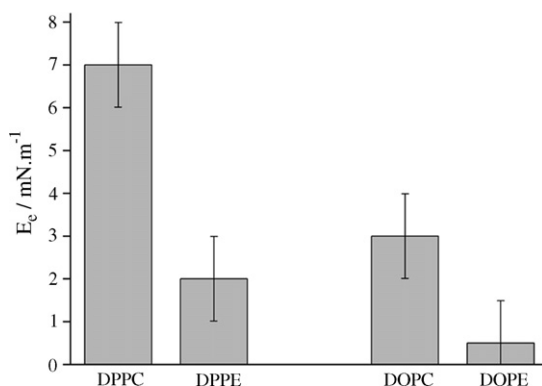

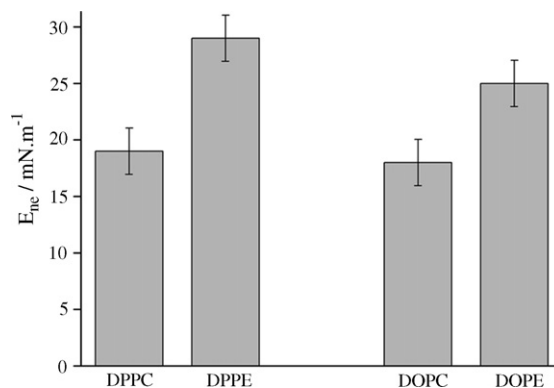Fig. 3. Experimental values of 'equilibrium' elasticity E_e.

Fig. 4. Experimental values of 'nonequilibrium' elasticity E_{ne}.

E_{ne} and τ, as a systematic comparison between PE and PC lipids, di-saturated (dipalmitoyl) or di-unsaturated (dioleoyl).

On the one hand, the conservative elasticity E_e, reflecting the intrinsic elasticity of the monolayer, clearly exhibits higher values for the PC molecules (Fig. 3). This behavior remains unchanged whatever the degree of saturation of the acyl
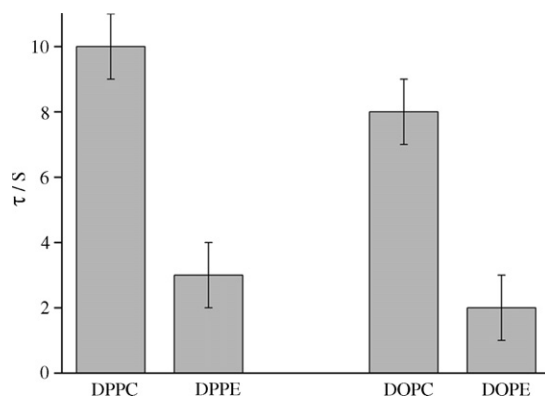

Fig. 5. Experimental values of relaxation time τ.

chains, e.g. $(E_e^{DPPC} - E_e^{DPPE}) \sim 5\,mN\,m^{-1}$ and $(E_e^{DOPC} - E_e^{DOPE}) \sim 2.5\,mN\,m^{-1}$. Moreover, a reversed and marked trend appears with the values of the elasticity term E_{ne} (Fig. 4). Important differences of E_{ne} values between two molecules differing only in their headgroups is disclosed, and also, whatever the saturation of the chains, e.g. $(E_{ne}^{DPPE} - E_{ne}^{DPPC}) \sim 10\,mN\,m^{-1}$ and $(E_{ne}^{DOPE} - E_{ne}^{DOPC}) \sim 7\,mN\,m^{-1}$. Finally, regarding the relaxation times τ (Fig. 5), once again a clear difference between the two kinds of molecule appears, which shows very low values of τ for PE molecules compared with PC ones $(\tau^{DPPC} - \tau^{DPPE}) \sim 7\,s$ and $(\tau^{DOPC} - \tau^{DOPE}) \sim 6\,s$. Although the lipids studied in this section only differ in the ethanolamine or choline headgroups, this structural difference has considerable implications in terms of intermolecular interactions and monolayer cohesion as regards our rheological approach. To be considered before discussing our results are the respective hydration states of PE and PC, essential in the understanding of the phenomena, and widely investigated in the literature. Indeed, early studies proposed theoretical approaches on providing evidence for the existence of hydration shells around ammonium hydrogens and non-esterified headgroup oxygens of PE lipids (Damodaran et al., 1992) strongly involved in the interactions of the head group with water. Likewise, computations of the energy required to bind water to the phospholipid headgroup have emphasized the significantly lower values for PC (in comparison with PE), and even for comparable water/water interactions (Port and Pullman, 1973). In addition, more recently (Dyck et al., 2005), evidence has been provided for the important differences in hydration existing between ethanolamine and choline headgroups (and therefore in the affinities of the amphiphiles for the aqueous sub-surface). Under experimental conditions similar to ours, for studying fully hydrated headgroups of DPPE and DPPC in Langmuir monolayers, they showed important results in investigating in detail the interaction of the headgroup with the surrounding water molecules by surface-sensitive X-ray scattering. Thus, at any given surface pressure π, for DPPE the amine fragment is more deeply embedded in the water phase than is DPPC, and thus it suggests higher hydration and stronger interactions of the DPPE headgroup with the water sub-surface. Quantitatively, the amine function in PE extends 1–2 Å into the sub-surface more than those of the PC molecules, which also induces considerable hydration of the lipid backbone and phosphate. As a result, the global hydration difference between PE and PC molecules is around 25%. The main explanation of these results is the better solubility in water of the PE

headgroup in comparison with the PC one due to the potential formation of hydrogen bonds of PE within the aqueous phase.

As regards the results provided by our rheology study of monolayers, it is to be considered the previously established assumptions that: (i) The monolayer is insoluble in the two phases (the amphiphiles are not ejected in bulk phases during the weak area perturbation, but rather are slightly displaced in the sub-surfaces); (ii) since relaxation occurs following only one relaxation time, the perturbation induces amphiphile displacement towards only one of the sub-surfaces; (iii) the dissipative term of the viscoelastic model only results in the monolayer/sub-surface interactions (and not in some reorganization of the monolayer). Thus, it emerges from the remarkable difference in the amphiphiles/sub-surface interactions between PE and PC headgroups, as seen in Fig. 4, and demonstrate that globally the present study is consistent with the literature. In this way, by correlating first the fact that the PE molecules provided in the literature to be largely more soluble in water than the PC ones, and secondly, that our experimental results show that the PE molecules exhibit higher affinities for the sub-surface, the amphiphile displacements resulting from a weak film deformation appear to be towards *the aqueous sub-surface*. This is confirmed by the behavior of the values of the relaxation times presented in Fig. 5, definitely shorter for PE molecules. The reestablishment of equilibrium after a weak perturbation remains easier for the more hydrated (PE) molecules, since their affinity for water is higher and their displacement towards the sub-surface (due to their equilibrium position) is shorter than for the PC ones. Furthermore, also to be taken into account are the values of the associated viscosity $\eta_{ne} = \tau E_{ne}$ which is greater for the methylated than for the non-methylated amine headgroup: $\eta_{ne}^{DPPC} = (190 \pm 40)\,mPa\,s\,m$, $\eta_{ne}^{DPPE} = (90 \pm 30)\,mPa\,s\,m$, $\eta_{ne}^{DOPC} = (140 \pm 30)\,mPa\,s\,m$ and $\eta_{ne}^{DOPE} = (50 \pm 30)\,mPa\,s\,m$. It is to be noted that this behavior remains unchanged when comparing saturated and unsaturated tails; therefore it is fundamentally induced by the type of headgroup.

Besides, the conservative term related to the pure elasticity of the monolayer (E_e in Fig. 3) exhibits inverse behavior. Phosphocholine headgroups induce better lateral intermolecular forces between phospholipids, and therefore the elasticity of the monolayer. The explanation is again provided by the relative position (and affinities) of the amphiphiles within the sub-surfaces: the higher the headgroup hydration (PE), the deeper the molecules embed themselves in the water sub-surface to the detriment of monolayer cohesion.

3.2. The influence of fatty acyl chain saturation

This section looks at changes in monolayer rheological behavior between phophospholipids which only differ in their (symmetrical) fatty acyl chain structure, i.e. between dipalmitoyl (DP) and dioleoyl (DO). The former molecules present fully saturated acyl chains, whilst those of the latter ones remain unsaturated. Marked differences appear as regards the cohesive lateral interactions within the monolayer, indicated in this paper with E_e (Fig. 3), to account for layer elasticity. Monolayers formed with saturated lipids exhibit higher elastic features than unsaturated ones, e.g. giving $(E_e^{DPPC} - E_e^{DOPC}) \sim 4\,\text{mN m}^{-1}$ and $(E_e^{DPPE} - E_e^{DOPE}) \sim 1.5\,\text{mN m}^{-1}$. On the other hand, the influence of the level of saturation on the dissipative terms and molecular interactions with the sub-surface is less evident. Only a slight trend is observed in Fig. 4, which shows that the unsaturations lower the interactions amphiphile/sub-surface. However, since the dissipative component of the monolayer rheological response essentially results in surfactant displacements between sub-surface and interfacial film, the influence of the tail saturation on the dissipative component remains very slight. Therefore these approaches for investigating the intermolecular forces existing between fully hydrated lipids in the monolayer present results that globally fit the general studies of such molecules that have already been widely described in the literature. In our experimental conditions the amphiphiles are free to rotate and diffuse in the monolayer plane, which appears comparable with the dynamic phenomena in liquid crystalline phases. It has been acknowledged for some time that in an L_α liquid crystalline phase, DPPC molecules, for instance, are free to rotate and diffuse in the plane of the lipid lamellae, as based on the results of IR, ^{1}HNMR, ^{13}C NMR and ^{31}P NMR spectroscopy (Lewis and McElhaney, 1996; Mendelsohn and Snyder, 1996; Chapman, 1965; Levine et al., 1972; Lichtenberg et al., 1975; Bloom et al., 1978; Gorenstein, 1984). The study of ^{2}H NMR spectra of specifically deuterated phospholipids have shown (Griffin, 1981) that in liquid crystalline phases, the molecules execute fast anisotropic motion, resulting in axially symmetric and narrow width powder patterns. Therefore, on the ^{2}H NMR time scale, the correlation times shorter than 10^{-6} s contain no direct information on the motion type. Such fast limit powder patterns have been suggested (Saupe, 1964; Seelig et al., 1977) as being linked to the so-called order parameter, and the specific order parameter of the principal axis is commonly used and noted S_{mol}. Hence, in the cases of saturated phospholipidic molecules such as DPPC, S_{mol}

has been shown to be dependent on the position of the CD_2 group, and its value gradually decreases when the deuterated carbon moves from the headgroup towards the acyl chain (Seeling and Seeling, 1974). Likewise, the raising of the temperature globally lowers the value of S_{mol} (Seeling and Seeling, 1974; Oldfield et al., 1978a,b; Davis, 1979), the reason being the extent of angular fluctuations that increase upon layer expansion. Besides, in the case of di-unsaturated acyl chains (e.g. DOPC) or even for one of them (e.g. POPC), a typical dip of the S_{mol} value appears at the location of the unsaturation (Seelig et al., 1977; Seelig and Waespe-Sarcevic, 1978), due to the average orientation of the C–D vector at the unsaturation point in comparison with the director. Thus, the differences appear in terms of decreasing mobility for unsaturated molecules since the double bond does not allow rotation; the layer expansion may also be stronger for molecules presenting unsaturation, since they induce a dip of S_{mol}. The level of unsaturation shows close relation to the notion of acyl chain order (Ho et al., 1995) and thus to lateral intermolecular forces, from which one can conclude that unsaturation increases lipid disorder.

The trends disclosed with the present rheological results appear to be consistent with these NMR studies, which lead us to assume that similar effects occur within our monolayers. All unsaturated DO molecules globally exhibit lower lateral interactions between amphiphiles within the monolayer, as a lower elasticity and E_e value, than saturated DP ones. In addition and finally, the difference between the values of E_e of DO and DP lipids, for PC and PE headgroups (reported as 4 and $1.5\,\text{mN m}^{-1}$, respectively), may probably come from the fact that S_{mol} determined from PE molecules is definitely higher than the corresponding values for PC headgroups (Blume et al., 1982; Marsh et al., 1983; Hubner and Blume, 1987; Ho et al., 1995). Apart from the influence of the PC/PE headgroups on elasticity, the higher order parameter of the PE molecules works against the effects due to the unsaturations. To summarize, the main effect induced by the presence of double bonds in the two acyl chains remains in monolayer cohesion and elasticity, reduced with the unsaturations. Their influence on the monolayer/sub-surface interactions appears nearly nonexistent, and so it is consistent with the fact that relaxation happens towards the sub-surfaces rather than within the monolayer, as well as with the fact that this so-called sub-surface is the aqueous one.

3.3. The influence of fatty acyl chain symmetry

Two monolayers exclusively made of asymmetrical phospholipids (either POPC or POPE) were investigated.

Table 1
Experimental values of E_e, E_{ne} and τ compared for POPC and POPE monolayers

	E_e (mN m^{-1})	E_{ne} (mN m^{-1})	τ (s)
POPC	3 ± 1	18 ± 2	10 ± 1
POPE	6 ± 1	22 ± 2	3 ± 1

Asymmetrical phospholipids are found in natural systems and bilayers and typically generate high levels of loss in the amphiphile order, and therefore considerably decrease the crystallization temperature of such assemblies. The comparison of E_e, E_{ne} and τ is shown in Table 1.

Firstly, the behavior of E_{ne} and τ appear similar to those obtained for di-saturated and di-unsaturated molecules, as well as their effects, due to the headgroup nature. Indeed, the PE molecules induce a trend that slightly increases E_{ne} as well as remarkably decreasing the relaxation time τ. Values compare well with those provided by di-saturated and di-unsaturated lipids (Figs. 4 and 5). Thus, from the discussions made in the previous sections relating to the influence of the headgroup type on monolayer/sub-surface interactions, the behavior of the monolayers towards the aqueous sub-surfaces remains unchanged in the present asymmetrical configuration of the acyl chains. The PE molecules therefore, once again, show higher affinities for the aqueous sub-surface in which they are considered to be more deeply embedded.

The basic difference arising from such a rheological study of POPC and POPE monolayers, is provided by the results of layer elasticity. The elastic compliance of the monolayers E_e for PC and PE asymmetrical amphiphiles exhibit a surprising inversed behavior in comparison with the above-studied monolayers, for di-saturated and di-unsaturated lipids, as shown in Fig. 3. Actually, E_e^{POPE} appears higher (for about 3 mN m^{-1}) than E_e^{POPC} it follows therefore that the lateral intermolecular forces and layer cohesion, this time, are unexpectedly enhanced by the ethanolamine headgroup.

A probable explanation comes from the competition between two opposite effects for inducing or not inducing order and organization within the lipidic layer, that is to say intermolecular forces and elasticity. The first one, noted (a) and presented above, is a loss in the global order of acyl chains (lipid layer) resulting from the level of chain unsaturation. The second and opposite effect, noted (b), concerns the strong relation that links the increase of the order of the lipid layer to headgroup hydration (and therefore headgroup nature), see (Blume et al., 1982; Marsh et al., 1983; Hubner and

Blume, 1987; Ho et al., 1995). Therefore a comparison of PC and PE shows that the ethanolamine headgroup provides a higher constant contribution for ordering the monolayer.

We can therefore consider that the substantial difference between the order in monolayers is the relative elastic compliance between PC and PE: $\Delta E_e = E_e^{PC} - E_e^{PE}$. In the previous sections, we show that a positive value of ΔE_e is the result of the relative position of the ethanolamine amphiphile embedded in the aqueous sub-surface that inhibits their *sensitivity* towards hydrophobic interactions and in addition to the effect that (a) outweighs (b). However, the weakening of the effect (a) with the level of unsaturation leads to effect (b), which can outweigh the (a) in the case of PO acyl chains. The illustration is provided by the values of ΔE_e for DP, DO and PO acyl chains so, respectively, (5 ± 2), (2.5 ± 2) and (-3 ± 2) mN m^{-1}. Finally, these results may suggest that the elasticity of the monolayers is not only related to the level of unsaturation but also to the symmetry within the fatty acyl chains. Thus, the disorder effect, created in pure monolayers, made of asymmetrical amphiphiles, appears even more important than the one generated by di-unsaturated acyl chains. Similar ideas have already been suggested (Blume, 1993; Rico-Lattes and Lattes, 1994).

4. Conclusion

This paper has compared intermolecular forces in monolayers between different sorts of phospholipids differing in headgroups (PC and PE) as well as in fatty acyl chain saturation and symmetry (DP, DO and PO). By using an interfacial rheological approach, we focused on the whole interactions between amphiphiles governing both monolayer cohesion due to the lateral forces within the lipid layer, and the interactions of the monolayer with the sub-surfaces, which proved to be essentially the aqueous one in our systems. Therefore, this study proposed results concerning the influence of the lipid headgroups and acyl chain structures: Concerning molecule hydration; the relative position of amphiphiles with regards to the interface; the order of the lipid layer; and finally on the synergy which can be created with the simultaneous arrival of these effects. The main results link the considerable difference in hydration between the PE (the most hydrated) and the PC headgroups to energy dissipation, that definitely leads to the intimate interactions between PE molecules and the water sub-surface. Secondly, the orders in lipid layers were disclosed in terms of layer elasticity, clarifying the extent that the differences in amphiphile structures involve. Finally, surprising behav-

ior induced by the asymmetrical chains was shown as reflecting the different opposite effects originating order and disorder in such lipid layers.

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