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ORIGINAL CONTRIBUTION

J. Sánchez-González
M.A. Cabrerizo-Vilchez
M.J. Gálvez-Ruiz

Chain dependence in phospholipid interactions: A thermodynamic study of mixed monolayers

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J. Sánchez-González
M.A. Cabrerizo-Vilchez
M.J. Gálvez-Ruiz (✉)
Grupo de Física de Fluidos y Biocoloides
Departamento de Física Aplicada
Universidad de Granada
18071 Granada
Spain
E-mail: mjgalvez@ugr.es

Abstract The interactions between three of the major phospholipids in biomembranes, distearoylphosphatidylethanolamine, distearoylphosphatidylcholine and sphyngomyelin, forming monolayers at the air–water interface, are studied. Following the Goodrich [1] thermodynamic formulation, a quantitative analysis on these interactions is carried out. The general conclusion reached is that, depending on the molecular structure of the lipid and the experimental temperature, significant interactions occur between lipid molecules. One hypothesis is the possibility that, the usual miscibility analysis, in mixed monolayers, could be inadequate to provide information on the interactions in the systems which the hydration forces are significant, is advanced.

Key words Miscibility – interaction parameter – lipids – mixed monolayers

Introduction

The analysis of the interactions between lipids is of great interest since they are the majority components, together with the proteins, of the biological membranes, being able to provide information on its functionality. In particular, a study of the phase and mixing behavior of these systems is a very useful way to understand some of the properties of bilayers in biomembranes [2]. For this reason, a considerable number of papers have considered the behavior of lipids as components of monolayers. Nevertheless, the characterization of the Langmuir and the Langmuir–Blodgett (LB) films of such molecules is still far from complete.

In this paper, an investigation examining the miscibility of three essential lipids, i.e., L-α-distearoylphosphatidylcholine (DSPC), L-α-distearoylphosphatidylethanolamine (DSPE) and sphyngomyelin (Sph) at different temperatures is presented.

In previous studies [3, 4] we reported the mixing behavior of DSPC and Sph. Now, simple monolayers of DSPE and mixed monolayers of DSPE–DSPC and DSPE–Sph at the air–water interface will be considered. The molecular structure of the DSPE and DSPC differs only in the polar head group, while that for DSPE and Sph, both hydrocarbon chains and head polar groups are different. Then, the results will be interpreted on the basis of the molecular structure of the monolayer components.

In order to carry out a quantitative analysis of the molecular interactions in the mixed monolayers, the thermodynamic formulation proposed by Goodrich [1] has been followed. The excess areas, free energies, entropies, and enthalpies of mixing and an interaction parameter [5] have been estimated for our systems as described elsewhere [3], by the application of the following relationships:

$$\Delta G^{\mathrm{E}} = \int_{0}^{\pi} \Delta a^{\mathrm{E}} \, \mathrm{d}\pi \,, \qquad (1)$$

where ΔG^{E} is the excess free energy, and Δa^{E} the excess area of mixing, given for:

$$\Delta a^{\mathrm{E}} = N_{\mathrm{A}}(a_{12} - x_1 a_1 - x_2 a_2) , \tag{2}$$

where N_{A} is the Avogadro number,

$$\Delta S^{\mathrm{E}} = -\left(\frac{\partial G^{\mathrm{E}}}{\partial T}\right)_{\pi, x} - N_{\mathrm{A}} \Delta a^{\mathrm{E}} \frac{\mathrm{d}\gamma_0}{\mathrm{d}T} , \tag{3}$$

where ΔS^{E} is the excess entropy of mixing and γ_0 is the surface tension of water.

$$\Delta H^{\mathrm{E}} = \Delta G^{\mathrm{E}} + T \Delta S^{\mathrm{E}} , \tag{4}$$

where ΔH^{E} is the excess enthalpy of mixing, and

$$\alpha = \frac{\Delta G^{\mathrm{E}}}{RT\, x(1 - x)} , \tag{5}$$

where α is an interaction parameter.

Experimental

Materials

In all the experiments the subphase was water, first double distilled in an all-Pyrex apparatus and then passed through a Millipore Milli-Q Reagent Water System for further purification. Conductivity of the water obtained after this process was always $\leq 10^{-4}\,\Omega^{-1}\,\mathrm{cm}^{-1}$ and its pH was 5.5–6.0. The monolayer components, DSPE, DSPC and Sph were supplied as analytical research grade by Sigma (USA). The spreading solvent was a 5:1 (v/v) mixture of chloroform and methanol (Merck AR grade, Germany). A 0.05% of amylalcohol was added to the lipid solutions in order to improve the spreading process.

Experimental device and method

π–a isotherms were performed using the previously described [3] Langmuir method. A computer-controlled film balance (Lauda FW-1) has been used.

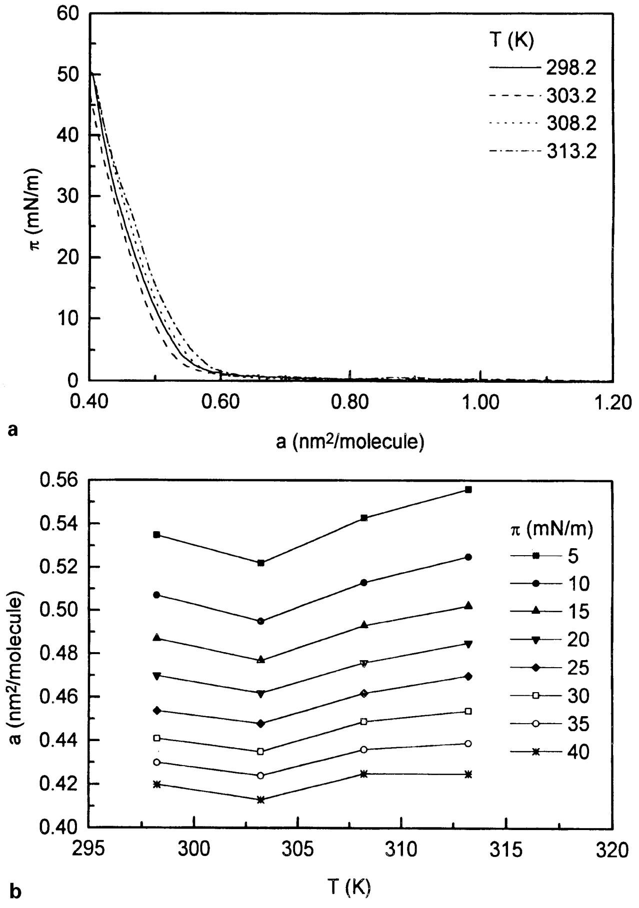
Before the compression of the monolayers, a 10 min lapse was estimated to be sufficient for evaporating the solvent without contamination of the films. The monomolecular films were compressed at a constant rate of $6.5 \times 10^{-4}\,\mathrm{m\,s}^{-1}$. The isotherms were recorded at constant temperature controlled by a Haake F3K thermostatic bath to ± 0.1 K.

Results and discussion

Simple monolayers of DSPE

In Fig. 1 we can examine the shape of experimental isotherms recorded by compressing monolayers formed by DSPE lipid molecules at different temperatures. We observe that the monolayers remain in the liquid condensed (LC) state in all the compression range and under different tested temperatures (298.2–313.2 K). Compared with early results [3, 4] obtained for a similar lipid, DSPC, the behavior with temperature is different. For DSPC monolayers, this effect has practically not been shown. Even, when the temperature increases form 298.2 to 303.2 K, a condensation effect is observed (Fig. 1b). We point out that the molecular structures for both lipids only differ in the head polar groups. Then, the results lead us to believe that, when the ethanolamine group, which is smaller than the choline group, is present in the lipid molecules, the hydration forces play an important role. Increasing the

Fig. 1 (a) Surface pressure–area isotherms for DSPE simple monolayers, (b) mean area values vs. temperature at several values of π



temperature, the dehydration process compensates the decrease of the van der Waals forces between the hydrocarbon chains, and the isotherms do not show the expected extension with temperature [6, 7].

Mixed monolayers of DSPE-DSPC

Figure 2 shows the experimental isotherms corresponding to simple and mixed films formed by DSPE and DSPC in different proportions, at different temperatures. It can be observed that mixed monolayers of these compounds show a somewhat intermediate behavior compared with that shown by pure components except at 313.2 K, when the DSPC monolayer undergoes a liquid expanded (LE)–LC phase transition [4].

The collapse pressure values are similar for both lipids and no significant deviations from these values have been found for the mixed monolayers.

In order to get information on the nature of the interactions between these lipids, in Fig. 3a the interaction parameter, α, is represented as a function of the composition of the mixture at 298.2 K and at different surface pressures. We observe that, on increasing the surface pressure, the interaction between the both lipids became more significant, as it was expected, since the intermolecular distances became shorter by the compression of the monolayers. Moreover, we found a maximum repulsive interaction when the proportion of the lipids is 10% DSPC–90%DSPE and a maximum attractive interaction for the 90%DSPC–10%DSPE mixture.

In principle, we could believe that this behavior is due to the hydration of the ethanolamine group in the DSPE hindering the approach of the molecules. However, we observe that the monolayers corresponding to DSPE are more condensed than those shown for DSPC in all the compression situations and for the experimental

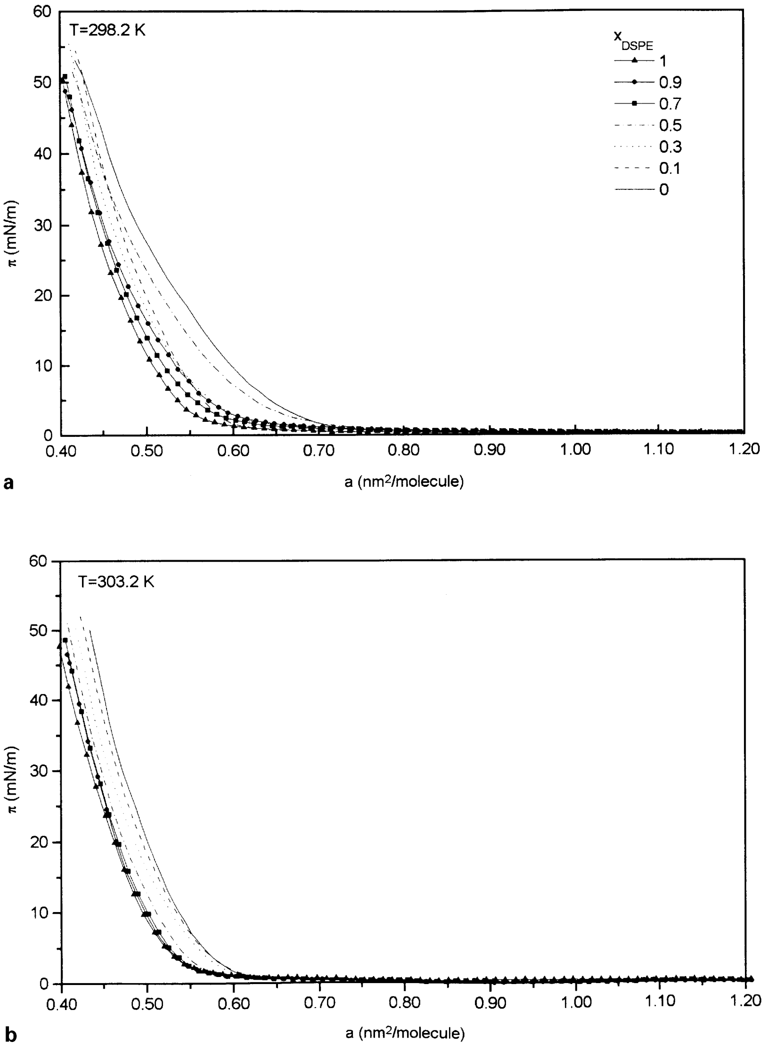
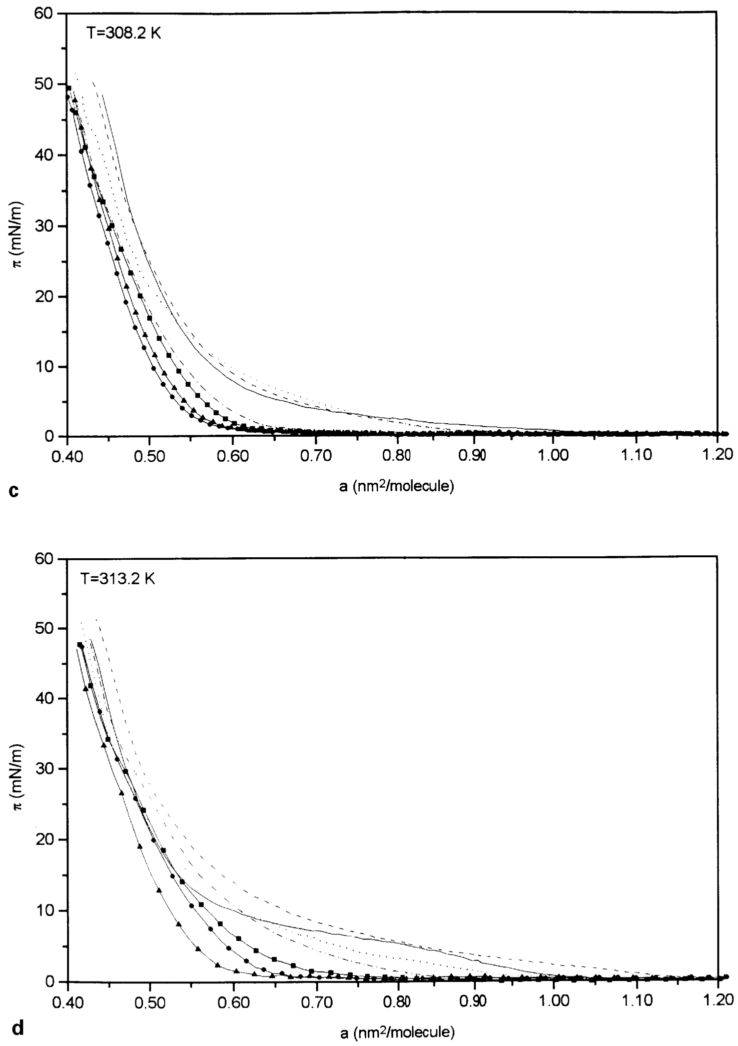


Fig. 2 π–a compression isotherms for DSPE–DSPC mixed monolayers at (a) 298.2 K, (b) 303.2 K, (c) 308.2 K and (d) 313.2 K

Fig. 2 (Continued)



temperature ranges. This means that for DSPE films, the attractive van der Waals forces between the hydrocarbon chains are more important than for DSPC films. In order to confirm this feature, a quantitative analysis is given.

The dispersion forces are known to play a very important role in lipid monolayers [8]. For straight chain hydrocarbon molecules, the dispersion forces can be determined from the following relationship [8, 9]:

$$W_d = -\,1.24 \times 10^3 \,\frac{N}{D^5}\,\text{kcal/mol}\,, \qquad (6)$$

where W_d is the interaction energy in kcal/mol per methylene group, N is the number of methylene groups in each chain and D is the chain separation distance. Considering a hexagonal packing of circular cross sections [8, 9], it is found that a(area/molecule) $= \sqrt{3}\,D^2/2$, i.e.:

$$W_d = -\,0.865 \times 10^3 \,\frac{N}{a^{5/2}}\,. \qquad (7)$$

From the π–a isotherms of DSPE–DSPC monolayers we have calculated W_d at different surface pressures. The values are shown in Table 1.

In fact, we demonstrate that the attractive van der Waals forces are more important for DSPE films than for DSPC monolayers. Then, in spite of the hydration of the ethanolamine groups, its smaller size, comparing with the choline groups, permits a close approach of the molecules. Moreover, it is also possible that water molecules around the ethanolamine groups screen the dipole moments of these groups hindering the electrostatic repulsive interactions, making the isotherms, again, more condensed.

Therefore, regarding again Fig. 3, we cannot conclude that the repulsive forces observed at a high proportion of

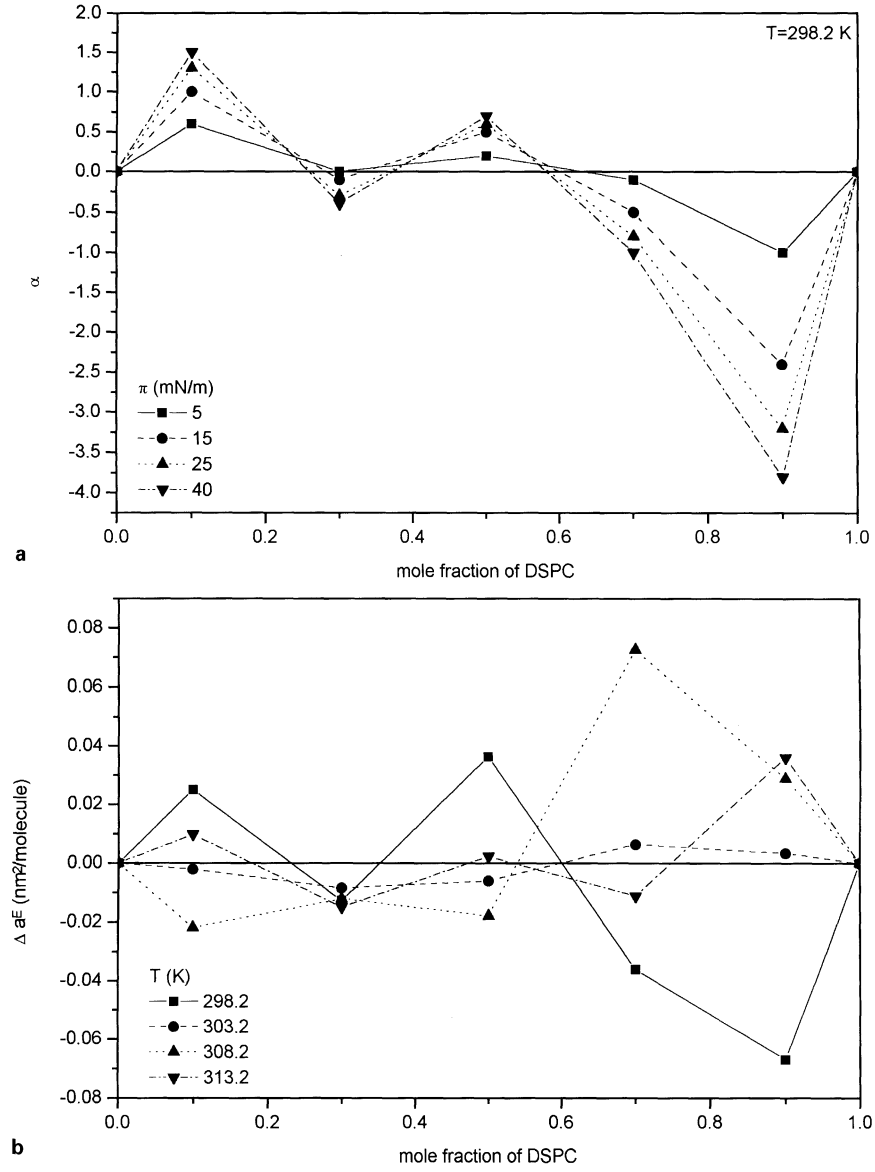


Fig. 3 (a) Interaction parameter versus mole fraction of DSPC in the monolayers, at 298.2 K, and at different surface pressures. (b) Excess area of mixing versus mole fraction of DSPC at different temperatures

Table 1 Dispersion forces, W_d (in kcal/mol), for DSPE and DSPC forming films at different temperatures and surface pressures

Temperature [K]	π [mN/m]	W_d [DSPE]	W_d [DSPC]
	5	−4.21	−2.66
298.2	20	−5.82	−4.15
	40	−7.70	−6.24
	5	−3.86	−1.45
313.2	20	−5.38	−4.84
	40	−7.52	−6.56

DSPE arise from the hydration of the polar group. We can consider that if the attractive interaction between DSPE molecules is large, they form a very high closely packed array, hindering the approach of DSPC molecules. Then, only the electrostatic repulsive interactions are marked clear. On increasing the proportion of DSPC in mixed monolayers, the interactions between both lipids assume an attractive character, since the dispersion forces between DSPC molecules are not sufficient to avoid an attractive interaction with the DSPE molecules.

In Fig. 3b, the excess area of mixing values versus the mole fraction of the DSPC in monolayers, are shown at different temperatures.

At a high proportion of DSPC, the deviations from the ideal behavior change from a negative to a positive character on increasing the temperature. In fact, when the temperature is increased, the mobility of hydrocarbon

Table 2 Excess entropy of mixing values (in J mol^{-1}K^{-1}) for DSPE–DSPC mixed monolayers, at different surface pressures and temperatures

$T = 298.2$ K

x_{DSPC}	$\pi = 5$ mN/m	15 mN/m	25 mN/m	40 mN/m
0.1	10	9.0	7.0	6.0
0.3	3.3	−8.0	−20	−26
0.5	13	7.0	−1.4	−4.4
0.7	−13	−37	−52	−57
0.9	−35	−75	−101	−119

$\pi = 15$ mN/m

x_{DSPC}	$T = 298.2$ K	303.2 K	308.2 K	313.2 K
0.1	9.4	8.1	7.0	10.5
0.3	−8.2	−7.4	−6.7	−4.5
0.5	7.2	4.9	5.3	9.7
0.7	−37.5	−34.7	−32.8	−32
0.9	−75	−71	−69.8	−65.6

chains is also increased, decreasing the van der Waals interactions.

On the contrary, when the mixture is rich in DSPE molecules, an increase in the temperature means that the repulsive interactions became attractive. This would mean that on increasing the temperature a dehydration effect occurs, favoring an approach of the molecules.

The excess entropy of mixing values for DSPE–DSPC mixed monolayers, at different surface pressures and temperatures are shown in Table 2. Also, we observe that on increasing the proportion of DSPC in the mixture, the attractive interactions between the film-forming molecules became dominant, ordering and reducing the possible number of the molecular configurations at the air–aqueous solution interface and then, reducing its entropy during the mixing process.

Mixed monolayers of DSPE–Sph

The π–a isotherms recorded by compression of simple and mixed monolayers forming by DSPE and Sph lipids at different temperatures are shown in Fig. 4. Again, we observe that mixed monolayers show an intermediate behavior compared with that shown by simple monolayers.

We found that the collapse surface pressure values of the Sph monolayers are slightly smaller than those for DSPE monolayers. This means that the asymmetric structure of the Sph molecules, containing a double bond, means that this lipid is expelled from the interface before DSPE lipid. No significant differences in the collapse surface pressure values for the mixed monolayers are observed. Nevertheless, we cannot conclude the immiscibility of both compounds.

With the aim to analyze the miscibility between DSPE and Sph lipids at the mentioned interface, in Fig. 5 we represent the thermodynamic interaction parameter as a function of the composition of the monolayer at different surface pressures. It can be observed that in all the compression range, the interactions between both kinds of molecules have a repulsive character. On the contrary, for the first system, the asymmetric molecules of Sph hinder the van der Waals attractive interactions between the nonpolar parts of the lipids, and the repulsive interactions that arise from the electrostatic forces between the polar groups of the molecules, are dominant. X-ray crystallography and neutron scattering [10, 11] experiments show that the head groups of phosphatidylcholines and phosphatidylethanolamines have similar orientations, essen-

Fig. 4 π–a compression isotherms for DSPE–Sph mixed monolayers at (a) 298.2 K, (b) 303.2 K, (c) 308.2 K and (d) 313.2 K

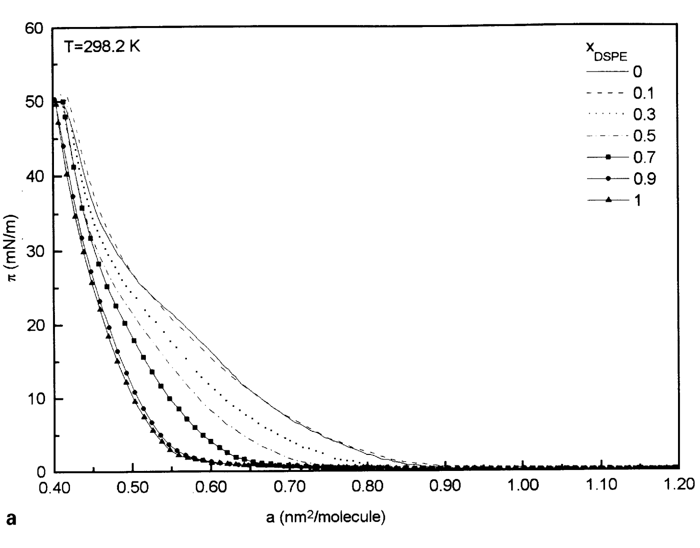
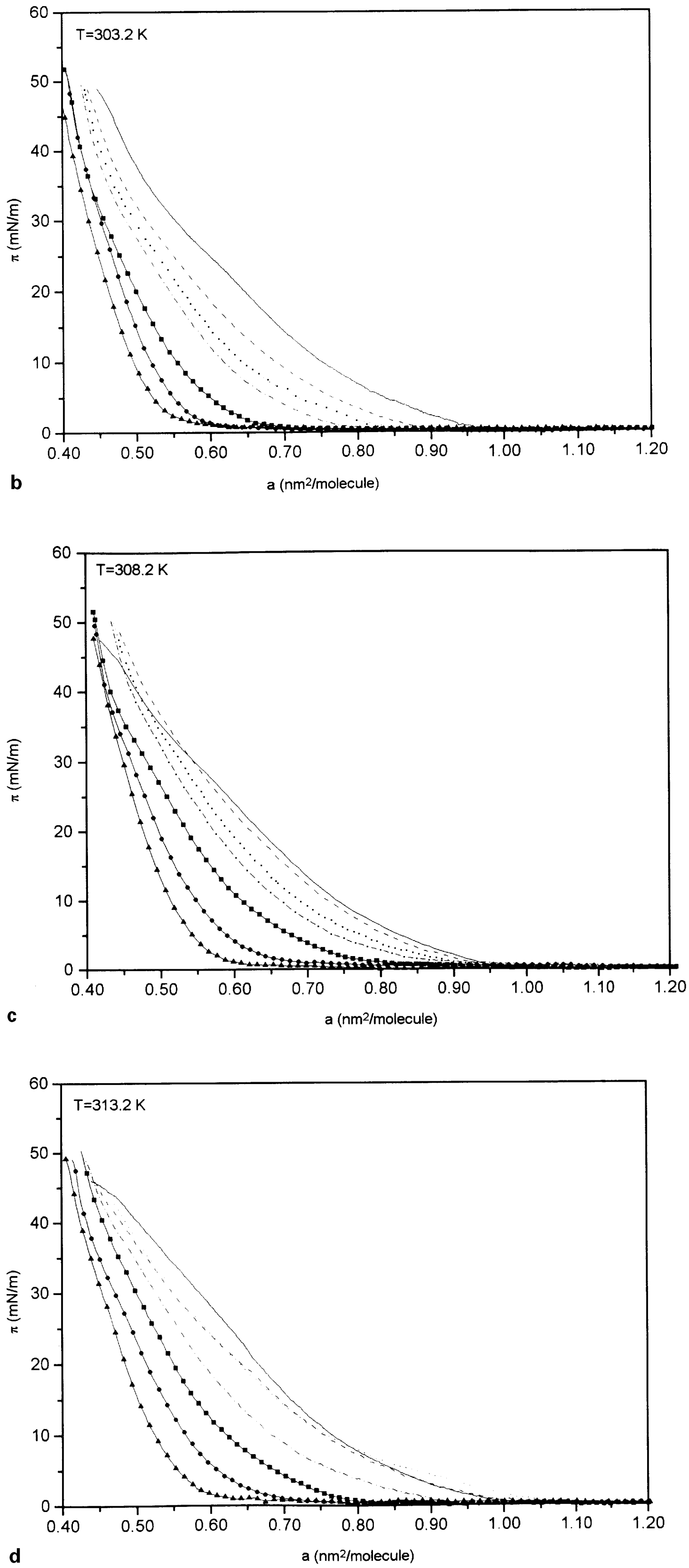


Fig. 4 (Continued)



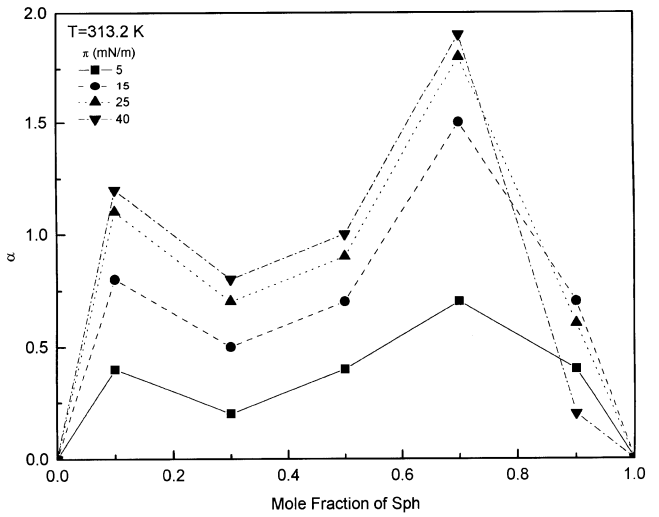


Fig. 5 Interaction parameter versus mole fraction of Sph in the monolayers, at 313.2 K, and at different surface pressures

tially parallel to the plane of the interface [12] and both of them possess an important dipole moment with two components, one "lateral" and another "normal". According to Gurkov et al. [12] we stipulate that if the lipid molecules cannot approach, the repulsion will prevail over the attraction.

The rest of the thermodynamic quantities of mixing (not shown) are explained in the same terms.

Conclusions

From the results obtained in this investigation we can conclude the following:

- When the ethanolamine group is present in the lipid molecules, the hydration forces play an important role in the behaviour of its monolayers.
- The interactions between the DSPE and DSPC lipids depend on the composition of the mixture. At high proportions of DSPC, the attractive van der Waals forces between the hydrocarbon chains are very significant. An increase in the temperature means a decrease of these interactions. On the contrary, if the mixed monolayers are rich in DSPE, the electrostatic repulsive interactions became dominant. In this case, on increasing the temperature, dehydration of the polar groups is responsible for a condensation effect in the films.
- In the mixed monolayers formed by DSPE–Sph, the same as the DSPC–Sph, the electrostatic repulsive interactions between polar groups dominate over those with attractive character between the hydrocarbon chains, due to the steric effects introduced by the Sph molecules.

Nevertheless, we point out that some authors [8, 13, 14] have shown that Eq. (2) does not describe the mixed films satisfactorily, due to the neglect of interaction between the subphase, water, and the film-forming components. Taking into account that for our systems the hydration effect is not negligible, we can further conclude that the relation given in Eq. (2) for such mixed films, could be inadequate to provide information on the mean interactions between both lipids, in agreement with early works [8, 13, 14], in spite of that this formulation is used by several authors.

It is apparent that further studies are required to confirm this hypothesis.

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References

1. Goodrich FC (1957) Proc 2nd International Congr Surface Activity, Vol 1, p 33
2. Dorfler H-D, Koth C, Retting W (1995) Langmuir 11(12):4803
3. Sánchez-González J, Gálvez-Ruiz MJ (1995) Progr Colloid Polym Sci 98:248
4. Sánchez-González J, Cabrerizo-Vílchez MA, Gálvez-Ruiz, MJ (1995) Short and long chains at interfaces, XXXth Moriond Workshop, Switzerland, Editions Frontieres, p 189
5. Queralto-Moreno A, Castro-Ruiz RN, Otero-Aenlle E (1980) An Quim 76:58
6. Gabrielli G, Puggelli M, Ferroni E, Carubia G, Pedochi L (1989) Colloids Surfaces 41:1
7. Gálvez-Ruiz MJ, Cabrerizo-Vílchez MA (1991) Colloids Surfaces 58:61
8. Birdi KS, Sørensen KE (1979) Colloid Polym Sci 257:942
9. Salem L (1962) J Chem Phys 37:2100
10. Dill KA, Stigter D (1988) Biochemistry 27:3446
11. Stigter D, Dill KA (1988) Langmuir 4:200
12. Gurkov TD, Kralchevsky PA, Nagayama K (1996) Colloid Polym Sci 274:227
13. Motomura K (1974) J Colloid Interface Sci 48:307
14. Motomura K, Sekita K (1974) J Colloid Interface Sci 48:319