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STABILITY AND INSTABILITY PROPERTIES OF AGGREGATION OF SINGLE CHAIN AMPHIPHILES INTO BINARY MIXTURES

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In a lysophospholipid binary mixture, there are three ways of association between the mixture components of single-chain amphiphiles: (a) between two identical molecules each of the first and second component (self-association process) and (b) between two different molecules (cross-association process). Association probabilities for three binary mixtures were analysed as functions depending on the electric dipole moments of the polar head groups. A 3-D view representation is most suitable for this analysis. The most important finding is that for certain values of the electric dipole moments there are molecular couples which have a maximum stability to the changes in the external electrolytic medium. This fact confirms the formation of clusters and their stability, which is equivalent to the existence of micro-heterogeneities within the lipid bilayers. On the other hand, there are unstable molecular associations, and this fact influences the appearance of some phase transitions. Generally, the increase of the electric dipole moment or the increase of the acyl-chain length of one component from a binary lipid mixture decreases the self-association probability between its own molecules, but it increases the self-association probability of the other mixture components. Furthermore, the cross-association probability has high values for any binary lipid mixture of single-chain amphiphiles. © *1997 Society for Mathematical Biology*

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1. Introduction.

Experimental studies concerning the properties of binary mixtures of phospholipids using differential scanning calorimetry (DSC), X-ray diffraction and electronic microscopy have been frequently performed during the last years and some of their results are particularly important. First, it was found that the molecular clusters occur in synthesized lipid bi-layers formed by binary mixtures (Jain, 1983). In some binary lipid mixtures, there is a phase separation of the molecular species, depending on the chain structure and the polar headgroups (Jorgensen *et al.*, 1993; Huang and Feigenson, 1993; Popescu, 1993). A unique phase transition was found using X-ray scattering on mono-layers composed of one single-chain amphiphile species (Helm *et al.*, 1991), whereas the DSC thermograms for various lipid binary mixtures show that at least one secondary transition appears additionally to the main transition (Huang and Mason, 1986).

Phase transition temperatures depend on the nature of mixture components, especially on the length of hydrophobic chains (Phillips *et al.*, 1970; Jain *et al.*, 1980; Huang and Feigenson, 1993). These phase transitions have been associated with the transitions between different organization patterns, which are characteristic for the liquid crystals (Huang and Mason, 1986). We were focused on finding those features of the cross-association probabilities that might be related to the typical states of binary mixtures (stable or unstable states). There is also a selective association process of the components in the lysophospholipid binary mixtures (Popescu, 1993, 1994; Movileanu and Popescu, 1995a, b, 1996). Biomembranes and lipid bi-layers are not static entities and their interfaces are not smooth (Hladky and Gruen, 1982; Miller, 1984; Popescu *et al.*, 1991). Lipid molecules perform lateral and perpendicular movements on the bi-layer surfaces.

Collective motions in lipid bilayers have been revealed by nuclear magnetic resonance (NMR) measurements (Stohrer *et al.*, 1991). This late result relates the selective association processes with the existence of clusters and the dynamic features of the lipid bi-layer structure. Data concerning the dependence of the association probabilities on some parameters of the binary mixture have been reported (Popescu, 1994; Movileanu and Popescu, 1995a, b). An analytic description of the association probabilities as a function of the two electric dipole moments (p_1 and p_2) would be more useful toward the understanding of these biological phenomena. This is the main purpose of the present paper, representing a mathematical extension of the previous ones.

2. Association Probabilities.

A binary mixture of amphiphile molecules with linear saturated chain can be characterized by the length of the hydrophobic chains (N_1, N_2), the cross-section area of the polar headgroups (a_1, a_2), the electric dipole moments of the polar headgroups (p_1, p_2) and the mole fraction x_1 (or $x_2 = 1 - x_1$) of one of the mixture components.

The length of the hydrophobic chain has been given by the number of methylene groups. The parameters concerning the short-chain component will be denoted by the index "1" and those concerning the long-chain component by the index "2". In a binary mixture organized as a mono-layer, two identical molecules can associate themselves with the association probabilities P_{11} and P_{22}, respectively, and two different molecules with the probability P_{12}. These association probabilities can be calculated using the following system (Popescu and Victor, 1990):

$$\frac{P_{11}}{P_{12}} = \frac{x_1}{x_2} \exp[-(U_{12} - U_{11})/RT], \tag{1}$$

$$\frac{P_{22}}{P_{12}} = \frac{x_2}{x_1} \exp[-(U_{12} - U_{22})/RT], \tag{2}$$

$$P_{11} + P_{12} + P_{22} = 1. \tag{3}$$

Solving this system, we have obtained the appropriate relation for the three association probabilities:

$$P_{11} = x_1^2 e_1 / (x_1^2 e_1 + x_2^2 e_2 + x_1 x_2), \tag{4}$$

$$P_{12} = x_1 x_2 / (x_1^2 e_1 + x_2^2 e_2 + x_1 x_2), \tag{5}$$

$$P_{22} = x_2^2 e_2 / (x_1^2 e_1 + x_2^2 e_2 + x_1 x_2), \tag{6}$$

where we used the following notations:

$$e_1 = \exp[-(U_{12} - U_{11})/RT], \tag{7}$$

$$e_2 = \exp[-(U_{12} - u_{22})/RT], \tag{7'}$$

$$U_{11} = -\left[\frac{p_1^2 N_A}{4\pi\varepsilon(2r_1)^3} + W_{11}\right], \tag{8}$$

$$U_{12} = -\left[\frac{p_1 p_2 N_A}{4\pi\varepsilon(r_1 + r_2)^3} + W_{12}\right], \tag{9}$$

$$U_{22} = -\left[\frac{p_2^2 N_A}{4\pi\varepsilon(2r_2)^3} + W_{22}\right], \tag{10}$$

with p_1, r_1 and p_2, r_2 denoting the electric dipole moment and the radius of the polar headgroup of the amphiphile molecule from the first and second species, respectively. In these equations, N_A, R, T and ε denote the Avogadro's number, the gas constant, the absolute temperature and the dielectric constant of water, respectively. U_{11}, U_{12}, U_{22} are coupling interaction energies between two identical neighbouring molecules (U_{11}, U_{22}) or different ones (U_{12}). The first term in equations (8)–(10) accounts for the interactions between the electric dipole moments. Since the electric dipole moments of lysophospholipids have no sterical restrictions, they are parallel to the mono-layer surface and can couple themselves in the most favourable energetic state. The second term in equations (8)–(10) is due to the van der Waals dispersion forces between hydrophobic chains. They are given by the following expressions (Movileanu and Popescu, 1995b):

$$W_{11} = \sum_{i,k=1}^{N_1} \frac{A}{\left[4r_1^2 + (i-k)^2\lambda^2\right]^3}, \tag{11}$$

$$W_{12} = \sum_{i=1}^{N_1} \sum_{k=1}^{N_2} \frac{A}{\left[(r_1 + r_2)^2 + (i-k)^2\lambda^2\right]^3}, \tag{12}$$

$$W_{22} = \sum_{i,k=1}^{N_2} \frac{A}{\left[4r_2^2 + (i-k)^2\lambda^2\right]^3}, \tag{13}$$

where $\lambda = 1.27$ Å is the length of the covalent bond C—C, which is perpendicularly projected to the mono-layer.

At low temperatures, the mono-layer is in gel phase and the lipid molecules are close enough so that it is right to suppose that the hydrophobic chains are parallel and all the covalent bonds are in trans-conformation. For the calculation of the van der Waals–London interaction energy, the assumption that each saturated hydrophobic chain is constituted by attraction centres localized on the acyl-chain axis has been made. Hamacker's constant was calculated so that each attraction centre is equivalent to a methylene group and is laid on the chain axis. Within the limits of the second-order perturbation theory, the hypothesis of local additivity of the dispersion energies is accepted (Salem, 1962). In the case of cylinders of uniform polarizability and non-zero radius, the adequate formula has been given by Langbein (1976). Using the more accurate formula of Langbein in the calculation of the van der Waals–London dispersion energy, we have a numerical effect of about 4% (an increase), in comparison with the value obtained by Salem's formula (Nagle and Wilkinson, 1978). Moreover, because the difference between the cross-pair formation energy and the like-pair formation energy appears in the computation of the association probabilities, the effect of this approximation on the association probabilities might be neglected. Therefore, we can assume the validity of the conclusions concerning the association probabilities, even though the non-additivity of the dispersion forces was not considered. The distance between any two attraction centres is less than 1000 Å, so that the retardation effects have not been considered here (Langbein, 1976). At the same time, the distance between the nearest attraction centres (about 7.14 Å) is much higher when compared with the dimensions of the attraction entities (atoms, covalent bonds), and the London's law ($W = -A/d^6$) is valid, where $A = 5.6 * 10^3$ Å^6 J mol^{-1}.

3. Results and Discussion. Association probabilities have been calculated for 36 binary lysophospholipid mixtures of single-chain amphiphile molecules and they can be displayed in a triangular matrix. Each element of the triangular matrix contains hydrophobic chain lengths of the two components. The elements of the matrix are: (N_1, N_2) with $N_1 \in [8, 10, \ldots, 22]$; $N_1 < N_2 \leq 24$, $N_2 \in [N_1 + 2, 24]$. Some of these considerations were presented in previous papers (Popescu 1993, 1994, Movileanu and Popescu, 1995a, b). The cross-section area for the polar headgroup of the first component is $a_1 = 40$ Å^2, and the second one is $a_2 = 60$ Å^2. We imagined a general procedure for the calculation of the optimal surface area of amphiphile molecules by using the hard-core method (Popescu and Victor, 1991). Values of 5, 10, 15, ..., 45 D were selected for the electric dipole moment for each component (1 D = 1 Debye = 3.33564×10^{-30} cm).

Generally, the electric dipole moment of amphiphile molecules depends strongly on the external aqueous medium that can modify both fixed effective electrical charges of the electric dipole moment and the distance between them (Movileanu and Popescu, 1995b). For this reason, a set of values for the electric dipole moments was chosen so that the real values of the lipid molecules fall in this interval. For instance, lysophosphatidylcholine has an electric dipole moment higher than 35 D.

A 3-D representation of the association probabilities as a function of the electric dipole moments of the mixture components (p_1 and p_2) gives an overall view of the influences on the association process. Of the 36 binary mixtures investigated, only three have been selected, in order to emphasize the specific influence of hydrophobic chains on the three association probabilities. These binary mixtures are (C08 : C10), (C08 : C24) and (C22 : C24).

3.1. *Cross-association probability.* By analysing the results obtained for all the binary mixtures, we found that the cross-association probability had a special behaviour with respect to the electric dipole moment and the length of the hydrophobic chain of each component. For this reason, we have decided to carry out both a theoretical study and a 3-D view on the cross-association probability as a function of the electric dipole moments of mixed amphiphile molecules.

First, we focused our attention on finding an easier way to describe the important features of the cross-association probability for all the single-chain binary mixtures. Since the cross-association probability depends on both electric dipole moments p_1 and p_2, we have successively analysed its dependence only on one electric dipole moment (considered as a variable), while the other one was considered a parameter, whose values were maintained constantly. Therefore, whenever p_1 is a variable, p_2 is a parameter, and vice versa. In order to find out the extreme values of P_{12}, it is necessary to solve the equation that derives from the annulment of its first-order differential equation with respect to the variable electric dipole moment ($\mathrm{d}P_{12}/\mathrm{d}p = 0$).

Following this procedure, we obtained the equations:

$$\frac{2\alpha_1}{\alpha_0}\frac{p_1}{p_2} - 1 = \exp\left(\alpha_1 p_1^2 - \alpha_2 p_2^2 - \gamma_0\right)$$

$$\text{for the variable electric dipole moment } p_1, \tag{14}$$

$$\frac{2\alpha_2}{\alpha_0}\frac{p_1}{p_2} - 1 = \exp\left(\alpha_2 p_2^2 - \alpha_1 p_1^2 + \gamma_0\right)$$

$$\text{for the variable electric dipole moment } p_2, \tag{15}$$

where the constants have the following meaning:

$$\alpha_1 = N_A/\left(4\pi\varepsilon(2r_1)^3\right); \qquad \alpha_2 = N_A/\left(4\pi\varepsilon(2r_2)^3\right), \tag{16}$$

$$\alpha_0 = N_A/\left(4\pi\varepsilon(r_1 + r_2)^3\right); \qquad \beta = 1/RT, \tag{17}$$

$$\gamma_1 = \beta(W_{12} - W_{11}); \qquad \gamma_2 = \beta(W_{22} - W_{12}); \qquad \gamma_0 = \beta(W_{22} - W_{11}). \tag{18}$$

These equations have been derived by retaining the first three terms in the Taylor's series. As far as the dependence of the cross-association probability on the variable electric dipole moment p_1 or p_2 is concerned, there are three distinct cases. To prevent a very long discussion, we arranged these distinct situations in Table 1.

Table 1. The dependence cases of the cross-association probability P_{12} on each of the two electric dipole moments when the other is considered as a parameter. In each case, the equations for the calculation of the critical values are given

	(a) Dependence on p_1; parameter: p_2 ($t_1 = u/\alpha_2$; $D_1 = 2[1 + \exp(\gamma_0) - 2u^*\exp(\gamma_0)]$)	(b) Dependence on p_2; parameter p_1 ($t_2 = u/\alpha_1$; $D_2 = 2[1 + \exp(-\gamma_0) - 2u^*\exp(-\gamma_0)]$)
Case 1	$\gamma_0 > -\ln(2u - 1)$	$\gamma_0 < \ln(2u - 1)$
Case 2	$-\ln[2(2u - 1)] < \gamma_0 < -\ln(2u - 1)$ $p'_{2c} = [t_1 \exp(\gamma_0)/(1 + D_1^{1/2})]^{1/2}$ $p''_{2c} = [t_1 \exp(\gamma_0/(1 - D_1^{1/2})]^{1/2}$	$\ln(2u - 1) < \gamma_0 < \ln[2(2u - 1)]$ $p'_{1c} = [t_2 \exp(-\gamma_0)/(1 + D_2^{1/2})]^{1/2}$ $p''_{1c} = [t_2 \exp(-\gamma_0)/(1 - D_2^{1/2})]^{1/2}$
Case 3	$\gamma < -\ln[2(2u - 1)]$ $p_{2c} = [t_1 \exp(\gamma_0)/(1 + D_1^{1/2})]^{1/2}$	$\gamma_0 > \ln[2(2u - 1)]$ $p_{1c} = [t_2 \exp(-\gamma_0)/(1 + D_2^{1/2})]^{1/2}$

In this paper, the above-mentioned mixed molecules possess the following parameters:

$$\alpha_1 = 7.1947\ \mathrm{D}^{-2}; \qquad \alpha_2 = 3.9163\ \mathrm{D}^{-2}; \qquad \alpha_0 = 5.2271\ \mathrm{D}^{-2}.$$

The constants γ_1, γ_2 and γ_0 are given in Table 2.

The inclusion of a certain binary mixture in one of the three cases is determined by γ_0, i.e. by the difference between the van der Waals interaction energies of like-pair formation. In other words, the hydrophobic chain lengths of the two components define the case where their binary mixture belongs. In the first case, there are two values of the variable electric dipole moment for which the cross-association probability P_{12} shows a minimum and a maximum regardless of the value of the parameter electric dipole moment. If γ_0 fulfils the condition for the second case (Table 1), then the parameter electric dipole moment takes two "critical" values (p'_c and p''_c). These values define the dependence ways of the cross-association P_{12} on the variable electric dipole moment. Therefore, if the parameter electric dipole moment (p_1, for example) takes values ranging within the interval $[p'_c, p''_c]$, then the cross-association probability P_{12} is a decreasing function on the variable electric dipole moment (p_2, in this situation). Finally, in the third case, the parameter electric dipole moment has only a "critical" value (p_c). The cross-association probability

Table 2. The values of some parameters calculated for the three binary mixtures used in this work. P_{11}^m, P_{12}^m and P_{22}^m are the mean association probabilities over both electric dipole moments

Binary mixture	P_{11}^m	P_{12}^m	P_{22}^m	γ_1	γ_2	γ_3
C08 : C10	0.2119	0.3529	0.4351	−0.3051	−0.1499	−0.4550
C08 : C24	0.2731	0.4460	0.2810	−0.2616	−0.5344	−0.2728
C22 : C24	0.0851	0.3158	0.5986	−1.1354	−0.6003	−1.7357

P_{12} decreases whether the parameter electric dipole moment takes higher values than its critical value and extreme values whether the parameter is less than p_c. The higher critical value decreases and the lower one increases when γ_0 decreases (the difference between the hydrophobic chain length is reduced).

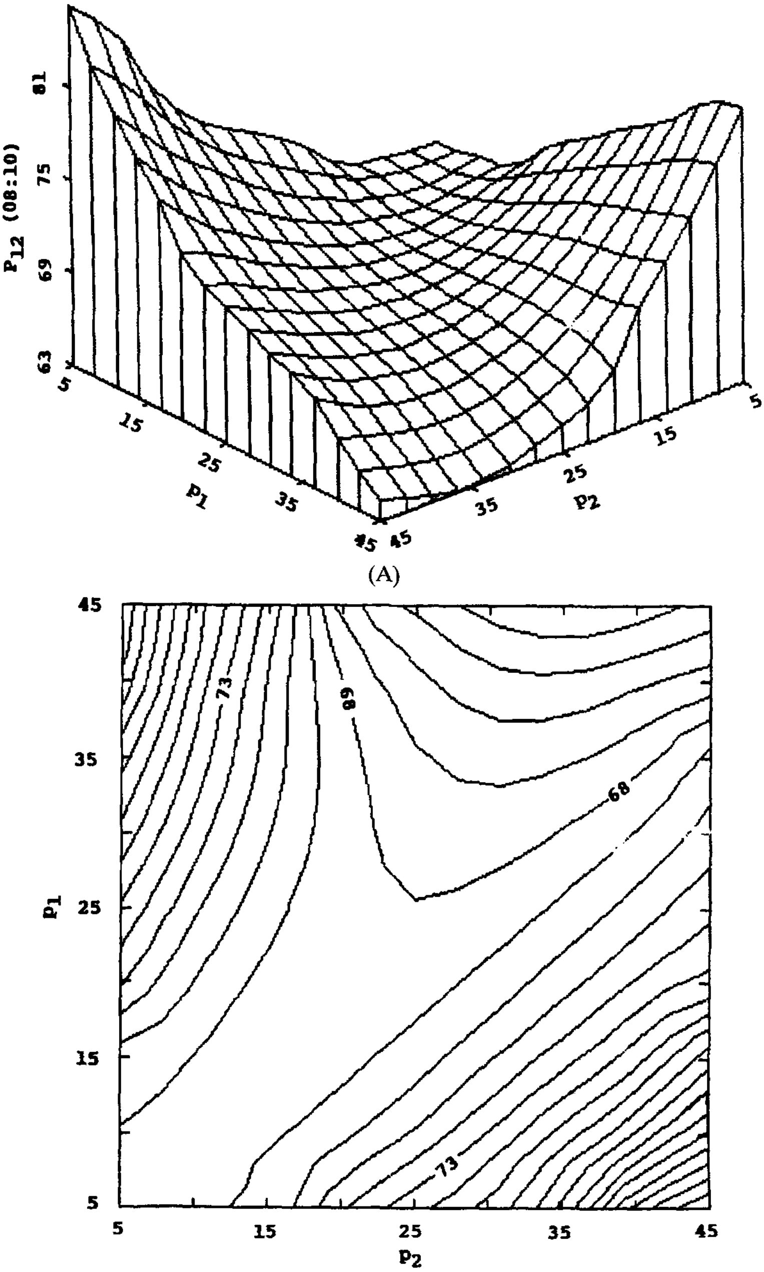
On the other hand, both abscissas of the extreme values increase and move toward one another when the parameter takes increasing values. For this reason, it is possible for the electric dipole moment values selected in this paper (that cover the range [5, 45] D) to fall inside or outside the range $[p_m, p_M]$. The term p_m is the electric dipole moment that corresponds to the minimum cross-association probability, and p_M is the electric dipole moment that corresponds to the maximum cross-association probability. For this reason, the function $P_{12}=f(p_1)$ has an inverse behaviour for lower values of p_2 than for its high values with respect to the monotony (Fig. 1A). In Fig. 1A, we observe that $P_{12}=f(p_2)$ behaves in the same way. For instance, when $p_1 = 5$ D, then $P_{12}=f(p_2)$ increases rapidly from 0.3450 and 0.4288, whereas for $p_1 = 45$ D, the cross-association probability P_{12} decreases from 0.2012 to 0.1585. For the same reason, the cross-association P_{12} may have only a minimum or only a maximum value on our plots, even though γ_0 obeys Case 2 (Table 1). Looking at Fig. 1A–C we observe that the 3-D view of the cross-association probability P_{12} as a function of both dipole moments is a surface resembling a square sheet folded approximately along one of its diagonals. This diagonal corresponds to equal dipole moments ($p_2 = p_2$). For all three mixtures chosen in this paper, the valley has a tendency from low values toward high values of the electric dipole moments. Its position and slope change with respect to the binary mixture. Actually, the projection of the valley background on the plane of the electric dipole moments (p_1, p_2) is a straight line:

$$p_1 = \sqrt{\frac{\alpha_1}{\alpha_2}}\left(u + \sqrt{u^2 - 1}\right)p_2, \tag{19}$$

with

$$u = \frac{\sqrt{\alpha_1 \alpha_2}}{\alpha_0} = 1.0155.$$

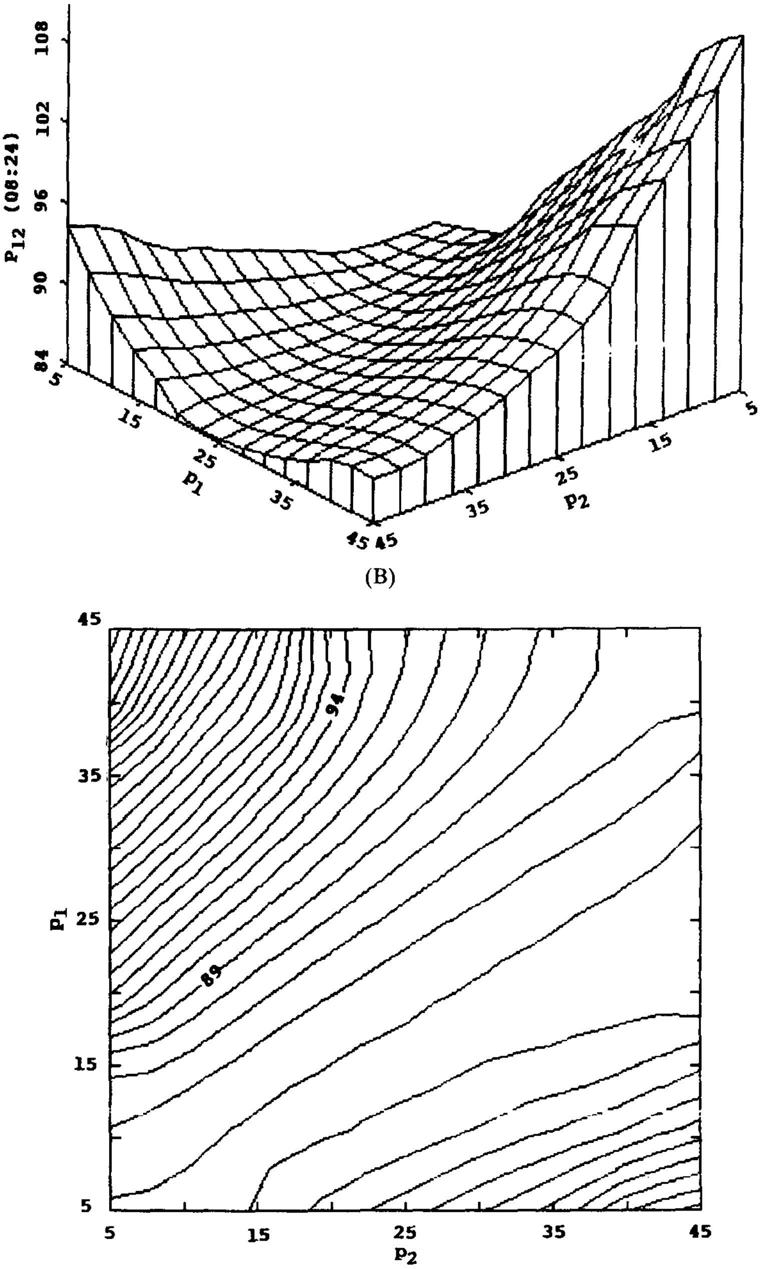
Additionally, a more detailed analysis was necessary to present the data (Fig. 1A–C) so that the curves connect points having the same probability magnitude (iso-probability curves), similar to the way isobars are plotted to create a synoptic map, and referred to as the "contour" of the data, or, simply, "the contour map". To meet the cases previously discussed in this paper, we should analyse in detail the 3-D view representations (or simply,



(A)

(a)

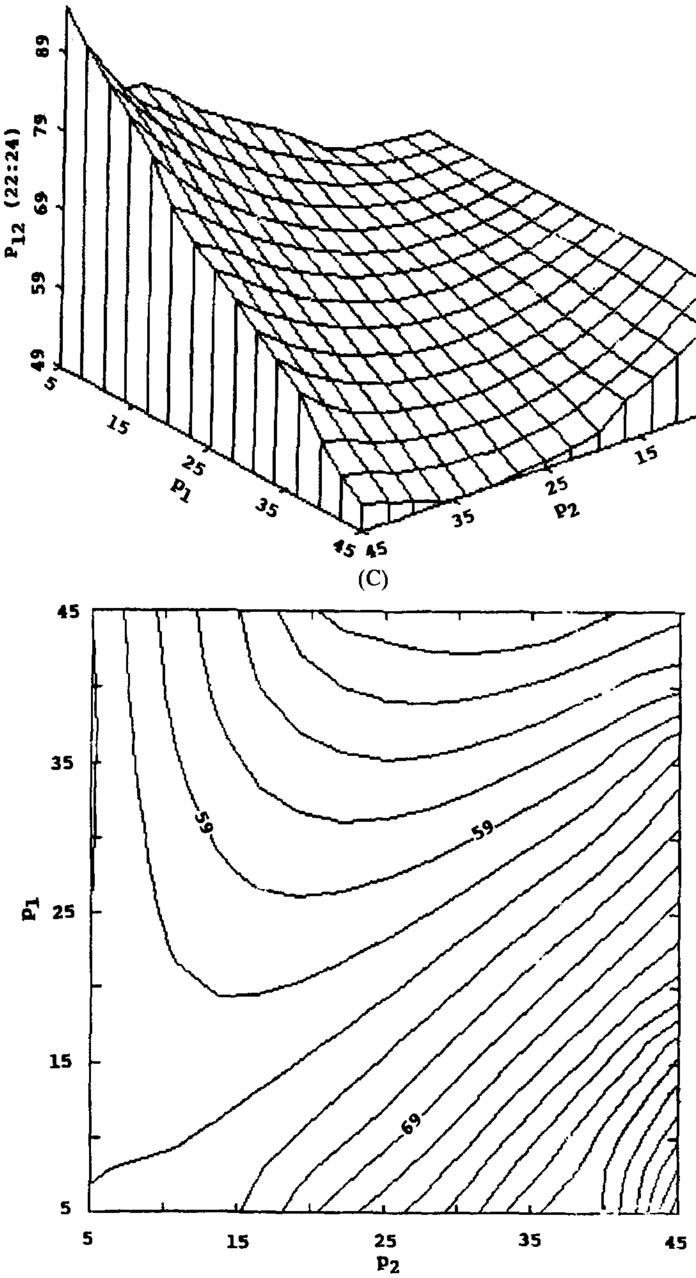
Figure 1. 3-D view representation of cross-association probability P_{12} as a function of electric dipole moments of both mixed components for following binary mixtures: (A) C08 : C10, (B) C08 : C24; (C) C22 : C24. For each 3-D view, iso-probability contour map was appended: (a) C08 : C10. P_{12} varies between 63.25 and 85.92 with step equal to 1; (b) C08 : C24. P_{12} varies between 84.21 and 110.55 with step equal to 1; (c) C22 : C24. P_{12} varies between 49.11 and 93.98 with step equal to 2. Electric dipole moments are given in Debye. Cross-association probabilities are multiplied by 200.



(B)

(b)

Figure 1. (*Continued*).



(C)

(c)

Figure 1. (*Continued*).

the 3-D view) and the appended contour maps for all binary mixture systems (Fig. 1A–C and a–c).

Looking at the three 3-D views of the cross-association probability P_{12}, we can easily find that the surface $P_{12}(p_1, p_2)$ modifies strongly whether the hydrophobic chain lengths are changed into a binary mixture. In Fig. 1A, the case when the first mixture component is comparable with the second one is shown ($N_1 = 8$, $N_2 = 10$). The cross-association probability P_{12} is a maximum for $p_1 = 5$ D and $p_2 = 45$ D. At the same time, P_{12} reaches higher values for a large electric dipole moment of the first mixture component and a small electric dipole moment of the second mixture component, or vice versa. Accordingly, the cross-association probability P_{12} is increased whether the difference between the electric dipole moments of the two mixture components is higher.

In the case of the binary mixture C08 : C10, the cross-association probability P_{12}, as a function depending on the electric dipole moment p_1, increases (but very slowly) if $p_2 < 14$ D; this function exhibits a very flat minimum if $14\text{ D} \leq p_2 < 18.13$ D; the cross-association probability P_{12} shows a minimum and a maximum if $18.13\text{ D} \leq p_2 < 19.43$ D, and it decreases if $p_2 \geq 19.43$ D (Fig. 1A and a). Taking into account the value α_0 for this binary mixture, then it falls in Case 2a (Table 1). Critical values of the parameter p_2 are $p'_{2c} = 0.3485$ D and $p''_{2c} = 0.9888$ D. For the binary mixture C08 : C24 (Fig. 1B and b), if $p_2 < 14$ D, then the cross-association probability has a steep increase, whereas in the range $14\text{ D} \leq p_2 < 37.8$ D, it has a minimum with an abscissa increasing with p_2 (it obeys approximately the law $p_{1m} = 2.85 + 20p_2/37$). For $p_2 \geq 37.8$ D, P_{12} shows a minimum value as well as a maximum value. This dependence is described by Case 1a (Table 1). The valley background is nearly parallel to the horizontal plane (Fig. 1B), as we can see from our contour map (Fig. 1b). Therefore, the cross-association probability P_{12} has the same minimum value for the values of p_1 and p_2 that obey the above equation. The last binary lysophospholipid mixture C22 : C24 relates to the condition of Case 3a. The critical value p_2 equals $p_{2c} = 0.5927$ D. Therefore, for the electric dipole moment magnitudes p_1 selected here, the cross-association probability P_{12} decreases for all fixed values of the parameter electric dipole moment p_2. Regarding the dependence of the cross-association probability P_{12} on the electric dipole moment p_2 (p_1 is a parameter in this situation), this has a minimum value whatever the fixed value of the dipole moment p_1 for the mixtures C08 : C10 and C22 : C24 (Case 1b). The abscissa magnitude p_{2m} increases with p_1. The valley orientation for the binary mixture C22 : C24 (Fig. 1C and c) is on the other part of the diagonal by comparison with its orientation for the binary mixture C08 : C10 (Fig. 1A and a). The cross-association probability P_{12} has a minimum value if $p_1 \leq 30.8$ D and increases for the other values of p_1 in the case of binary mixture C08 : C24 (Case 2b). Critical values of the parameters p_1 are $p'_{1c} = 0.2547$ D and $p''_{1c} = 0.5589$ D.

3.2. *Self-association probability.* The surfaces representing the self-association probability P_{11} on both electric dipole moments p_1 and p_2 for all homologous binary lipid mixtures are nearly like those from Fig. 2 (plotted for the binary mixture C08 : C24). It means that the lengths of the hydrophobic chains have a weak influence on the way P_{11} depends on the electric dipole moments. The self-association probability surfaces P_{11} for the homologous binary mixtures are shifted to one another with an additive quantity. The shift depends on the hydrophobic chain lengths. The above observations are available for the self-association probability surface P_{22} as well. For these reasons, we have presented here a 3-D plot for the self-association probability for only a binary mixture.

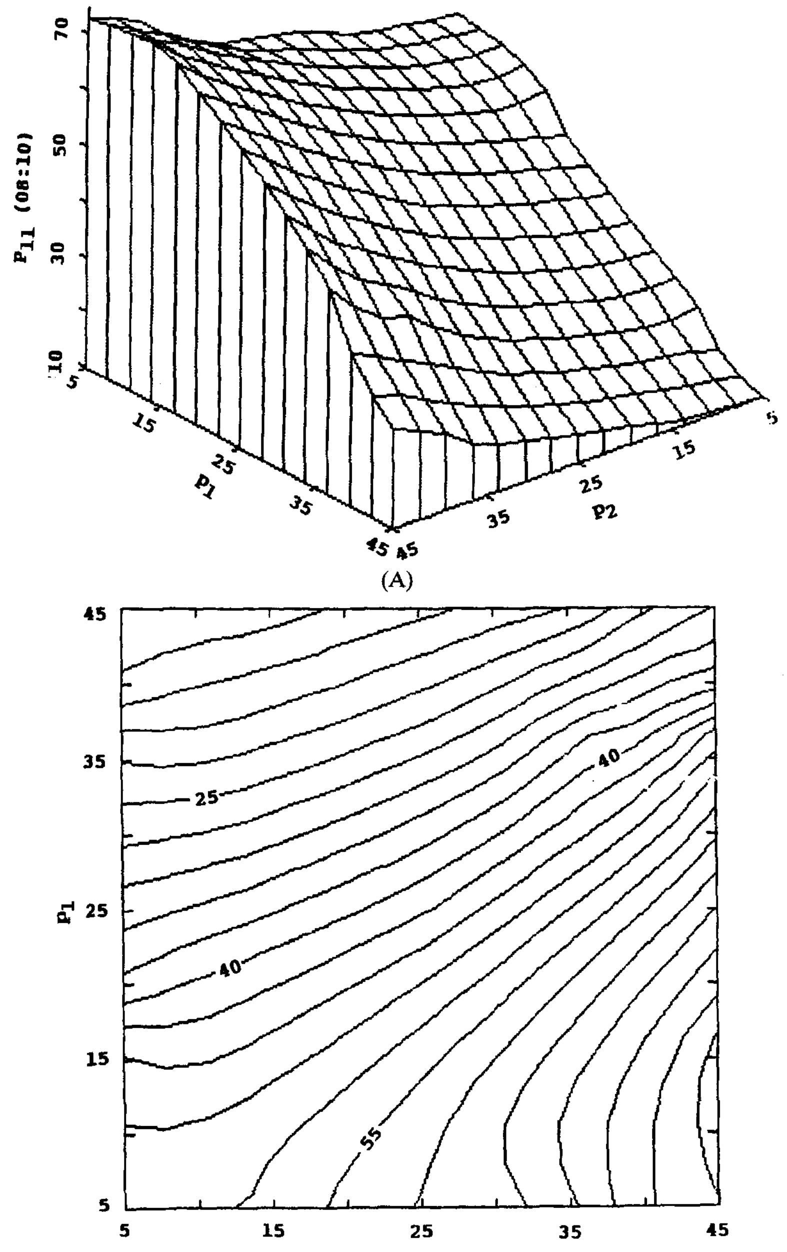
A surface describing the dependence of self-association probability on the electric dipole moments consists of two domains:

(a) a planar zone, corresponding to the almost straight lines in the contour map (Figs. 2a and 3a);
(b) a curved zone, corresponding to the curved lines in the contour map that shows the appearance of a peak (Figs. 2a and 3a).

For an easier analysis of the self-association probabilities on the electric dipole moments, we describe equations (4) and (6) in the following way (with the condition $x_1 = x_2 = 0.5$):

$$P_{11} = P_{12}e_1; \qquad P_{22} = P_{12}e_2 \tag{20}$$

The plots from Figs. 2 and 3 together with the corresponding contour maps show us that an increase of the electric dipole moment promotes the self-association of the other component molecules (whatever their electric dipole moment is) and inhibits the self-association of lipid molecules in its own phase for any binary mixture. For instance, the self-association probability P_{11} is an increasing function of p_2 for any constant value p_1 as well as of p_2 (as a function depending on p_1). If we study the association probability of two identical molecules as a function of their own electric dipole moment, then the following conclusion should arise: for a certain magnitude of the electric dipole moment of amphiphile molecules belonging to one phase, their self-association probability could have a maximum value. For instance, for $p_1 < 10$ D, the self-association P_{11}, as a function depending on p_1, has a maximum value (Fig. 2), whereas P_{22} has a maximum value as a function depending on p_2 ($p_2 < 25$ D, Fig. 3). Let us compare the iso-probability maps for P_{11} with the one for P_{22} for each binary lipid mixture: Fig. 2a with Fig. 3a. The places of the iso-probability lines corresponding to the planar region (nearly straight lines on the contour map) and those of the curved region (parabolic lines) appear interchanged on the P_{11} chart compared to P_{22}. This is an effect of the association probability symmetry with respect to the electric dipole mo-



(A)

(a)

Figure 2. 3-D view representation of self-association probability of shorter hydrophobic chain molecules P_{11} versus both their electric dipole moments for binary mixtures: (A) C08:C10. For this surface, we showed iso-probability contour map (a). P_{11} varies between 10.12 and 74.20 with step equal to 3. Electric dipole moments are given in Debye. Self-association probabilities of shorter hydrophobic chain molecules are multiplied by 200.

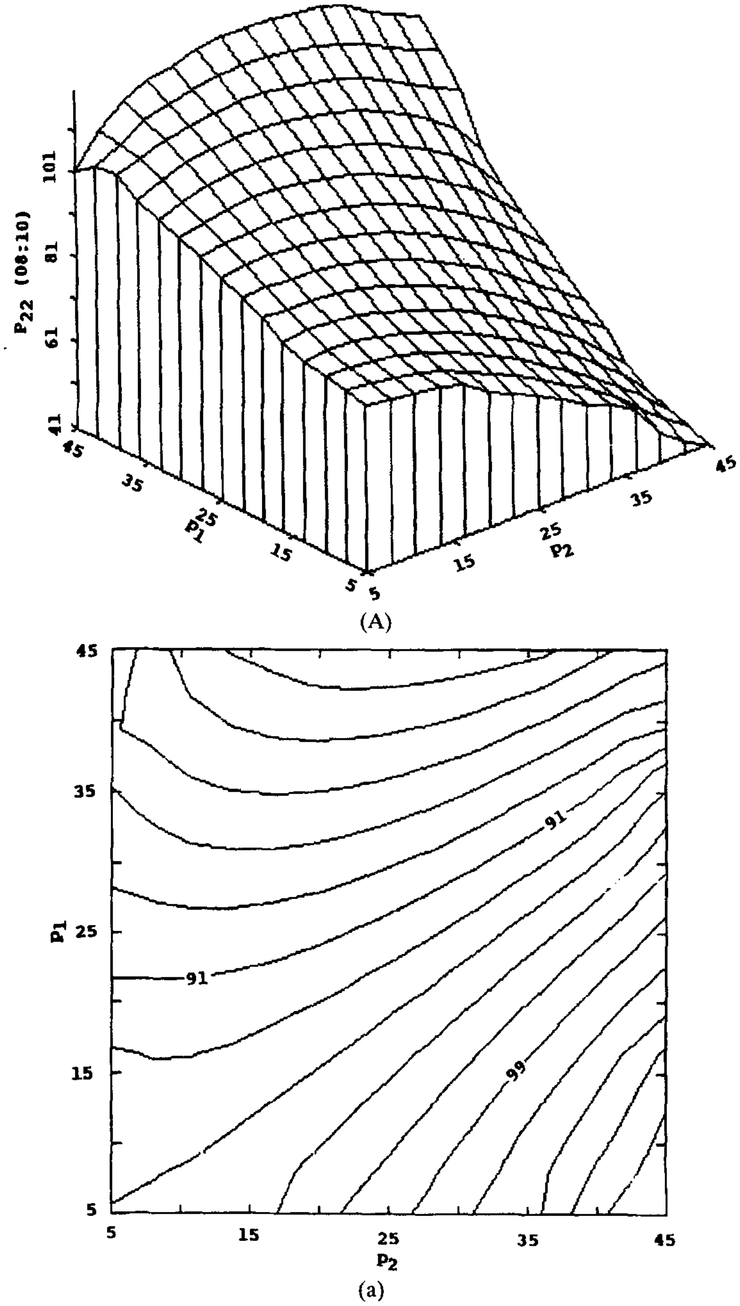


Figure 3. 3-D view representation of self-association probability P_{22} of longer hydrophobic chain molecules versus both their electric dipole moments for binary mixtures: (A) C08 : C10 and its corresponding contour map (a). P_{22} varies between 41.03 and 120.3 with step equal to 5. Electric dipole moments are given in Debye. Self-association probabilities of longer hydrophobic chain molecules are multiplied by 200.

ments p_1 and p_2. Because the two mixture components are different from each other by their acyl-chain length and the size of the polar headgroups, the symmetry is compared to the line separating the two types of iso-probability. Actually, for each binary lipid mixture, this line does not change its place on the contour maps of the self-association probabilities P_{11} and P_{22}.

3.3. *Hydrophobic chain effect.* These three binary mixtures were also selected in order to emphasize the hydrophobic chain influence on the association probabilities. In this respect, we have paid attention to the change and the shift of each surface. The mean association probabilities (P_{ij}^m, $i, j = 1,2$) for each of the three binary mixtures have been evaluated as well (Table 2). The mean association probability is, actually, the mean distance from the respective surface to the horizontal plane of the electric dipole moments (p_1, p_2) (for instance, see the 3-D views). Making a comparison between the results regarding the mean association probabilities for the binary mixtures C08 : C10 and C08 : C24, we can see the effect of the increase of the longer hydrophobic chain. As far as an analysis of the binary mixtures C08 : C24 and C22 : C24 is concerned, we pursue the effect of the increase of the shorter hydrophobic chain. Comparing the surfaces for different binary mixtures (Figs. 1A–C, 2A and 3A, as well as the corresponding contour maps) and P_{ij}^m from Table 2, and taking into account the scale factor introduced by the computer program, we find the same remark as in the case of the mean association probabilities: the increase of the longer hydrophobic chain from 10 to 24 methylene groups promotes an increase of the association probabilities P_{11} and P_{12} and a decrease of the association probabilities P_{22}; the increase of the shorter hydrophobic chain from 8 to 22 methylene groups has an opposite effect on each association probability compared with the increase of the longer hydrophobic chain. Some observations should be made: (1) the self-association probability P_{11} is drastically diminished by the increase of the longer hydrophobic chain; (2) the dispersion forces strongly affect the dependence of the cross-association probability P_{12} on the electric dipole moments of each component by comparing the dependence of the self-association probabilities P_{11} and P_{22} on the electric dipole moments.

4. Concluding Remarks. By analyzing formulas (4)–(10) we observe that the amphiphile molecule association processes by one of the three ways (P_{11}, P_{12} and P_{22}) is determined by the relative magnitude of the interaction energy between the two different molecules (P_{12}) and between the two identical molecules, and not by the absolute value of the interaction energy between two molecules in each of the possible pairs. In other words, neither the dipole–dipole interaction energy nor the interaction energy through dispersion forces between two different or identical molecules play the leading role in the association processes. Obviously, γ_1 and $-\gamma_2$ have opposite signs and they increase as the difference between the hydrophobic chain lengths increases. In the present stochastic inter-molecular interaction model, we have not introduced the energy term that comes from the mismatch in the hydrophobic chain lengths (Sperotto and Mouritsen, 1991; Fattal and Ben-Shaul, 1993; Jorgensen *et al.*, 1993). It is reasonable to understand that the non-ideality of mixing increases with the increase of the chain length difference of the two lipid components. Very recently, Nibu *et al.* (1995) showed that the mixing behaviour of homologous phospholipids might be understood in terms of the polar headgroup contribution to the overall inter-molecular interaction. However, the non-ideal nature of mixing for different phospholipids due to the mismatch in the hydrophobic lengths could lead to a progressively increasing local ordering, while the acyl-chain length difference is increased (Jorgensen *et al.*, 1993). If the difference between the hydrophobic chain lengths is constant, then γ_1 and γ_2 increase with the increasing value of the hydrophobic chain lengths. If the electric dipole moments p_1 and p_2 are equal, then the differences between the dipole–dipole interaction energies are small, but not zero, since the cross-section areas of the polar headgroups are different. Taking into account these remarks and the relations (4)–(6) with respect to the electric dipole moments, then the slope of the surface in Fig. 3A might be easily explained. For steeply different electric dipole moments, the dipole influence largely exceeds the dispersive influence. The formulas (4)–(6) for the association probabilities are symmetrical to the electric dipole moment interchanges. By comparing the iso-probability plots from Figs. 2a and 3a, we found that the places of the iso-probability lines corresponding to the planar region (nearly straight lines) and those of the curved region (parabolic lines) appear interchanged on plot P_{22} compared with plot P_{11}. There are the electric dipole moment values (those for which an association probability points to the maximum or minimum magnitude) for which the associated amphiphiles are stable to the changes of the electric dipole moments. At the same time, there are electric dipole moment values (the abscissas of the inflexion point) for which the association processes are very sensitive to any change of the electric dipole moments. Taking into account that the electric dipole moment magnitude of the polar headgroup may be influenced by the external aqueous medium (Movileanu and Popescu, 1995b), we conclude that, in certain conditions, the internal bi-layer dynamics should be correlated with the molecular dynamics of the external aqueous medium. A mixture of fatty acid and lysophosphatidylcholine forms planar bi-layers, even though the separate components form micelles (Jain *et al.*, 1980). Therefore, it is clear that a mixture of lysophospholipids, the formed pairs are similar to double-chain phospholipids having a double polar headgroup, which have a good fit to the outside mono-layer of liposomes. Phospholipids represent the major component of the biological membranes. We have dealt with the association probability model for binary mixtures, since an association of three mixture components from a ternary system is not probable (Popescu and Victor, 1991).

The selective association processes have multiple biological implications. The association processes of lysophospholipids account for the appearance of micro-clusters separated by micro-interfaces (Jain, 1983; Popescu, 1993, 1994; Jorgensen *et al.*, 1993). The authors think that the differential scanning calorimetry (DSC) traces of a single-chain binary mixture should contain four endothermic peaks (one of them due to the dipole–dipole interaction) and their position is strongly dependent on the nature of the mixture components. Each of the other three peaks is necessary to correspond to one of the three association ways of mixed single-chain lipids. The increase of the hydrophobic chain length of one component from a binary mixture promotes shifting the two peaks toward the higher temperatures. On the other hand, the selective association processes might stimulate the appearance of phospholipid domains into bi-layer membranes. Different classes of phospholipids can accumulate into bi-layers due to the specific interactions between them. Phospholipid domains modulate the spatial organization patterns into biological membranes (Norris, 1992). Some membrane phenomena may be influenced by this mechanism of association, as in the flip-flop diffusion of phospholipids through bi-layer lipid membranes (Norris, 1989; Movileanu *et al.*, 1996) or cell cycle events (Norris, 1992). At a critical packing density of phospholipids, the appearance of the internal defects of the bi-layer, or even the breakdown of the lipid bi-layer, is very probable.

REFERENCES

Fattal, D. R. and A. Ben-Shaul. 1993. A molecular model for lipid-protein interaction in membranes: the role of hydrophobic mismatch. *Biophys. J.* **65**, 1795–1809.

Helm, C. A., P. Tippman-Krayer, H. Mohwald, J. A. Nielsen and K. Kjaer. 1991. Phases of phosphatidylethanolamine monolayers studied by synchrotron X-ray scattering. *Biophys. J.* **60**, 1457–1476.

Hladky, S. B. and D. W. R. Gruen. 1982. Thickness fluctuations in black lipid membranes. *Biophys. J* **38**, 251–258.

Huang, C. and J. T. Mason, 1986. Structure and properties of mixed-chain phospholipid assemblies. *Biochim. Biophys. Acta* **864**, 423–470.

Huang, J. and G. W. Feigenson. 1993. Monte Carlo simulation of lipid mixtures: finding phase separation. *Biophys. J.* **65**, 1788–1794.

Jain, M. K., C. J. A. Van Echteld, F. Ramirez, J. de Gier, G. H. de Haas and L. L. M. Van Deenen. 1980. Association of lysophosphatidylcholine with fatty acids in aqueous phase to form bilayers. *Nature (London)* **284**, 486–487.

Jain, M. K. 1983. Non-random lateral organization in bilayers and biomembranes. In *Membrane Fluidity in Biology*, R. Aloia (Ed), Vol. 1. London: Academic Press.

Jorgensen, K., M. M. Sperotto, O. G. Mouritsen, J. H. Ipsen and M. J. Zuckermann. 1993. Phase equilibria and local structure in binary lipid bilayers. *Biochim. Biophys. Acta* **1152**, 135–145.

Langbein, D. 1976. *Springer Tracts in Modern Physics*. Berlin: Springer.

Miller I. R. 1984. Energetics of fluctuation of lipid bilayer thickness. *Biophys. J.* **45**, 643–645.

Movileanu, L. and D. Popescu. 1995a. Aspects of self- and cross-association hydrophobicity in a single chain binary mixture. *Acta Biochim. Polon.* **42**, 89–96.

Movileanu, L. and D. Popescu. 1995b. Differential length effects in a binary mixture of single chain amphiphiles in planar monolayers. A three-dimensional approach. *BioSystems* **36**, 43–53.

Movileanu, L. and D. Popescu. 1996. Global ratio of efficiency in a single chain binary mixture. *J. Biol. Syst.* **4**, 425–432.

Movileanu, L., D. Popescu, G. Victor and G. Turcu. 1996. Selective association of phospholipids as a clue for the passive flip-flip diffusion through bilayer lipid membranes. *BioSystems*, to appear.

Nagle, J. F. and D. A. Wilkinson. 1978. Lecithin bilayers. Density measurements and molecular interactions. *Biophys. J.* **23**, 159–175.

Nibu, Y., T. Inoue and I. Motoda. 1995. Effect of headgroup type on the miscibility of homologous phospholipids with different acyl chain lengths in hydrated bilayer. *Biophys. Chem.* **56**, 273–280.

Norris, V. 1989. Phospholipid flop-out controls the cell cycle of Escherichia coli. *J. Theor. Biol.* **139**, 117–128.

Norris, V. 1992. Phospholipid domains determine the spatial organization of the Escherichia coli cell cycle: the membrane tectonics model. *J. Theor. Biol.* **154**, 91–107.

Phillips, M. C., B. D. Ladbrooke and D. Chapman. 1970. Molecular interactions in mixed lecithin systems. *Biochim. Biophys. Acta* **196**, 35–44.

Popescu, D. and G. Victor. 1990. Association probabilities between the single chain amphiphiles into a binary mixture in planar monolayers (I). *Biochim. Biophys. Acta* **1030**, 238–250.

Popescu, D., C. Rucareanu and G. Victor. 1991. A model for the appearance of statistical pores in membranes due to self-oscillations. *Bioelectrochem. Bioenerg.* **25**, 91–103.

Popescu, D. and G. Victor. 1991. Calculation of the optimal surface area for amphiphile molecules using the hard core method. *Biophys. Chem.* **39**, 283–286.

Popescu, D. 1993. Association probabilities between the single chain amphiphiles into a binary mixture in planar monolayers (II). *Biochim. Biophys. Acta* **1152**, 35–43.

Popescu, D. 1994. Selective association processes of single chain amphiphiles in monolayer films. *Biophys. Chem.* **48**, 369–381.

Salem, L. 1962. Attractive forces between long saturated chains at short distances. *J. Chem. Phys.* **37**, 2100–2113.

Sperotto, M. M. and O. G. Mouritsen. 1991. Monte Carlo simulation studies of lipid order parameter profiles near integral membrane proteins. *Biophys. J.* **59**, 261–270.

Stohrer, J., G. Grobner, D. Reimer, K. Weisz, C. Mayer and G. Kothe. 1991. Collective lipid motions in bilayer membranes studied by transverse deuteron spin relaxation. *J. Chem. Phys.* **95**, 672–678.

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