Determination of the effect of a local anesthetic on hydrocarbon chain order in membranes: a statistical mechanical approach

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Abstract. A statistical mechanical formalism is developed for the study of rigidity changes of a dymiristoylphosphatidylcholine bilayer induced by the inclusion of the local anesthetic benzyl alcohol. In the model, packing constraints play a central role and the solution is treated both in the ideal and the Huggins approximations. The dependence of the phospholipid chain order parameter on the alcohol mole fraction is determined, as well as an estimation on the change produced on the bilayer thickness. The results are in good agreement with available NMR experiments and X ray measurements. The analysis serves as an indication that the main site of action of the benzyl alcohol is the hydrocarbon bilayer.

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1. Introduction

In recent years the development of theoretical models for the study of structural and conformational properties of amphiphilic aggregates has received considerable attention [1,2]. Much interest has been dedicated to the study of phospholipid bilayers [3,4,5], because of their relevance in the understanding of biological membranes among other reasons. In this work we are concerned with the behavior membranes undertake in the presence of anesthetics. In particular, we propose a theoretical formalism for the study of the changes in the hydrocarbon chain order of a lecithin bilayer (dymiristoylphosphatidylcholine, DMPC), induced by the inclusion of a local anesthetic (benzyl alcohol, BA). One of the main reasons for choosing this problem is the availability of relevant experimental results [6].

Our purpose is to calculate the dependence of an order parameter S (which is a measure of the degree of rigidity of the phospholipid chains) on the concentration of BA. We also estimate the change in the bilayer thickness due to the BA. Our calculation is carried out using a statistical mechanical formalism. The model we adopt for the DMPC was first introduced by Marsh [7] in his study of the fluidity of lecithin bilayers. In this model the conformational properties of the acyl chains are determined within the rotational isomeric approximation developed for polymer

solutions [8]; headgroup and interchain interactions are taken into account in terms of packing configurational restrictions.

The scheme of the calculation is the following: with our statistical mechanical model we obtain the free energy per mole of the DMPC bilayer in the absence of BA, and the value P_t of the average *a priori* probability of a having a trans bond in the acyl tail of the lipid chain. We then consider the change in the free energy due to the inclusion of the BA as a solvent, treating the solution both in the ideal approximation and within the Huggins [9] approximation. We then obtain, through the statistical mechanical formalism, the change induced on P_t . With the new P_t we evaluate the order parameter S. Since P_t is a function of the DMPC concentration, we obtain the sought dependence of S on concentration. We can then use this result to estimate the changes in the bilayer thickness when the BA is added.

The presentation of this paper is the following: in Section 2 we define properly the bilayer model we use for our calculation. Section 3 is mainly concerned with the determination of the fundamental quantity P_t as a function of the BA mole fraction. In Section 4 we analyse the behavior of the order parameter and in Section 5 we discuss our results.

2. The model

Phospholipid bilayers are complex many body systems for which a wide variety of models have been developed [1,2,4]. The most general potential for these systems may be expressed as a sum of surface and core terms. In the phospholipid, two sections can be clearly distinguished: the polar headgroup and the acyl chain; in terms of these components, the surface contribution consists of water-head, head-head, head-chain, and chain-water interactions. The core terms includes inter- and intra-chain interactions. Another feature which has proven to be relevant in the description of these systems is the aggregate geometry (*e.g.*, interface geometry and smoothness characteristics, packing conditions). Given the complexity of the system, all the theoretical treatments involve some degree of approximation; depending on the problem under study, some interactions are ignored and others are treated within a mean-field approximation.

Most theoretical efforts on the structural properties of membranes have focused on the gel/liquid-crystal phase transition [5], the formation of the aggregates [1] and protein inclusion [10,11]. However, to our knowledge, for the problem of anestheticmembrane interactions only a few theoretical models have been developed (e.g. O'Leary [12] and references there in). Most of these models [13,14,15] fall into a group, often referred to as the Marcelja-type models [16,17], in which excluded volume interactions between different acyl chains are accounted for in a mean-field way and the details of packing are ignored. Another model [10,18] for lipid-anesthetic effects is of the Nagle type [19], in which the excluded volume interactions between the acyl chains are accounted for exactly and long range van der Waals interactions are treated in mean-field. In both cases, anesthetics are assumed to interact either with the acyl group or the headgroup, or both.

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The model we shall be using has a more *a priori* character than the statistical theories mentioned above. As a stated in the introduction, we adopt the approach set up by Marsh, in which the headgroup and interchain interactions are incorporated as "packing constraints". This type of assumption has proven to be useful in various treatments of membrane properties (*c.f.* Gelbart [1]). Within this scheme, the potential for the system is the sum of single chain conformational energies, expressed in terms of the rotational isomeric description of polymer chains, with the appropriate modifications due to the constraints.

Based on the discussion forwarded by Nagle [5], the bilayer is treated as the sum of independent monolayers, and no distinction is made between the chains belonging to the same lipid or to different ones. Our starting point coincides with the "random coil" description proposed by Gruen [20], in which it is argued that, above the gel/liquid-crystal phase transition temperature, the intermolecular disorder of the acyl chains is very similar to that in bulk liquid n-alkane. However it should be stressed that in our approach, instead of introducing a set of mean-field consistency equations we adopt the configurational constraints proposed by Marsh [7].

In the rotational isomeric approximation, the carbon-carbon (C-C) bonds of the acyl chain are in one of three energetically favored states: t (trans), g^+ (gauche plus) or g^- (gauche minus). The partition function Z_n of a polymer chain of nidentical bonds (with free end bonds) is given by

$$Z_{n} = \sum_{\text{conf}} \prod_{i=2}^{n-1} U_{\xi\eta;i} \equiv (1,0,0) \begin{pmatrix} U_{tt} & U_{tg^{+}} & U_{tg^{-}} \\ U_{g^{+}t} & U_{g^{+}g^{+}} & U_{g^{+}g^{-}} \\ U_{g^{-}t} & U_{g^{-}g^{+}} & U_{g^{-}g^{-}} \end{pmatrix}^{n-2} \begin{pmatrix} 1 \\ 1 \\ 1 \end{pmatrix}$$
$$\equiv (1,0,0) \mathbb{U}^{n-2} \begin{pmatrix} 1 \\ 1 \\ 1 \end{pmatrix}, \tag{1}$$

where \sum is the sum over all possible configurations of the chain, *i* represents a C–C chain bond, $U_{\xi\eta}$ is the conditional probability for a bond to be in a state ξ , given that its neighbor is in a state $\eta(\xi, \eta \in \{t, g^+, g^-\})$, and \mathbb{U} is the transfer or statistical weight matrix. It follows from (1) that the evaluation of Z_n can be envisaged as a Markov process.

For our purposes we are primarily interested in the calculation of the *a priori* probability P_t of finding a trans state in the chain, averaged over the internal bonds. If we define $P_{t,i}$ as the average probability for the i^{th} bond to be in a state t, we have

$$P_t = (n-2)^{-1} \sum_{i=2}^{n-1} P_{t;i},$$
(2)

which can be expressed in terms of the partition functions of smaller chains (see

Appendix) by

$$P_t = (n-2)^{-1} \sum_{w=2}^{n-1} \frac{Z_w Z_{w-i+1}}{Z_n}.$$
(3)

In order to define our phospholipid model explicitly we now follow the argument given by Marsh [7] in his study of the liquid crystal transition of lecithin bilayers, in which packing conditions produce steric hindrances that restrict the probability transition between different states of the system: a t bond can be followed by a bond in any state, but a g^{\pm} bond can only be followed by a t bond. Under these assumptions

$$\mathbf{U} = \begin{pmatrix} 1 & \sigma & \sigma \\ 1 & 0 & 0 \\ 1 & 0 & 0 \end{pmatrix},\tag{4}$$

where $\sigma = \exp -\{\beta [E_{g\pm} - E_t]\}$, with $\beta = 1/kT$, k = Boltzmann's constant and $(E_{g\pm} - E_t)$ is the energy difference between a g^{\pm} and a t state.

In a phospholipid bilayer there is a rigidity gradient along the distance perpendicular to the surface of the bilayer. The bonds of the fatty acid near the polar headgroup are more rigid than the ones at the center of the bilayer. Marsh incorporates this feature by considering the first 3 carbon bonds closer to the headgroup to be in a rigid trans conformation. For our calculation of the partition function Zand of P_t for one of the hydrocarbon chains of the DMPC, we adopt this measure. The only free parameter we are left with in the model is the value of the energy difference $(E_{g\pm} - E_t)$. For this we use the value of 750 cal/mole, which corresponds to a best fit of the problem treated by Marsh. We should also point out that the last carbon bond of the chain is left free of steric hindrances.

3. Determination of P_t as a function of DMPC mole concentration

If we calculate P_t as a function of the BA mole concentration, we will be able to determine the variation of the order parameter with the inclusion of the alcohol. An important quantity is the partition function Z of the acyl chain. In general, for the evaluation of the partition function Z_n of Section 2, it is convenient to express it in terms of the eigenvalues λ_{ξ} of the matrix \mathbb{U}

$$Z_n = \sum_{\xi=1}^3 \Gamma_\xi \lambda_\xi^{n-2},\tag{5}$$

where

$$\Gamma_{\xi} = A_{1\xi} \sum_{\eta=1}^{3} B_{\xi\eta}, \tag{6}$$

and $A_{1\xi}$ are the first row components of the matrix \mathbf{A} that diagonalizes \mathbf{U} , $B_{\xi\eta}$ are the elements of matrix $\mathbf{B} \equiv \mathbf{A}^{-1}$ and λ_{ξ} are the elements of matrix $\Lambda \equiv \mathbf{A}^{-1}\mathbf{U}\mathbf{A}$.

Using the value of U given by Eq. (4), we obtain, after some straightforward matrix algebra, the following results for our problem: $\lambda_1 = (1 + \sqrt{1 + 8\sigma})/2$, $\lambda_2 = 1 - \lambda_1$, $\lambda_3 = 0$, $\Gamma_1 = \lambda_1/(2\lambda_1 - 1)$ and $\Gamma_2 = 1 - \Gamma_1$.

With the above expressions and the results of the Appendix, we arrive at

$$Z = Z(r) = (2\lambda_1 - 1)^{-1} [\lambda_1^{r+2} - (1 - \lambda_1)^{r+2}],$$
(7)

$$P_t = \frac{\Gamma_1}{r\lambda_1(1+\gamma\epsilon^r)} \left\{ r(1+\gamma^2\epsilon^{r-1}) + \frac{2\gamma(\epsilon^r-1)}{\epsilon-1} \right\},\tag{8}$$

where ϵ , r and γ have been defined in the Appendix.

Under the assumption of the model stated in Section 2, for the myristic acid r = n - 4 = 10 (the total number of bonds are 14, the first three are fixed in the t state and the end bond is rotationaly free). It should be stressed that within the approximations intrinsic to the model, Eqs (7) and (8) are exact expressions, valid for a finite number of bonds. Usually, most analytical calculations are done in the limiting case of r going to infinity (c.f. Appendix).

To calculate the dependence of Z on BA concentration we evaluate the change Δg produced on the Gibbs free energy per mole of lipid, assuming an ideal solution behavior [21], *i.e.*

$$\Delta g = g(X_{\rm L}) - g(1) \equiv RT \ln(X_{\rm L}),\tag{9}$$

where $X_{\rm L}$ is the lipid mole fraction, $g(X_{\rm L})$ the free energy per mole of lipid (*i.e.* the lipid chemical potential) and R the universal gas constant.

From standard statistical mechanics, we have that $g(X_{\rm L}) = -RT \ln Z_{\rm T}(X_{\rm L})$, $Z_{\rm T}(X_{\rm L})$ being the total partition function for the the whole lipid chain at $X_{\rm L}$. (Depending on whether a process takes place at constant pressure of at constant volume, the free energy defined by $-RT \ln(Z)$ will respond to the Gibbs (G) or the Helmholtz (F) free energy, respectively. For our problem we used the Gibbs free energy because we are dealing with an experiment done at constant pressure and we envisage the mol fraction change in terms of a volume change; besides, since $\Delta G = \Delta F$ in an ideal solution, the distinction between the two free energies is somewhat irrelevant for our purposes). Since in our model we do not consider headgroup/acyl-chain interactions, the partition function for the phospholipid factorizes as $Z_{\rm T}(X_{\rm L}) = Z(X_{\rm L})Z_{\rm HG}$, where $Z_{\rm HG}$ is the partition function of the headgroup. Under these conditions we

have from (9) that

$$\frac{Z(1)}{Z(X_{\rm L})} = X_{\rm L}.$$
 (10)

Notice that implicit in Eq. (10) is the assumption that the BA will have an influence mainly on the hydrocarbon chains of the DMPC bilayer, *i.e.* we have used the hipothesis that Z_{HG} is independent of X_{L} . A discussion on this point, which is a matter of controversy in the study of the mode of interaction of anesthesics, will be relegated to Section 5.

From Eqs. (8), (9) and (10) we can now calculate the new value $P_t(X_L)$ of the average *a priori* probability that a carbon bond in the acyl chain is in a trans state at a given BA mole fractions X_{BA} (= 1 - X_L).

4. Order parameter results

Once $P_t(X_L)$ is known, we can evaluate the order parameter [4,5] $S_i = \frac{1}{2}(\langle 3 \cos^2 \theta_i \rangle - 1)$ where θ is the momentary angle between the direction of the *i*th acyl chain segment (counted from the headgroup) and the normal to the bilayer surface; $\langle \rangle$ indicates a time average. Following the procedure outlined by Hubbell and Mc-Connell [26] we obtain for S_{σ} :

$$S_{\sigma}(X_{\rm L}) = P_t^3 - \frac{3}{8}P_t^2(1 - P_t) + \frac{7}{16}P_t(1 - P_t)^2, \tag{11}$$

where P_t is a function of X_L . (Hubbell and McConnell determine S_i calculating the probability of having a chain configuration with one gauche bond, two gauche bonds and so forth and then evaluating the $\langle .. \rangle$ time average for each type of configuration; as they mention, the calculation is in itself straightforward but somewhat tedious to mention in detail).

Expression (11) is similar to S_3 in [26] since the first three bonds of our model are in a t state. There is however a slight difference because we exlcude chain configurations with g^+g^+ and g^-g^- sequences which were allowed in Hubbell's work.

In Fig. 1 we plot, as a function of the mole fraction ratio X_{BA}/X_L , the values of S_{σ} determined theoretically at a temperature T = 311 K by Eq. (11) and those determined from the quadrupole splitting measurements carried out at the same temperature by Turner and Oldfield [6]. The relation between the quadrupole splitting Δ_{ν} and S_i is [23,24]

$$S_i = \frac{8}{3} \left(\frac{h}{qQe^2} \right) \Delta_{\nu},\tag{12}$$

where h is Plank's constant, Q is the electric quadrupole moment of the D (deuterium) nucleus, and q the electric field gradient due to the bonding electrons at the



FIGURE 1. Plot of the order parameter S_{σ} of DMPC for various values of the BA mole fraction $X_{BA}(=1-X_L)$, where X_L is the lipid mole fraction). The full circles are experimental values [6], the error bar comes from the uncertainty reported in [6] for $X_L = 1$. We have assigned this uncertainty to all the measurements. The solid line is the least squares best fit for the circles (slope $m_e = -0.018$). The triangles are the ideal solution theoretical values. The dashed line is the best fit for these values (slope $m_t = -0.026$). The crosses are theoretical results calculated within the Huggins approximation. The broken line is the corresponding best fit (slope $m_H = -0.031$). The crosses for X_{BA}/X_L equal to 0.909 and 0.83 are not shown since they overlap, within the scale of the figure, with the triangles.

 i^{th} chain segment. The quantity $(e^2 q Q)/h$ is the quadrupole coupling constant and takes the value of 170 kHz for most carbon deuterium bonds. In writing Eq. (12), the chain rotation around the surface normal has been considered to be isotropic [1,4,23]. For the case of NMR experiments this assumption appears to hold since the averaging time scale is slow enough for the average position of the chain skeleton to be normal to the bilayer surface, and the average C–D bond position perpendicular to the bilayer normal [25,26,17,23] (this consideration implies that the parameter S_0 of Hubbell's work is equal to 1).

Both experimental and theoretical sets of values lie on a straight line (the experimental correlation is 0.996, while the theoretical one is -0.976). The experimental slope, determined by least squares, is $m_e = -0.018$ and the theoretical one is $m_t = -0.026$. If we use the error bar reported by Turner and Oldfield [6] as the maximum experimental error and take into account the standard deviation, the value of the theoretical slope falls within the statistical and experimental uncertainty.

A further quantity which can be compared with experiment is the change in the effective length of the hydrocarbon chain in the bilayer $\Delta \langle L \rangle$, due to the BA. Using the expression for $\langle L \rangle$ given by Stockton *et al.* [23,24], we have

$$\Delta \langle L \rangle (X_{\rm L}) = \frac{1.25}{2.125} \sum_{j} \left[S_j(1) - S_j(X_{\rm L}) \right], \tag{13}$$

where the sum is taken over all the acyl chain C-C bonds and S_j is the appropriate generalization of $S_6(X_L)$ for the j^{th} carbon bond. From Eq. (13) we predict a bilayer

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thickness reduction of 0.85 ± 0.059 Å for $X_{\rm L} = 0.25$ (the uncertainty comes from using an upper bound expression for S_j for the last 4 bonds). Experimentally, X ray and NMR measurements [6,27] show a decrease in the bilayer thickness of the order of 1Å.

We have also carried out our calculation considering a Huggins [9,28] type approximation in which the relative size of the components of the solution is taken into account by substituting in Eq. (9) the lipid mole fraction with the lipid activity $A_{\rm L}$ given by

$$A_{\rm L} = \frac{\alpha X_{\rm L}}{1 + (\alpha - 1)X_{\rm L}} \exp\left[\frac{-(\alpha - 1)(1 - X_{\rm L})}{1 + (\alpha - 1)X_{\rm L}}\right],$$

where $\alpha = 258/89$ is the quotient of the molar volume of tetradecane over the molar volume of benzene [29]. With this substitution the final results are good but less encouraging than those for the ideal solution formulation, the slope m_H (Fig. 1) is for this case -0.031 with a correlation of -0.980.

5. Discussion

Considering the simplicity of the model here presented, the coincidence with the available experimental data is indeed very good. We are therefore led to believe that up to a certain extent some of the assumptions and approximations involved in the model illustrate aspects of the actual behavior of the bilayer. We maintain that the calculation serves as evidence that the BA acts mainly on the hydrophobic region of the bilayer. This is a point of interest in the current literature on anesthetics [27,30–34] that has prompted various alternative theoretical treatments [12,15,20,30]. Furthermore, our findings support that, for the case of biological membranes, the lipid matrix appears to be an important site of action of the BA, within the hydrophobic region.

As we remarked in Section 4, in our calculation we exclude a direct interaction between the acyl chain and the headgroup with the partition function factorization, and between the BA and the headgroup when we consider Z_{IIG} independent from X_{L} . However, secondary effects of the BA on the headgroup can be incorporated by modifying the rigidity of the first 3 C-C bonds closer to it. Preliminary results on this approach indicate that the predictions of the model with regards to solution properties improve considerably; *i.e.*, the theoretical slope m_t approaches the experimental value m_e . We leave the discussion of these results to a future report.

From the point of view of statistical mechanical modelling we are well aware that the literature on this subject has evolved dramatically in the past few years. As mentioned in the introduction there is a wide variety of treatments which involve various degrees of complexity. In this respect our approach is a useful first order approximation to the problem, supported by an encouraging comparison with experiment. Two basic ingredients of our formulation are the ideal solution assumption and the role of the packing constraints. Both assumptions are closely related. The fact that we chose the potential for the phospholipid bilayer as an assembly of single chain potentials subject to restrictions is a type of independeant "quasi-particle" approximation consistent with an ideal solution description. In fact, the results of the Huggins approximation show that improvements to our model should be incorporated consistently, *e.g.* if we consider explicitly interchain interactions this would produce an energy change that could compete with the entropy modification introduced with the Huggins theory, improving the final results.

The final picture that emerges from this work is that an independent "quasiparticle" description for the bilayer, for which the energy available from the dilution of the BA is mainly incorporated in the internal energy of the "quasi-particle", is adequate for the problem under study. In our formulation the bilayer changes its rigidity because the internal flexibility of the hydrophobic chain is enhanced. Notice that we do not attribute the change to a global modification of the packing constraints, these are left invariant with dilution. Our findings support this picture but are by no means conclusive in this respect. The analysis of alternative experiments on other systems focussing on other properties is necessary in order to draw more definite conclusions on a bigger variety of systems and to establish the limit of applicability of our assumptions. It is evident that many systems will most likely involve other mechanisms. So far, the model works reasonably well for the case of the DMPC-BA solution, with regard to the order parameter measurements of Oldfield and Turner [6] and may throw light on systems closely related to this case.

Finally, it is worthwhile mentioning that our results have been obtained essentially without fitting any parameters, since the only parameters involved are the three initial trans bonds and the value for the $(E_{g\pm} - E_t)$ energy difference, both of which originated from fits done by Marsh in the context of a different problem related to the fluidity of lecithin bilayers.

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Appendix

In this Appendix we follow the formulation set up in Flory's book [8]. For a polymer of n bonds with free ends, the probability $P_{t,i}$ can be expressed by

$$P_{t,i} = Z_n^{-1} \mathbf{J}^* \left[\prod_{l=2}^{i-1} \mathbf{U}_l \right] \mathbf{U}_{i,t} \left[\prod_{j=i+1}^{n-1} \mathbf{U}_j \right] \mathbf{J}, \tag{A1}$$

where $\mathbf{J}^* = (1,0,0), \ \mathbf{J} = \begin{pmatrix} 1\\1\\1 \end{pmatrix}$, and $\mathbf{U}_{i,t} = \begin{pmatrix} 1 & 0 & 0\\1 & 0 & 0\\1 & 0 & 0 \end{pmatrix}$ ensures that the *i*th link is in a trans state.

Since

$$\mathbb{J}\mathbb{J}^* = \mathbb{U}_{i,t},\tag{A2}$$

then

$$P_{t,i} = Z_n^{-1} \mathbf{U}^* \left[\prod_{l=2}^{i-1} \mathbf{U}_l \right] \mathbf{U} \mathbf{U}^* \left[\prod_{j=i+1}^{n-1} \mathbf{U}_j \right] \mathbf{U}.$$
(A3)

From (1), (2) and (A3), Eq. (3) follows directly.

If the hydrocarbon chain of n bonds has its first q bonds in a trans state and the last bond free, then, for $i \ge q$:

$$Z^{-1}P_{t,i} = \mathbf{J}^* \left[\prod_{l=q+1}^{i-1} \mathbf{U}_l \right] \mathbf{J}\mathbf{J}^* \left[\prod_{j=i+1}^{n-1} \mathbf{U}_j \right] \mathbf{J} = \mathbf{J}^* \mathbf{U}^{i-q-1} \mathbf{J}\mathbf{J}^* \mathbf{U}^{n-i-1} \mathbf{J}, \quad (A4)$$

with the total partition function of the acyl chain given by $Z = \mathbb{J}^* \mathbb{U}^{n-q-1} \mathbb{J}$.

If we define $\Omega_m \equiv \mathbb{J}^*\mathbb{U}^m\mathbb{J}$, then the average probability P_t of having a *t* state in any of the $r \equiv n - q - 1$ bonds of the hydrocarbon chain that are described by the rotational isomeric approximation is

$$P_t = \frac{1}{(n-q-1)Z} \sum_{i=q+1}^{n-1} P_{t,i} = \frac{1}{(n-q-1)Z} \sum_{i=q+1}^{n-1} \Omega_{i-(q+1)} \Omega_{n-i-1}.$$
 (A5)

Note that the normalizing factor Z is the same for the whole acyl chain as for the r link subchain since the fixed t bonds do not increase the number of configurations. We shall therefore refer from now on to Z as the partition function $Z(r) = Z_{r+2}$ of the r link subchain.

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Since $\Omega_m = Z_{m+2}$ defined by Eq. (1), using Eq. (5) we arrive at

$$\Omega_m = \Gamma_1 \lambda_1^m (1 + \gamma \epsilon^m + \gamma_1 \epsilon_1^m), \tag{A6}$$

with $\gamma = \Gamma_2/\Gamma_1$, $\gamma_1 = \Gamma_3/\Gamma_1$, $\epsilon = \lambda_2/\lambda_1$ and $\epsilon_1 = \lambda_3/\lambda_1$.

Substituting (A6) into (A5) and using the values for λ_1 , λ_2 , λ_3 , Γ_1 and Γ_2 given in Section 3, after some algebra, we arrive at

$$Z = Z(r) = (2\lambda_1 - 1)^{-1} [\lambda_1^{r+2} - (1 - \lambda_1)^{r+2}],$$
(A7)

$$P_t = \frac{\Gamma_1}{r\lambda_1(1+\gamma\epsilon^r)} \left\{ r(1+\gamma^2\epsilon^{r-1}) + \frac{2\gamma(\epsilon^r-1)}{\epsilon-1} \right\}.$$
 (A8)

If we take the large r limit, then from (A6), (A7), and (A8), we obtain the usual asymptotic expressions (Flory [8]) $Z_m = \Omega_{m-2} \cong \Gamma_1 \lambda_1^{m-2}$ and $P_t = \Gamma_1 / \lambda_1$.

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Resumen. Se desarrolla un formalismo mecánico estadístico para el estudio de los cambios en la rigidez de una bicapa de dimiristoil-fosfatidilcolina, inducidos por la disolución del anestésico local, alcohol bencílico, en la bicapa. Las constricciones de empaquetamiento juegan un papel central en el modelo. La solución se trata como ideal y dentro de la aproximación de Huggins. Se determina la dependencia del parámetro de orden del fosfolípido con la fracción molar del alcohol y se obtiene una estimación del cambio producido en el grosor de la bicapa. Los resultados obtenidos concuerdan satisfactoriamente con determinaciones experimentales de RMN y rayos X. El análisis realizado sirve como indicio de que el principal sitio de acción del alcohol bencílico es la bicapa de hidrocarburo.