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A general model for the interaction of foreign molecules with lipid membranes: drugs and anaesthetics

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A general microscopic interaction model is proposed to describe the changes in the physical properties of phospholipid bilayer membranes due to foreign molecules which, to different degrees, partition between the membrane phases and the aqueous environment. The model is a multi-state lattice model for the main phase transition of lipid bilayers and the foreign molecules are assumed to intercalate as interstitials in the lattice. By varying the model parameters, the diversity in the thermodynamic properties of the model is explored using computer-simulation techniques which faithfully take account of the thermal fluctuations. The calculations are performed in both the canonical and the grand canonical ensembles corresponding to the cases where the concentration of foreign molecules in the membrane is either fixed or varies as the external conditions are changed. A classification of the diverse thermal behaviour, specifically with regard to the phase diagram, the specific heat, the density fluctuations, and the partition coefficient, is suggested with a view to rationalizing a large body of experimental measurements of the effects of different foreign molecules on membrane properties. The range of foreign molecules considered includes compounds as diverse as volatile general anaesthetics like halothane, cocaine-derived local anaesthetics like procaine, calcium-channel blocking drugs like verapamil, antidepressants like chlorpromazine, and anti-cancer agents like adriamycin.

I. Introduction

Considerable effort in biochemical, physiological, and pharmacological research is devoted to the characterization of the transfer of small foreign molecules between biological membranes and their aqueous environment [1]. These membrane-associated transfer-processes are important everywhere in biological systems and they play a crucial role for the action of commonly used drugs, such as antibiotics, tranquillizers, and anaesthetics. The molecules in question include compounds with substantial structural and physico-chemical differences. It is widely assumed that the biologial activity of these compounds arises as a result of binding to active sites in membrane-bound proteins, while the lipid background is considered to play a more passive role [2]. However, experimental work on artificial lipid bilayers or model membranes has demonstrated that the structural properties of these membranes may be strongly affected by the presence of membrane-associated molecules [3]. This is most significantly reflected in changes in the main phase transition characteristics of pure lipid bilayers. Well known examples are short-chain alcohols [4] and a range of anaesthetics [5] which generally cause a depression of the transition temperature without significantly affecting the transition enthalpy.

The present study is concerned with the physico-chemical properties of these model membrane systems.

Abbreviations: DMPC, dimyristoyl phosphatidylcholine; DPPC, dipalmitoyl phosphatidylcholine; DSC, differential scanning calorimetry.

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A simple but general microscopic model for the interaction of foreign molecules with phospholipid bilayers is proposed and analyzed by use of computer-simulation techniques. The model is an extension of a model previously advanced by De Verteuil et al. [6] to describe lipid-anaesthetic mixtures. Despite its simplicity, the extended model displays a thermal behavior sufficiently complex to encompass experimental observations for a surprisingly large class of different foreign molecules.

Small foreign molecules can interact physically with lipid membranes in two fundamentally different ways: (i) they can enter the membrane as substitutional impurities taking the place of a lipid molecule (examples include cholesterol, fatty acids, and long-chain alcohols), and (ii) they can intercalate between the flexible acyl chain of the lipids as interstitial impurities (examples include most general and some local anaesthetics as well as short hydrocarbons). Whether a given foreign compound should be regarded as a substitutionally or interstitially embedded molecule is not necessarily related to its molecular size but rather to its amphipathic nature and to its specific interaction with the polar-head group region of the lipid bilayer. In addition to the distinction between substitutional and interstitial impurities, foreign molecules should also be distinguished according to relative solubility in the membrane and aqueous phases as described by a partition coefficient which is often temperature-dependent. From the point of view of thermodynamics, a large partition coefficient would suggest a description using the canonical ensemble, whereas a small partition coefficient corresponds to the grand canonical ensemble. Obviously, for many compounds it is difficult to make a sharp distinction between the substitional and interstitial impurity case, i.e., between the canonical and grand canonical description. However, in all cases the foreign molecules will influence physical properties of the lipid bilayer. In particular the lipid properties at the gel-to-fluid transition (the main transition) will be strongly affected in a way which intimately reflects the mode of interaction between the foreign molecules and the lipid-membrane phases. It is the hypothesis of the present paper that these different modes of interaction and the corresponding manifestations in the lipid membrane properties may provide a useful way of characterizing a large group of molecular compounds interacting with membranes.

A specific example of a foreign molecule which has a dramatic effect on the thermodynamic properties of lipid membranes is cholesterol [7,8]. Other examples are steroids which form an extensive class of cholesterol derivatives. These molecules are generally more hydrophilic than cholesterol and consequently associated with a pronounced partitioning between the aqueous phase and the membrane. For those steroids which have their multiple hydrocarbon ring structure buried in the hydrophobic core of the membrane phase, a complicated phase behaviour should be expected on the basis of the structural relationship to cholesterol. The physical effects of these molecules on the lipid membrane indicate that they should be considered as substitutional impurities in the lipid matrix. For such a substitutional mixture the entropy of mixing plays a dominant role for the phase behaviour. The foreign molecules on which we focus our attention in this study can be treated as non-substitutional (interstitial) impurities. This has important implications for the thermal properties of the systems due to the absence of an entropy of mixing. A range of different molecules are candidates for such a description, e.g., volatile general anaesthetics like enflurane, halothane and ether, which are deeply buried in the hydrophobic membrane core, or molecules which preferentially bind to the headgroup region of the membrane and only insert a (small) hydrophobic moity into the hydrocarbon region of the membrane.

A considerable body of literature exists on experimental measurements of the physical effects of foreign molecules interacting with membranes. We shall make no attempt to review this literature. Rather we concentrate on some selected studies and offer a general discussion of some of the problems related to the interpretation of the experimental data. In contrast to the experimental literature, only a few theoretical models have addressed the interaction of foreign molecules with membranes. Most of these models are devoted to specific substitutional impurities, such as cholesterol and certain integral proteins (for a recent review, see for example Ref. 9). The case of interstitial impurities has been the subject of very few theoretical papers [6,10.11] of which the only one using a truly microscopic interaction model is that of De Verteuil et al. [6]. This latter model forms the basis of the present study which is the first to provide, via a computer-simulation approach, a quantitatively reliable description in the region of the main transition of phospholipid bilayers.

In a study of the effects of drugs and anaesthetics on lipid membranes and in particular on biological membranes, an important question comes up as to which concentration regime of the foreign molecules is the relevant one. This is an old question which has also been addressed recently in relation to volatile anaesthetics [12] where it was pointed out that so-called clinical concentrations only have little meaning. In the present work we will be considering fairly high global concentrations in the membrane since we want to reveal very clearly the physical effects on the membrane. Furthermore, we are going to argue that it may not necessarily be the global concentration which is the interesting quantity from the point of view of physiological effects since the dynamic lateral heterogeneity of the cooperative membrane assembly will lead to regions with local concentrations which is much higher than the global concentration.

II. Model and statistical ensembles

We have used the ten-state Pink model [13] to describe the main transition of saturated diacyl phospholipid bilayers. This model takes into account the acyl-chain conformational statistics and includes the dispersive forces between the chains in a detailed manner. The excluded volume effect is accounted for by assigning each acyl-chain to a site in a triangular lattice. The translational degrees of freedom are therefore ignored in this description. Furthermore, the two monolayers of the bilayer membrane are considered as independent. The acyl-chain conformations are represented by ten single-chain states α, each described by a cross-sectional area A_α, an internal energy E_α, and an internal degeneracy D_α. $\alpha = 1$ corresponds to the all-*trans* conformation of the acyl chain which is characteristic of the gel phase. $\alpha = 10$ represents D_{10} degenerate acyl-chain conformations which are highly excited due to *gauche* rotations. The remaining eight intermediate states represent low-energy conformations, which are selected from considerations of optimal packing of the acyl chains and low conformational energy [13].

As suggested in an earlier mean-field study [6] the impurities are considered as interstitially intercalated between the flexible acyl chains of the membrane and therefore their contribution to the exluded volume effect can be ignored. Hence, in the lattice model the impurities are situated in the center of the triangles formed by three neighbouring lipid acyl chains, cf. Fig. 1. The sites available for the impurities thus form a honeycomb lattice embedded in the triangular lattice for the acyl chains. The requirement that the lipid chains and the interstitial molecules occupy separate lattices precludes mixing and thereby ensures the absence of an entropy of mixing. The interstitial molecules are assumed to interact with their three neighbouring lipid acyl chains as well as with other interstitial molecules in neighbouring occupied interstitial sites. The full model Hamiltonian then takes the form

$$\mathscr{H} = \mathscr{H}_{\mathrm{LL}} + \mathscr{H}_{\mathrm{LA}} + \mathscr{H}_{\mathrm{AA}}$$

$$= \sum_{i,\alpha} (E_\alpha + \Pi A_\alpha) \mathscr{L}_{i\alpha} - \frac{J_{\mathrm{LL}}}{2} \sum_{i,j,\alpha,\beta} I_\alpha I_\beta \mathscr{L}_{i\alpha} \mathscr{L}_{j\beta}$$

$$- \sum_{i,\alpha} \sum_{\ell} J_{\mathrm{LA}}^{\alpha} I_\alpha \mathscr{L}_{i\alpha} \mathscr{L}_{\ell}^{\mathrm{A}} - \frac{J_{\mathrm{AA}}}{2} \sum_{\ell,k} \mathscr{L}_{\ell}^{\mathrm{A}} \mathscr{L}_{k}^{\mathrm{A}} \qquad (1)$$

where $\mathscr{L}_{i\alpha}$ is a lipid chain state occupation variable, $\mathscr{L}_{i\alpha} = 0{,}1$ with $\Sigma_\alpha \mathscr{L}_{i\alpha} = 1$, and $\mathscr{L}_{\ell}^{\mathrm{A}} = 0{,}1$ is an interstitial site occupation variable. The interaction energies are expressed in terms of nematic factors I_α for the acyl-chain states [14] and interaction constants J_{LL}, J_{LA}^{α}, and J_{AA}, with indices referring to lipid–lipid, lipid–foreign molecule, and foreign molecule–foreign molecule interactions. In the present work we have taken J_{LA}^{α} to be independent of the specific acyl-chain state and to have an effective dependence on the phase of the lipid membrane only, i.e., $J_{\mathrm{LA}}^{\mathrm{gel}} = J_{\mathrm{LA}}^{\alpha}$, $\alpha = 1, 2, \ldots, 9$; and $J_{\mathrm{LA}}^{\mathrm{fluid}} = J_{\mathrm{LA}}^{10}$. In principle, specific interactions between the lipid chains and the foreign molecules may be allowed for by other choices of J_{LA}^{α}. Since J_{LL} ($= 0.70985 \cdot 10^{-13}$ erg) and Π ($= 30$ dyn/cm) are fixed at their values for the pure lipid bilayer (DPPC), the present model is specified by the three parameters $J_{\mathrm{LA}}^{\mathrm{gel}}$, $J_{\mathrm{LA}}^{\mathrm{fluid}}$, and J_{AA}. For a pure DPPC bilayer model the main phase transition temperature is $T_{\mathrm{m}} = 314$ K. It should be remarked that the interaction parameters of the model in Eqn. 1 are effective parameters only and that they may each represent several aspects of the details in the molecular interactions. Furthermore, they are relative parameters in the sense that they refer to some chosen energy scale of the Hamiltonian. Hence they can be positive or negative depending on whether the interaction in question is *relatively* attractive or repulsive.

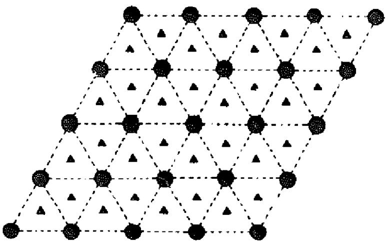

Fig. 1. Schematic representation of the triangular lattice (solid circles) of lipid acyl chains and the interstitial lattice (solid triangles) available for the interstitial foreign molecules.

In general, the number of interstitial molecules is not a conserved quantity since some partitioning between the membrane and the aqueous phase usually takes place. If the membrane/water partition coefficient K is not too large and the lipid/water ratio is small, the impurity concentration in the aqueous phase can be considered as constant. Under these conditions, which apply to many experimental systems, a treatment of the interstitial molecules in the context of the grand canonical ensemble is appropriate. The effective Hamiltonian then takes the form

$$\mathscr{H}_{\mathrm{grand}} = \mathscr{H} - \mu \sum_{\ell} \mathscr{L}_{\ell}^{\mathrm{A}} \qquad (2)$$

where μ is the chemical potential for the interstitial impurities. This description is appropriate for volatile general anaesthetics which partition between the membrane phase, the aqueous phase, and the gas phase. In this case the concentration of general anaesthetics in the membrane can be controlled by the partial pressure of anaesthetics in the gas phase via multiple phase equilibria. If the membrane/water partition coefficient is extremely large, the concentration of interstitial molecules in the membrane is almost constant and a description within the canonical ensemble is more appropriate. In the most general case, for which neither the canonical nor the grand canonical ensembles are applicable, the volume of the water is an important parameter and three-dimensional phase separation phenomena are expected. However, the description of the full three-dimensional membrane-water system can often be reduced to that of the membrane alone by varying specific system parameters, such as pH, thereby restoring the approximate validity of one of the ensembles.

In this work the concentration of foreign molecules in the membrane is given by

$$x = \frac{2\langle \mathscr{L}_i^A \rangle}{1+2\langle \mathscr{L}_i^A \rangle} \tag{3}$$

x is the natural concentration variable in our lattice representation of the model since there are two interstitial sites for each lipid acyl chain. The conventional molar concentration, y, may be derived from Eqn. 3 in the case of a diacyl lipid

$$y = \frac{2x}{1+x} \tag{4}$$

It should be noted that, since foreign molecules can only occupy interstitial sites, the mixture of lipids and foreign molecules under consideration is not a conventional binary mixture. Indeed, the range of variation for x is $0 \le x \le \frac{2}{3}$. The two concentration measures are the same in the low-concentration limit.

III. Model parameters and phase diagrams

The experimental data on the interaction of small hydrophobic molecules with lipid membranes has in most cases a character which makes a direct comparison with results from model calculations cumbersome. This makes it difficult to evaluate the validity of the model and its underlying assumptions. The reason for this is that only in exceptional cases reliable experimental data are available on both thermal properties (e.g., specific heat) and partition coefficients for a wide range of temperatures and compositions. Therefore, rather than aiming at describing a particular molecular species, we have chosen some representative sets of model parameters and then extracted information about general features of the model. The results obtained give a basis for a broad discussion of experimental data as well as proposals for new experimental investigations.

The fundamental characterization of models of membranes containing foreign molecules is provided by the related equilibrium phase diagram. Different model parameters lead to different phase diagrams. However, the interaction of most foreign molecules with lipid membranes gives rise to a depression of the transition temperature (freezing-point depression). Therefore we focus on the two generic types of phase diagram shown schematically in Fig. 2 even though the results would be equally applicable to systems for which the impurities elevate the transition temperature. In the case of models with substitutional impurities, the entropy of mixing usually leads to a coexistence region as shown in type I of Fig. 2. In the case of models with interstitial impurities, both types of phase diagram shown in Fig. 2 are possible. However, a wide coexistence region can only occur if there is a strong attractive interaction between the interstitial molecules. This in nontheless not a sufficient condition for having a wide coexistence region.

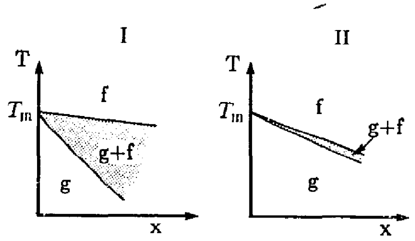

Fig. 2. Schematic representations of two different types of phase diagram for a lipid membrane with foreign molecules. Type I: Substantial freezing-point depression and a wide coexistence region. Type II: Some freezing-point depression and a narrow coexistence region.

The parameters in Eqn. 1 are selected by exploring the phase behaviour of the model in the mean-field approximation [6]. An example of a mean-field phase diagram is shown in Fig. 3a. In this example, the model of Eqn. 1 was used in conjunction with the canonical ensemble corresponding to a fixed concentration of water-insoluble molecules. The phase behaviour of the model can therefore be described by an (x,T)-diagram. Typical experimental paths in this diagram correspond to vertical thermal scans at a fixed concentration x. As previously stated, the phase separation is driven exclusively by the interactions in the system since the entropy of mixing of the two components is zero. Clearly, ideal solution theory arguments do not apply here. If the concentration of the foreign molecules is not fixed, but the system is in contact with a reservoir of foreign molecules, the control variables of the system are the temperature T and the chemical potential μ of the impurities. This representation is relevant under suitable experimental conditions for partly water-soluble impurities. Fig. 3a shows three paths through the phase diagram corresponding to three fixed values of μ. The paths are again obtained from a mean-field analysis of Eqn. 1. The temperature variation of x for fixed μ reflects the dependence of the partition coefficient on

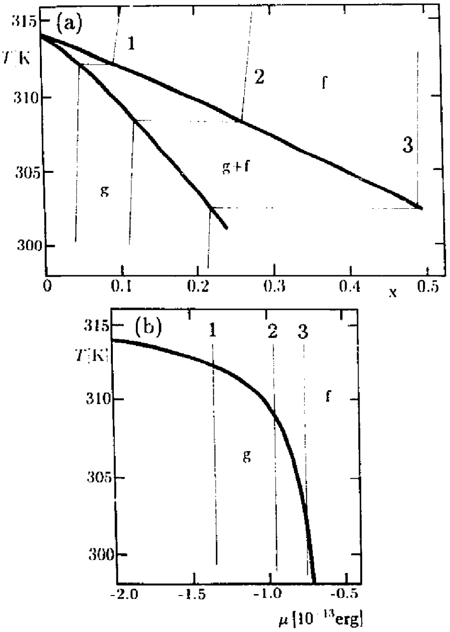

Fig. 3. (a). Phase diagram (x, T) within the mean-field approximation for the model in Eqn. 1 with parameters $J_{LA}^{gel} = -0.1 \cdot 10^{-13}$ erg, $J_{LA}^{fluid} = 0.5 \cdot 10^{-13}$ erg, and $J_{AA} = 0.45 \cdot 10^{-13}$ erg. g and f denote the gel and fluid lipid phases. Three constant chemical potential paths, cf. Eqn. 2, are shown corresponding to $\mu = -1.35$ (1), -0.95 (2), and -0.75 (3) in units of 10^{-13} erg. (b). The corresponding phase diagram in the (μ,T)-plane is shown with an indication of the constant μ-paths taken in the (x,T)-plane.

the temperature. In a measurement of thermal response functions, a representation of the phase behaviour in terms of a (T,μ)-diagram is therefore more appropriate. In this grand canonical description, the main phase transition is a transition line, cf. Fig. 3b, because of the dimensional reduction of the coexistence region when passing from a representation in terms of thermal densities to a representation in terms of intensive thermodynamic variables. The main transition thus remains sharp as impurities are added to the system. In contrast, in the canonical ensemble, the transition broadens when crossing the coexistence region.

Mean-field results such as those presented in Fig. 3 allow us to choose sets of model parameters which lead to phase diagrams of the generic types in Fig. 2. These parameter sets are then used for the computer simulations reported below.

IV. Computer-simulation techniques

Previous studies of the multi-state models for lipid membrane phase transitions [9] demonstrated that mean-field theory gives a misleading picture of the main transition due to the suppression of fluctuations with characteristic length-scales much larger than the intermolecular distances. Therefore, computer-simulation techniques are required for a proper comparison of the physical properties of multi-state models with experimental data. In principle, computer simulation allows for an accurate determination of thermal expectation values, taking full account of the thermal density fluctuations. In addition, it gives access to the microconfigurations of the system which provide information about the lateral organization of the membrane components. Phenomena like membrane heterogeneity and lipid-domain formation, which probably are of biological relevance, can therefore be characterized for different system parameters.

The computer-simulation techniques used in this work are of the Monte Carlo type [9]. The simulations are carried out on a triangular lattice of $N = L \times L$ sites for the acyl-chains and a honeycomb lattice of $2N$ sites for the interstitial molecules. The lattices are subject to toroidal periodic boundary conditions in order to minimize finite-system effects. All the lipid sites are occupied, while the degree of honeycomb lattice occupation depends on the molar concentration of foreign molecules in the membrane. Equilibrium is attained using Glauber dynamics for the lipids (single-chain excitations) and Kawasaki exchange dynamics for the interstitial molecules (lateral diffusion of foreign molecules). For the simulations in the grand canonical ensemble, the exchange of the foreign molecules between the aqueous reservoir of molecules and the membrane is simulated by Glauber dynamics.

V. Computer-simulation results

Selected equilibrium results from computer simulations of the model in Eqns. 1 and 2 are described in this section. The simulations are performed for the same set of model parameters in both the canonical and the grand canonical ensembles corresponding to the limits of a large and a small partition coefficient, respectively. Furthermore, non-equilibrium effects due to slow kinetics in the transfer of the foreign molecules between the aqueous phase and the membrane are briefly described.

V.A. Phase behaviour of type I

The major part of the results described below corresponds to a set of model parameters, $J_{LA}^{gel} = -0.1 \cdot 10^{-13}$ erg, $J_{LA}^{fluid} = 0.5 \cdot 10^{-13}$ erg, $J_{AA} = 0.45 \cdot 10^{-13}$ erg, which leads to a phase diagram of type I in Fig. 2. Results for type-II phase diagrams will be reported only briefly. The behaviour of the thermodynamic quantities observed in traversing the phase diagram for the canonical ensemble resembles at first sight that for binary lipid mixtures [15] and lipid bilayers with substitutional im-

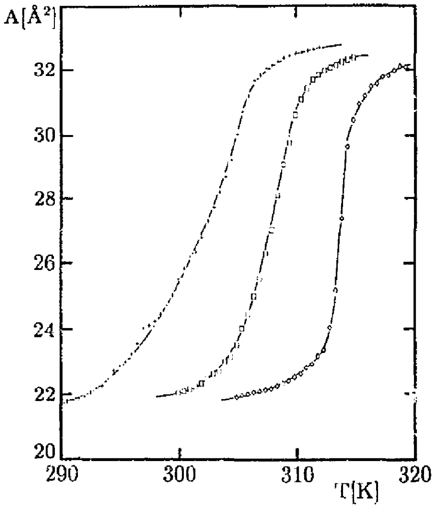

Fig. 4. Computer simulation data (canonical ensemble) for the average cross-sectional membrane area per lipid chain, $A(T)$, shown as a function of temperature for three different fixed concentrations, x, of foreign interstitial molecules. ◇, $x=0$; □, $x=0.2$, +, $x=0.33$. The phase diagram is of type I (Fig. 2) and the model parameters are $J_{\rm LA}^{\rm gel}=-0.1$, $J_{\rm LA}^{\rm fluid}=0.5$, and $J_{\rm AA}=0.45$ in units of 10^{-13} erg.

purities [16]. The temperature dependence of the average cross-sectional area per lipid molecules, $A(T)$, is shown in Fig. 4 for three fixed concentrations of interstitial impurities as the temperature is varied through the transition region. Fig. 4 clearly reflects a phase diagram similar to that of Fig. 3a and $A(T)$ varies linearly in the coexistence region between values of $A(T)$ at the upper and the lower phase boundaries. Note that the break in $A(T)$ at the phase boundaries becomes more pronounced for increasing x. The enthalpy displays a similar behaviour. The total enthalpy change, ΔH, observed in crossing the transition region is nearly independent of x, in contrast to the case of substitutional impurities [16] where ΔH decreases with increasing x.

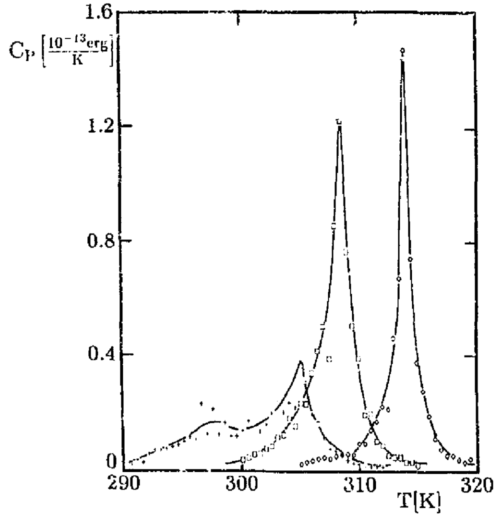

Fig. 5. Computer simulation data (canonical ensemble) for the specific heat per lipid molecule, $C_P(T)$, shown as a function of temperature for three different fixed concentrations, x, of foreign interstitial molecules. ◇, $x=0$; □, $x=0.2$; +, $x=0.33$. The model parameters are $J_{\rm LA}^{\rm gel}=-0.1$, $J_{\rm LA}^{\rm fluid}=0.5$, and $J_{\rm AA}=0.45$ in units of 10^{-13} erg.

The specific heat $C_P(T)$, which is shown in Fig. 5 for three concentrations x, is dominated at high concentrations by two peaks corresponding to the phase boundaries. In the coexistence region $C_P(T)$ has contributions from the bulk-phase fluctuations and from the transfer of material between the two phases as the temperature is varied.

We now turn to the grand-canonical simulation results for the same model with the same parameters. Fig. 6 shows the cross-sectional area per lipid chain, $A(T)$, as a function of temperature for three different values of the chemical potential μ. The main transition is signalled by jumps in $A(T)$ at a temperature $T_m(\mu)$ which decreases with increasing μ. Note that the jump in $A(T_m)$ becomes sharper as μ increases. This behaviour is in contrast to that seen in the canonical

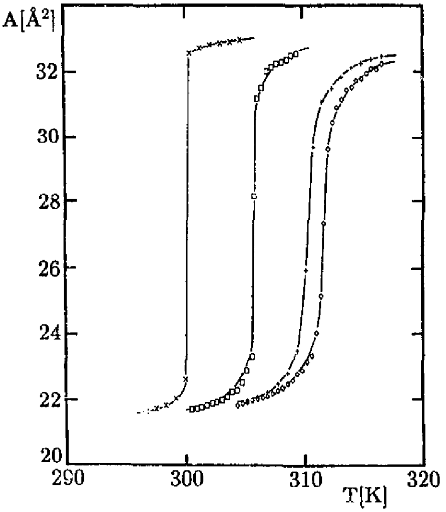

Fig. 6. Computer simulation data (grand canonical ensemble) for the average cross-sectional area per lipid chain, $A(T)$, shown as a function of temperature for three different chemical potentials, μ, of the foreign interstitial molecules. ◇, $\mu=-\infty$ (pure system), +, $\mu=-1.51$; □, $\mu=-0.94$; ×, $\mu=-0.75$ in units of 10^{-13} erg. The model parameters are $J_{\rm LA}^{\rm gel}=-0.1$, $J_{\rm LA}^{\rm fluid}=0.5$, and $J_{\rm AA}=0.45$ in units of 10^{-13} erg.

ensemble, cf. Fig. 4, and the enthalpy change in the transition region is almost independent of μ. The variation of $A(T)$ through the transition region reflects the disordering of the acyl chains since the area is reciprocally related to the acyl-chain orientational order parameter [17].

In the grand canonical ensemble the concentration of the foreign interstitial molecules in the membrane changes abruptly at the transition. This is shown in Fig. 7 for a series of different values of μ. A determination of the asymptotic values of x at the transition using the two pure-phase branches of the (x,T)-curves provides in principle the phase boundaries in the (x,T)-phase diagram. This phase diagram is shown in Fig. 7. Under appropriate conditions, which are discussed in Section VI, $x(T)$ is proportional to the membrane-water partition coefficient.

The lateral density fluctuations in the acyl-chain order are altered by the presence of the interstitial foreign molecules in the membrane. This is reflected in the behaviour of thermal response functions like the specific heat. $C_P(T)$ is shown in Fig. 8 for the pure lipid bilayer system as well as for two finite values of μ. The broad peak at T_m for pure DPPC-bilayers reflects the weak first-order nature of the main transition [18]. The extended wings in $C_P(T)$ around T_m for the pure bilayer are strongly reduced in the presence of interstitial molecules. The microscopic phenomena related to the changes in $C_P(T)$ are illustrated in Fig. 9 where the average cluster size, $\bar{\ell}(T)$, is shown for values of μ

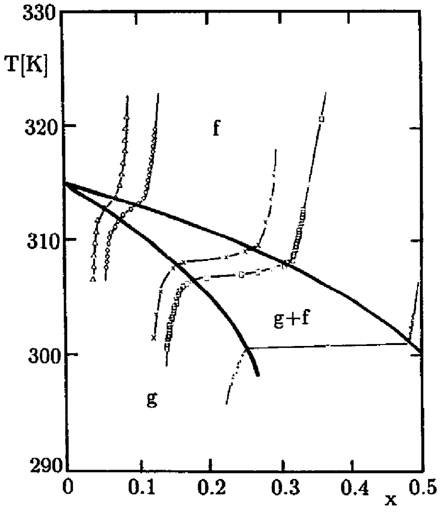

Fig. 7. Computer-simulation data (grand canonical ensemble) for the temperature dependence of the concentration, x, of foreign interstitial molecules shown for a series of different fixed chemical potentials μ. △, $\mu = -1.51$; ◇, $\mu = -1.35$; ×, $\mu = -1.0$; □, $\mu = -0.94$; □, $\mu = -0.75$ in units of 10^{-13} erg. The underlying phase diagram is indicated in heavy solid lines. g and f denote the gel and fluid lipid phases. The model parameters are $J_{LA}^{gel} = -0.1$, $J_{LA}^{fluid} = 0.5$, and $J_{AA} = 0.45$ in units of 10^{-13} erg.

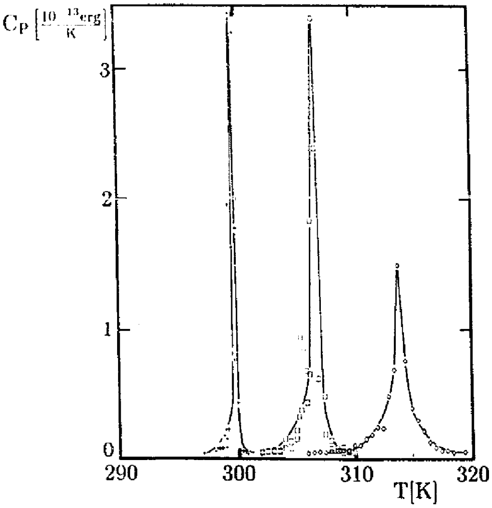

Fig. 8. Computer simulation data (grand canonical ensemble) for the temperature dependence of the specific heat per lipid chain, $C_P(T)$, for different values of the chemical potential μ of the interstitial foreign molecules. ◇, $\mu = -\infty$ (pure system), □, $\mu = -0.94$; +, $\mu = -0.75$ (in units of 10^{-13} erg). The model parameters are $J_{LA}^{gel} = -0.1$, $J_{LA}^{fluid} = 0.5$, and $J_{AA} = 0.45$ in units of 10^{-13} erg.

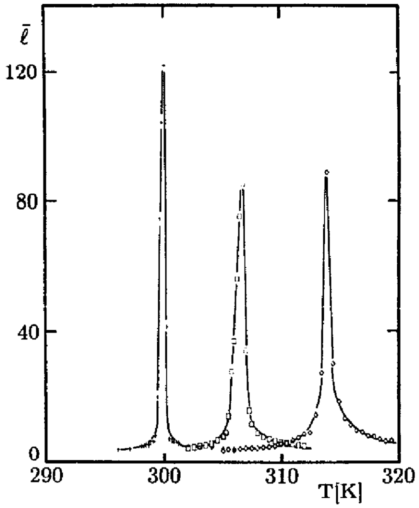

Fig. 9. Computer simulation data (grand canonical ensemble) for the temperature dependence of the average cluster size $\bar{\ell}$ (in units of lipid acyl chains) for different values of the chemical potential μ of interstitial foreign molecules. ◇, $\mu = -\infty$ (pure system), □, $\mu = -0.94$; +, $\mu = -0.75$ (in units of 10^{-13}) erg. The model parameters are $J_{LA}^{gel} = -0.1$, $J_{LA}^{fluid} = 0.50$, and $J_{AA} = 0.45$ in units of 10^{-13} erg.

corresponding to Fig. 8. The clusters in question are domains of gel-like lipids in a fluid bulk phase or domains of fluid-like lipids in a gel bulk phase. The cluster-formation phenomena are clear microscopic manifestations of the lateral density fluctuations which become long range at the weak first-order (pseudo-critical) main transition [18,19,20]. Fig. 9 shows that the average domain size is dramatically reduced away from $T_m(\mu)$ as μ is increased.

The computer-simulation results presented so far correspond to thermodynamic equilibrium. Under some experimental circumstances thermodynamic equilibrium may not be reached because the exchange kinetics for the transfer of foreign molecules between the membrane and the aqueous phase is too slow to keep up with the applied changes in the chemical potential. In that case one may observe non-equilibrium effects which interpolate between the canonical and grand canonical ensembles. An example of such an effect is demonstrated in Fig. 10 in which the specific heat, $C_P(T)$, and the concentration of interstitial molecules in the membrane, x, is shown under non-equilibrium condiditions. These non-equilibrium conditions are provided by initializing the system in a state below the phase transition (at 290 K), keeping μ fixed, and then heating at a rate which is too fast for the system to equilibriate at any given temperature, i.e., the membrane lags behind in its uptake of foreign molecules from the aqueous phase. It is seen that the non-equilibrium specific heat in Fig. 10a has an appearance which is intermediate between the equilibrium specific heat functions in the grand canonical ensemble (Fig. 8) and in the canonical ensemble (Fig. 5). Under such non-equilibrium conditions, the properties of the system will be very dependent on the particular thermal treatment.

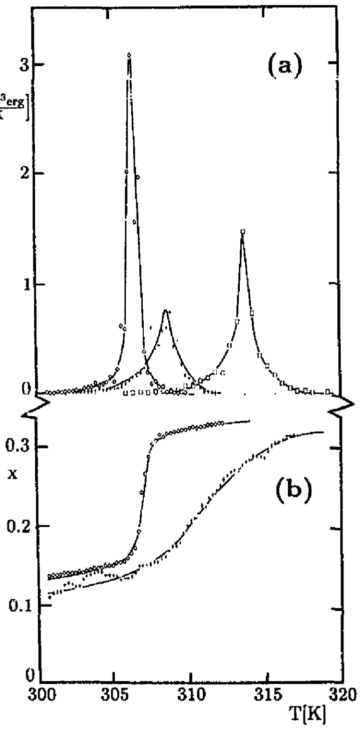

Fig. 10. Computer simulation data (grand canonical ensemble) for a membrane system under non-equilibrium conditions (+) caused by a slow exchange of foreign molecules between the membrane and the aqueous phase. (a) Specific heat $C_P(T)$ per lipid molecule. (b) Concentration x of foreign molecules in the membrane. Data are also shown for the corresponding equilibrium situation (◇) at a chemical potential $\mu = -0.94 \cdot 10^{-13}$ erg. For comparison the equilibrium specific heat for the pure system is also shown (□). The model parameters are $J_{LA}^{gel} = -0.1$, $J_{LA}^{fluid} = 0.50$, and $J_{AA} = 0.45$ in units of 10^{-13} erg.

V.B. Phase behaviour of type II

The results presented in Figs. 2–10 for both the canonical and grand canonical ensembles were based on a single choice of model parameters which lead to a phase diagram with substantial freezing-point depression and a wide coexistence region. The various results have demonstrated that, even though the interactions of the foreign interstitial molecules are governed by a particular Hamiltonian, these interactions give rise to different behaviours for the experimentally accessible thermodynamic quantities depending on the experimental conditions, i.e., whether canonical or grand canonical emsemble conditions apply and whether the system is subject to a non-equilibrium condition due to slow exchange of the foreign molecules between the membrane and the aqueous phase. In Fig. 11 we show a comparison between data obtained in the grand canonical and the canonical ensembles for a different choice of model parameters which lead to a phase diagram of type II (cf. Fig. 2) with some freezing-point depression but only a narrow coexistence region. Compared with the previous set of model parameters, only J_{LA}^{gel} is changed, i.e., $J_{LA}^{gel} = -0.05 \cdot 10^{-13}$ erg, corresponding to a weaker relative repulsion between the gel-like lipid acyl-chain conformations and the foreign molecules. Also for this set of model parameters the transition enthalpy is almost independent of μ and x. The data show that for this type of phase diagram the thermal scans in the specific heat are not sufficiently strong indicators of the underlying ensemble, i.e. from specific-heat data alone it is not possible to discern whether the membrane exchanges foreign molecules with the aqueous phase. That it indeed does so is seen in the insert of Fig. 11b which shows that the concentration in the membrane (and hence in many cases the partition coefficient) changes dramatically in going through the transition.

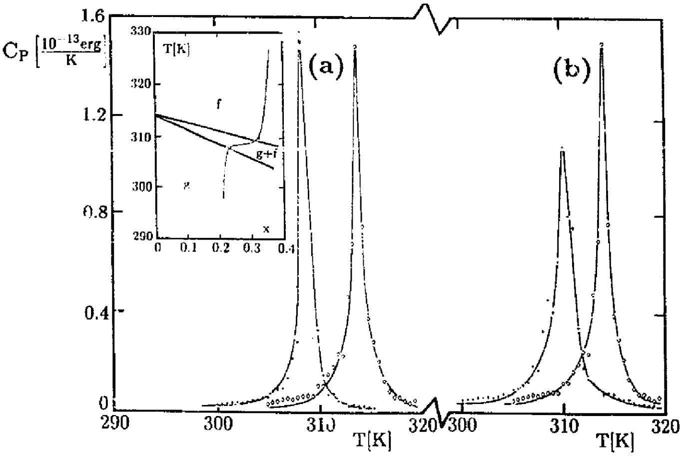

Fig. 11. Comparison between computer-simulation data for the specific heat $C_P(T)$ (+) obtained in the canonical and grand canonical ensemble for a set of model parameters $J_{LA}^{gel} = -0.05$, $J_{LA}^{fluid} = 0.50$, and $J_{AA} = 0.45$ in units of 10^{-13} erg. (a): canonical, $x = 0.2$. (b): grand canonical, $\mu = -0.94 \cdot 10^{-13}$ erg. A comparison with $C_P(T)$ for the pure membrane (◇) is provided. The inset shows $x(T)$ for fixed μ with the heavy solid lines indicating the phase boundaries. g and f denote the gel and fluid lipid phases.

Finally, it should be mentioned that the degree of freezing point depression for a phase diagram with a narrow coexistence region (type II) does not significantly influence the results and that the specific heat is quite similar to that of the pure lipid bilayer by analogy with Fig. 11.

VI. Interpretation of experimental data

VI.A. Choice of ensemble

As pointed out in Section II, the interpretation of the experimentally observed effects of foreign molecules on membrane properties depends critically on being able to identify the underlying statistical ensemble. In this subsection we address the question of the proper choice of ensemble in relation to a number of specific experiments. Membrane components and membrane additives like drugs display a wide range of solubilities. In a general description of these systems, the membrane, the aqueous environment and (for components like volatile general anaesthetics) the gaseous phase must all be taken into account. The various phases require different treatments:

(i) The gaseous phase. This phase plays an important role for small volatile membrane components. Ideal gas theory can usually be applied and the chemical potential for a substance α is then expressed as

$$\mu_\alpha^{gas} = \mu_{\alpha,0}^{gas}(T) + RT \ln P_\alpha, \tag{5}$$

where P_α is the partial pressure of the substance. The standard chemical potential $\mu_{\alpha,0}^{gas}(T)$ is only weakly temperature dependent in the temperature range of interest.

(ii) The aqueous phase. The theoretical description of the solution of polar or non-polar compounds in water is a difficult and largely unresolved problem. For very small concentrations, the chemical potential of the component α is

$$\mu_\alpha^{aq} = \mu_{\alpha,0}^{aq}(T) + RT \ln c_\alpha, \tag{6}$$

where c_α is the concentration of the component α in the aqueous phase. However, highly non-ideal mixing behaviour is expected for polar solvents like water. Eqn. 6 can be improved by the addition of virial expansion terms based on a microscopic interaction model for the aqueous solution. Since reliable models do not exist for polar liquids, another approach must be taken. If the aqueous mixture is miscible, there is a unique monotonic relationship between μ_α^{aq} and c_α with $\partial\mu_\alpha^{aq}(c_\alpha,T)/\partial c_\alpha > 0$. Therefore, in this case, μ_α^{aq} characterizes the overall concentration of component α in the aqueous phase.

(iii) The membrane. The chemical potential μ_α^{mem} of component α in the membrane is modelled by the Hamiltonian in Eqn. 1.

Under equilibrium conditions the relation $\mu_\alpha^{gas} = \mu_\alpha^{aq} = \mu_\alpha^{mem}(x_\alpha,T)$ is fulfilled. μ_α^{gas} is irrelevant for the description of membranes with non-volatile compounds. On the other hand, for volatile compounds, the gaseous phase serves as a reservoir for the membrane with respect to component α with the chemical potential controlled by the temperature and the partial pressure, P_α. Similarly for strongly water-soluble compounds, the solubility of component α in the aqueous phase relative to the membrane can simply be parameterized by a chemical potential. An equivalent measure is the membrane/water partition coefficient which will be considered later in this section.

We now consider two main groups of membrane soluble components, volatile and non-volatile compounds.

VI.A.1. Volatile compounds

This group of molecules includes several general anaesthetics like halothane and ether. The concentration of general anaesthetics in the membrane is usually controlled by a partial pressure. The equilibrium between the gaseous phase, the aqueous phases, and the membrane assumes equality of the chemical potentials of the components. In this description, the gaseous phase simply constitutes a reservoir for the membrane of anaesthetic molecules α controlled by P_α or μ_α^{gas}. The grand canonical approach is therefore appropriate. The solubility of the species α in water plays no role in this description, but it is of course important for the determination of the membrane/water partition coefficient.

According to the discussion in Section III we expect the main transition to occur at a well-defined temperature and to be signalled by a single peak in the specific heat. This is indeed observed in DSC-measurements for varying partial pressure of halothane [5,21]. The peak in C_P at the transition temperature is broadened as P_α is increased. One possible explanation for this may be slow exchange kinetics for the transfer of halothane between water, the membrane, and the gaseous phase when compared with the characteristic time of measurement. The non-equilibrium conditions therefore give rise to a smearing of the transition as demonstrated in the model calculations depicted in Fig. 10. Another explanation for the broadening of the transition could be a hidden conserved gaseous volume in the experiment. Finally, the broadening may indicate strongly enhanced fluctuations as the halothane is added.

VI.A.2. Non-volatile compounds

Partly soluble membrane components can be characterized by a membrane/aqueous-buffer partition coefficient

$$K = \frac{c_\alpha^{\text{mem}}}{c_\alpha^{\text{aq}}} \frac{N_\alpha^{\text{mem}}}{N_\alpha^{\text{aq}}} \cdot \frac{V_\alpha^{\text{aq}}}{V_\alpha^{\text{mem}}} \tag{7}$$

where N_α^{aq} and N_α^{mem} are the total numbers of molecules of species α respectively in the aqueous phase and in the membrane. V_α^{aq} and V_α^{mem} are the corresponding volumes. The description outlined in Section VI.A.1 for the partitioning of volatile general anaesthetics in the membrane can be extended to non-volatile compounds when one of the following conditions is met

$$N_\alpha^{\text{mem}} \ll N_\alpha^{\text{aq}} \text{ or } K \ll V_\alpha^{\text{aq}}/V_\alpha^{\text{mem}} \tag{8}$$

and the aqueous mixture is miscible. Under these conditions the concentration of foreign molecules in the aqueous phase is only weakly affected by changes in the concentration in the membrane, e.g., at the main transition.

The above conditions are typically fulfilled in experimental systems involving certain local anaesthetics like procaine ($K_{\text{DPPC}} = 50$ at pH = 7.5) [22] and a range of drugs like the anticancer agent adriamycin ($K_{\text{DPPC}} = 40$ at pH = 7.4) [23]. For these systems, the second inequality of Eqn. 8 is obeyed for a sufficiently dilute membrane solution. The partition coefficient K varies with temperature, solution concentration, pH, and lipid composition. The quoted values of K indicate therefore only the order of magnitude of the partition coefficient in a pH-neutral solution. When neither of the conditions in Eqn. 8 are strictly fulfilled, traces of three-dimensional phase separation will appear, leading to a broadening of the phase transition. This is likely to happen in a great variety of experimental systems [23,24]. Alternative explanations for broadening of the transition are considered below.

If

$$N_\alpha^{\text{mem}} \gg N_\alpha^{\text{aq}} \text{ or } K \gg V_\alpha^{\text{aq}}/V_\alpha^{\text{mem}} \tag{9}$$

the exchange of solutes between the membrane and the aqueous phase plays a minor role and cooperative phenonema take place in the membrane under the conditions of almost fixed concentration of impurities, i.e., a canonical ensemble description is appropriate. This does not prevent three-dimensional phase separation from occurring after a sufficiently long equilibration time in the real vesicle system. However, the average thermal properties can still be described by the two-dimensional canonical-ensemble treatment. A large class of poorly water-soluble drugs belongs to this category, including antidepressants like chlorpromazine ($K_{\text{DPPC}} = 3400$ at pH = 7.0 and $T = 50\,^\circ\text{C}$) [25] and the calcium-channel antagonist nimodipine ($K_{\text{DPPC}} = 128\,000$ at pH = 7.4) [26].

In most experimental situations, neither of the conditions given in Eqns. 8 and 9 is strictly met and the interaction of the foreign molecules with both the membrane and the aqueous phases must definitely be taken into account. However, for a given drug, K can often be modified substantially by changing ionic strength or pH [27,28] which makes it possible to approach one of the limits Eqn. 8 or Eqn. 9 thereby permitting a clean study of the interactions of the foreign molecules with the membrane. Alternatively, the water solubility of the foreign molecules may be altered by appropriate substitution or addition of suitable molecular residues. For example, Bae et al. [26] have studied the interaction of a whole series of adriamycin derivatives with DPPC bilayer membranes using DSC experiments. For this series of compounds, the whole spectrum of K-values from Eqn. 8 to Eqn. 9 is represented. The observed DSC scans for this series have the characteristics of the model calculations in Section V ranging from the canonical to the grand-canonical description, cf. Figs. 5 and 8.

VI.E. Choice of microscopic model

As pointed out in Section I, a proper interpretation, in terms of a microscopic interaction model, of an experiment on the physical effects of foreign molecules interacting with membranes, has to resolve the question as to whether the foreign molecules predominantly act as substitutional or interstitial impurities in the bilayer membrane. The most striking difference between these two types of impurity action is that substitutional impurities will in general reduce the changes in thermal densities at the transition (e.g., as the enthalpy) whereas interstitial impurities will leave these changes almost unaffected.

It is important to realize that the size of the foreign molecule is not the only quantity which determines whether a foreign molecule acts as a substitutional or an interstitial impurity. As an example we consider the series of calcium-channel antagonists, diltiazem, verapamil, nimodipine, and nisoldipine. The effects of this series on the thermotropic behaviour of DMPC membranes have recently been studied by DSC experiments [26]. These four drugs of comparable sizes are moreover comparable to the size of a lipid molecule. However, the interaction of diltiazem and verapamil with the membranes hardly affects the ΔH of the transition, whereas the addition of nimodipine and nisoldipine gives rise to a pronounced decrease in ΔH. X-ray and neutron-scattering studies [26] suggest that the two pairs of compounds differ in their position within in the membrane. While diltiazem and verapamil are buried deeply in the hydrocarbon core of the membrane, nimodipine and nisoldipine are located closer to the polar membrane/water interface. Hence, the latter two compounds strongly affect the packing of the lipid chains and consequently influence the cooperativity of the chain-melting transition. These membrane components must therefore be considered as substitutional impurities. Diltiazem and verapamil, on the other hand, intercalate nicely between lipid acyl chains and act as interstitial impurities if the concentration in the membrane is not too large.

Another group of membrane active compounds which also can be described as interstitial impurities includes headgroup-active agents like procaine and morphine which interact only weakly with the lipid hydrocarbon chains. ΔH is almost independent of concentration for these two compounds [24,29]. For cocaine and morphine derivatives, the change in ΔH depends on the size of the hydrophobic moity buried in the membrane. A more detailed description of these cases, as well as the interaction of small polar molecules like certain alcohols with the membrane, requires a theoretical treatment which is outside the scope of the present paper.

VII. Conclusions

In this paper we have presented a discussion of the key elements required for a description of the interaction between foreign molecules and lipid bilayer membranes on the basis of a simple but general microscopic interaction model. The interactions manifest themselves macroscopically in changes in the physical and thermodynamic properties of the pure bilayers as various amounts of foreign molecules enter the membrane. In particular, the characteristics of the main gel-to-fluid bilayer phase transition are dramatically changed in the mixed system. For a characterization of the thermodynamic behaviour of the system we have pointed out that the phase diagram and the variation of response functions (e.g., the specific heat) as well as the membrane/water partition coefficients seen in crossing the phase diagram are important indicators of the molecular interactions.

The basic hypothesis of this paper is that a proper description of the interaction of foreign molecules with lipid membranes is dependent on the following considerations: (i) whether or not the foreign molecules act as substitutional or interstitial impurities, and (ii) whether or not the concentration of the foreign molecules in the membrane is effectively constant or not (canonical or grand canonical ensemble). In case (i), the case of interstitial impurities usually implies that the transition enthalpy ΔH does not vary with concentration, whereas ΔH decreases in the case of substitutional impurities. In case (ii), a knowledge of the thermal variation of the partition coefficient is essential for determining the appropriate ensemble. As shown by our model calculations, information concerning just the specific-heat profiles across the phase diagram is not sufficient to settle the question about the ensemble. Also, under certain experimental conditions neither the canonical nor grand canonical ensemble descriptions are fully appropriate. For example, the transfer of molecules between the membrane and the aqueous phase may be slow on the experimental time scale with the result that the system is subject to a non-equilibrium condition which places it in between the two ensembles. In such cases it is important to tune the experimental system parameters to bring the system into one or the other ensemble. In the case of the canonical ensemble, the temperature-composition phase diagram can readily be determined. For the grand canonical ensemble, this diagram can only be determined when the temperature variation of the partition coefficient is also known. These are important considerations when studying the interaction between a given drug and a membrane, e.g. when the drug molecule has a small hydrophilic or charged group which makes it (partly) soluble in water. In that case the measured thermodynamic properties of the water-membrane system are not solely a signal of the lipid–drug interactions but the partitioning (via the ensemble) comes in as well. In such a case it is necessary to tune the experimental parameters to isolate the effects of the lipid–drug interactions.

The microscopic interaction model presented in this paper can easily be extended to account for more specific interactions between the foreign molecules and the lipid acyl chains. Such specific interactions could for example resemble a selective coupling between a drug molecule and a particular chain conformation, e.g., a kink excitation. If such a coupling is present, it would have a dramatic effect on the fluctuations of the system since the drug molecule would then control the formation of interfaces between lipid domains and the bulk phase in the neighborhood of the transition. Preliminary results on an extended model of this type [30] have confirmed this picture and have demonstrated that the partition coefficient can develop non-classical behaviour by displaying a maximum near the transition. Such a maximum has been observed experimentally in membranes with local anaesthetics like cocaine derivatives [22] and in lipid bilayers doped with certain insecticides like lindane [31].

Finally, we make a brief remark concerning the global concentration values used in the present model study and those which may be relevant for physiological studies of anaesthetics and drugs. In the present work quite high concentrations were considered. These may seem large compared to the global concentrations quoted for assays used for pharmacological purposes. However, this need not be the case since the membrane at the site of action may contain the drug in a much larger concentration than is globally present in the entire assay [12]. Furthermore, specific interactions may lead to a strong local accumulation of drugs within the membrane due to a coupling to the lateral density fluctuations [30].

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