Determination of Partition Coefficient by the Change of Main Phase Transition

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Abstract. The molar partition coefficients of amphiphilic additives, e.g. local anesthetics, between the aqueous phase, the liquid crystal and the gel phase of lipid membrane can be determined based on a combination of phase transition data obtained at high and low concentrations of the lipid in aqueous phase. The data obtained at high lipid concentration allow to find the phase diagram lipid-additive in the aqueous environment. The combination of this diagram with data obtained at low lipid and additive concentrations provides direct information on the concentration of anesthetics in the lipid and thus allows the calculation of the partition coefficient.

Key words: Partition coefficient — Phase diagram — Lipid bilayer — Phase transition — Density meter

Introduction

The partition coefficient allows to compute the concentrations of additive molecules like anesthetics or poisons in different phases of lipid membrane in water environment, if the concentrations of the additive and the lipid are known. More possibilities to define partition coefficients have been published. We will use the set of definitions based on ratios of molar fractions, which are convenient for the description of partitioning in lipid membranes with two phases – gel and liquid-crystalline (fluid):

$$K_{p,fw} = \frac{N_{af}}{N_{af}+N_{pf}} \Big/ \frac{N_{aw}}{N_{aw}+N_w}, \tag{1}$$

$$K_{p,gw} = \frac{N_{ag}}{N_{ag}+N_{pg}} \Big/ \frac{N_{aw}}{N_{aw}+N_w}, \tag{2}$$

$$K_{p,gf} = \frac{N_{ag}}{N_{ag}+N_{pg}} \Big/ \frac{N_{af}}{N_{af}+N_{pf}} = \frac{K_{p,gw}}{K_{p,fw}}, \tag{3}$$

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where $K_{p,fw}$ ($K_{p,gw}$) is the partition coefficient of the additive distribution between the fluid (gel) phase and water, and $K_{p,gf}$ is the partition coefficient of the additive distribution between the gel and liquid-crystalline phases; N_{af} (N_{ag}) is the number of moles of the additive in the membrane in the fluid (gel) phase, N_{pf} (N_{pg}) is the number of moles of the lipid in the fluid (gel) state; N_{aw} is the number of moles of the additive in water, and N_w is the number of moles of water. The definitions 1–3 are compatible with those used in the papers Kaminoh et al. (1989), Gallová et al. (1992, 1995)

Partition coefficients can be measured by different physical methods allowing to detect the additive in lipid or in water environment. The applied methods, e.g. spectrophotometry, fluorescence, sound velocity, light diffraction, ESR, etc. depend on the nature of the additives measured. Obviously, these methods need the lipid to be separated by sedimentation or centrifugation, if the method used is not specific to the environment of the drug (Lissi et al. 1980, 1990; Welti et al. 1984; Inoue et al. 1990; Babincová and Hianik 1994). The above mentioned methods are obviously very laborious and the obtained results depend on the separation, that may not be perfect or by co-sedimentation of the solute.

If the method used is sensitive to the concentration of the additive in one phase (lipid or water environment) no separation is necessary. Lissi et al. (1990) described a procedure applicable whenever it is possible to measure a property that depends on the extent of partitioning. This method is suitable for use with spectroscopic techniques. A similar procedure has also been described by Ondriáš et al. (1983) and Šeršeň et al. (1989) who estimated the partition coefficient by the perturbation effect of surfactants to the order parameter measured by ESR. The partition coefficient have been obtained by fitting the experimental data assuming that the order parameter of the spin probe depends linearly on the local anesthetic concentration in the lipid phase.

The concentration of the additive in the water environment can be measured by surfactant ion-selective electrodes. This approach has been applied to estimate partition coefficients by Uhríková et al. (1995). The biological effect, e.g. inhibition of some processes in the membrane by the applied drug, can also be used to determine partition coefficient (Šeršeň 1995). In this case, the effect is registered at different concentrations of the drug tested and the partition coefficient is computed for that concentration in the membrane at which the effect is observed.

A special case of the effect depending on the extent of partitioning are changes of the main phase transition in the lipid membranes. Kaminoh et al. (1988) have described the theoretical approach to this effect. A formula was derived for the reduction of a temperature of main phase transition in dependence on the partitioning between the anesthetic and the lipid in the fluid or in the gel phase.

The method presented in this paper is based on the analysis of changes using the phase diagram in the region of the main phase transition. From the experimental data, the begin and the end of phase transition are extracted and they are subsequently localized on the phase diagram. This approach has some advantages, and it differs from that used by Lissi et al.(1990) or Kaminoh et al. (1988). The

described method does not depend on theoretical assumptions, and it allows to obtain partition coefficients in systems where phase transition is observed and is affected by the content of the additive in the membrane.

Materials and Methods

Description of the method

Fig. 1 schematically illustrates the phase diagram lipid – additive in the region of the main phase transition. The horizontal coordinate represents the molar ratio $X_a = (N_{af} + N_{ag})/N_p$, where $N_p = N_{pf} + N_{pg}$ is the total number of lipid moles, and $N_{af} + N_{ag}$ is the number of moles of the additive in the membranes.

In Fig. 1, **g** denotes the gel phase, **f** is the fluid phase, and **f+g** represents the mixed fluid and gel phases. Three possible cases are shown on this diagram showing the route of the system during heating (Suezaki et al. 1990 and Jørgensen et al. 1993):

1) additive concentration in the lipid is constant,

2) phase transition runs at a constant temperature,

3) combination of the above mentioned marginal routes with a change of both concentration and temperature during the phase transition.

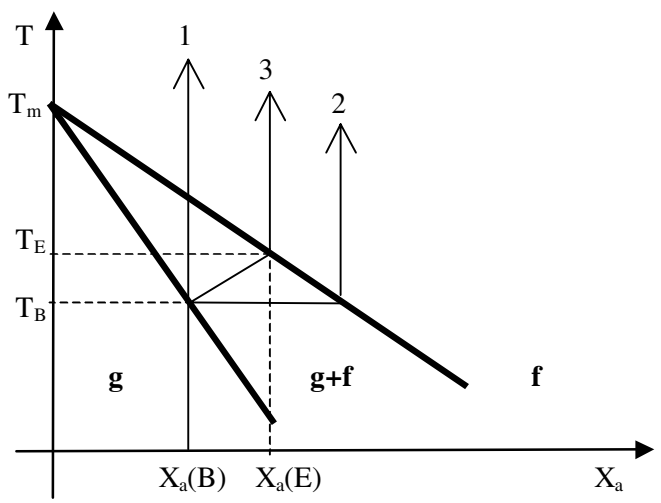

Figure 1. Phase diagram for the lipid/additive system in water environment. T is temperature, T_m is temperature of main phase transition of pure lipid, X_a is the molar fraction of the additive in the lipid membrane, **g** is the sign of the gel phase, **f** is the sign of the liquid-crystal (fluid) phase, **f+g** is the sign of the mixed phase, 1,2 and 3 are routes of heating for different concentrations of lipid (1 – high amount of lipid, 2 – negligible amount of lipid, 3 – medium amount of lipid), B is the index for the begin of phase transformation, E is the index of the end of phase transition for medium amount of lipid in sample.

The first marginal route occurs in the case of a high amount of lipid in the sample, when almost all the additive is in the lipid. In the case of the second marginal route, the amount of the lipid in the sample is so negligible that the additive is in the water solution. In this case, the water solution of the additive is a reservoir with unlimited capacity. In the third case, the amounts of the additive in water as well as in lipid are comparable.

According to Suezaki et al. (1990), the difference between the actual temperature T in the region of the main phase transition and the temperature $T_m$of the main phase transition of pure lipid, $\Delta T_m = T - T_m$, can be written as

$$\Delta T_m = -\frac{RT_m^2}{\Delta H}(1-\kappa)X_{am}\frac{1}{\kappa + \gamma(1-\kappa) + \dfrac{1}{P_{af}\cdot X_p}} \tag{4}$$

where ΔH is the enthalpy of the main phase transition, $\kappa = N_{ag}N_{pf}/(N_{pg}N_{af})$, the molar ratio $X_{am} = N_a/N_p$, $N_a = N_{af} + N_{ag} + N_{aw}$, $\gamma = N_{pf}/(N_{pf} + N_{pg})$ is the melted fraction of the membrane, $P_{af} = (N_{af}N_w/(N_{pf}N_{aw})$, $X_p = N_p/N_w$. The quantities P_{af} and κ in equation (4) are also partition coefficients, but they are defined as the ratio of the molar ratios in two compared environments, rather than as the more frequently used ratios of molar fractions, like in Eq. (1–3).

Suezaki et al. (1990) analyzed equation (4). According to this analysis, high or low amounts of lipid in the sample can be regarded as the value $P_{af}X_p$. When factor $P_{af}X_p$ is large compared to unity, then almost all additive molecules are adsorbed to the membrane and the number of additive molecules in the aqueous phase is nearly depleted; this is the case when the heating or cooling are described by the first marginal route (Fig. 1). When factor $P_{af}X_p \ll 1$, then the initial temperature of phase transition becomes equal to the temperature of the end of the phase transition, as described by Suezaki et al. (1990) (case No. 2). In the third case, factor $P_{af}X_p$ is comparable to unity and then experimental data show a widening of the phase transition and a lowering of the temperature of the main phase transition with the increasing concentration of the additive in the membrane.

The first route in the phase diagram in Fig. 1 is very convenient for the determination of the boundaries of the phase diagram. It should be based on thermodynamic measurement such as with differential scanning calorimeter (DSC), or on measurement of density in a sample with small amounts of water. In this case, in fact near all the additive is in the lipid phase, and $X_a = X_{am}$. The liquidus and solidus points of the phase diagram are found as the points of the begin (B) and the end (E) of phase transition. The details of this procedure for the DSC measuring are described in the paper by Dörfler et al. (1990).

Direct computation of the partition coefficient is possible if $P_{af}X_p$ of the sample is close to unity and if the phase diagram of the studied system was previously prepared. The thermodynamic measurement allows to obtain the temperatures of the begin and the end of the phase transition, and the corresponding molar ratios X_a(B) and X_a(E) can be directly read from the liquidus and solidus lines. The

knowledge of X_a, N_a and N_p is enough for simple calculation of the missing molar values and the partition coefficients. The problem of the measurement of the partition function is reduced to the problem of measuring the begin and the end of the phase transition.

By applying Eq. 4 the boundaries of the phase transition can be measured in a new, more precise way. We present it on an example of density measurement, but it can be simply modified for DSC experiments as well. The melted fraction γ in the region of phase transition can be expressed by neglecting the volume of phase boundaries between the gel and the liquid-crystalline phase as

$$\gamma = \frac{v_p - v_p^g}{v_p^f - v_p^g} \tag{5}$$

where v_p is the lipid volume measured in the region of the main phase transition, v_p^g is the lipid volume in the gel (solid) state, and v_p^f is the lipid volume in the fluid state. The lipid volumes v_p^g and v_p^f are usually linear functions of temperature and they are measurable by the temperatures below or above the main phase transition region. In the region of the main phase transition they can be obtained by linear extrapolation. Using Eq. 5 we can rewrite Eq. 4 to the form

$$\frac{1}{\Delta T_m} = \frac{\Delta H}{RT_m^2} \frac{\kappa + \dfrac{1}{P_{af} X_p} + (1-\kappa)\dfrac{v_p - v_p^g}{v_p^f - v_p^g}}{(1-\kappa) X_{am}}. \tag{6}$$

thus the value of $1/\Delta T_m$ is a linear function of the volume of the lipid in the region of the main phase transition. The begin of phase transition is at the temperature where $\gamma = 0 \Leftrightarrow (v_p = v_p^g)$, and the end of phase transition is at the temperature, where $\gamma = 1 \Leftrightarrow (v_p = v_p^f)$.

The application of the described method will be illustrated by the example of the distribution of the local anesthetics heptacaine in dipalmitoylphosphatidylcholine membranes.

Chemicals

Synthetic dipalmitoylphosphatidylcholine (DPPC) was obtained from Fluka and was used without any purification. The local anesthetic heptacaine prepared as described by Čižmárik and Borovanský (1975) was kindly provided by Professor J. Čižmárik, Faculty of Pharmacy, Comenius University, Bratislava. Water was triply distilled, once from alkaline potassium permanganate solution.

Preparation of samples

The stock solution with the highest concentration of the local anesthetic was used to prepare samples with the desired concentrations of the local anesthetic. Appropriate amounts of this solution were added to ∼6.6 mg of DPPC in a small glass flask, and the final concentration of the sample was obtained by adding degassed water to 2.6

ml. This sample was heated to 58 ℃ and homogeneous dispersion was obtained by intensive stirring during 10 minutes. After subsequent degassing, the sample was filled into the measuring chamber in the buoy of the density meter. The measuring started after 24 h stabilization at 30 ℃. This procedure is comparable with that described in the classical papers of Nagle and Wilkinson (1978, 1982), and our previous experiments have demonstrated that for the system lipid-water the results are the same as obtained by the method described by Melchior et al. (1980).

The weights of DPPC, heptacaine and the used solutions were determined on Sartorius balances with a sensitivity $\sim 1 \times 10^{-5}$ g. The weights were corrected for the content of water in the chemicals, the correction factors were previously established by drying under vacuum.

Density meter and measuring

The density meter used to measure specific volumes was designed and built in our laboratory as described by Bánó and Bán (1994). It is well suitable for the measuring of densities of small amounts of solids, solutes or dispersed materials in liquid environment at constant temperature or in scanning regime.

The principal part of our density meter is a glass buoy (Fig. 2) , which is immersed in water and balanced by electromagnetic force. The lower part of the buoy contains a chamber for the measured sample, the upper closed part is empty. The chamber is closed by glass stopper containing a permanent magnet. The attraction force between the magnet and the coaxial coil located under the buoy is used for equilibration. Automatic check of the equilibrium position is done by electro-optical system. The density can be calculated in real time from the equilibrium force.

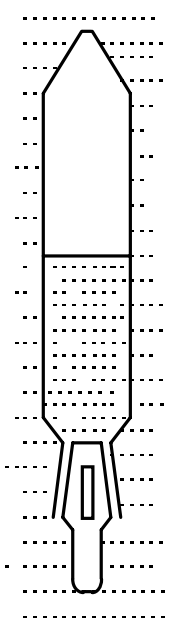

Figure 2. The buoy of the density meter. In the lower part there is chamber with the sample closed by a glass stopper. The stopper containes a permanent SmCo magnet.

The chamber is not absolutely tight because a small amount of liquid remains in the gap between the glass parts. In the scanning regime, the solvent slowly flows around the stopper. As a result, some solvent is lost, approx. 0.5 % in the temperature range used in our experiments. The "baseline" obtained in independent measurement scan of the solvent sample at the same scanning rate is used for comparison with measured data with the aim to avoid the shift caused by the above described effect as well as by some more

minute effects. Nevertheless, also in this case the negligible measurement errors occur due to the transport of the substance around the stopper. These errors have been analysed in our previous paper (Bánó and Bán 1994) and in the presented experiments they were below the threshold of the measurement sensitivity. The measurement accuracy at the equilibrium force is $\approx 1.5 \times 10^{-8}$ N. This means that the liquid density is measured with an accuracy of ≈ 1 mg/l in the 1.47 ml chamber, the sensitivity of temperature measurement is 1 mK. The reproducibility of the apparatus was checked many times by measuring thermal expansion of DPPC vesicles in water environment. The results were in good agreement with the data published earlier by Nagle and Wilkinson (1978, 1982) and Melchior et al. (1980).

Results

Fig. 3 shows examples of measured specific volumes of lipid in the sample for different concentrations of heptacaine. The results were obtained in the scanning mode at the scanning rate of 4 mK/min. There were 3.4 mmol/l of DPPC in each sample. The results obtained for pure DPPC ($X_{am} = 0$) show sharp changes of specific volume at the temperature of the main phase transition ($T_m = 41.4\,$℃) and at the temperature of the pretransition (34.3℃). These temperatures and the half-width of main phase transition ($\Delta T_{1/2} = 120$ mK) were obtained by the method

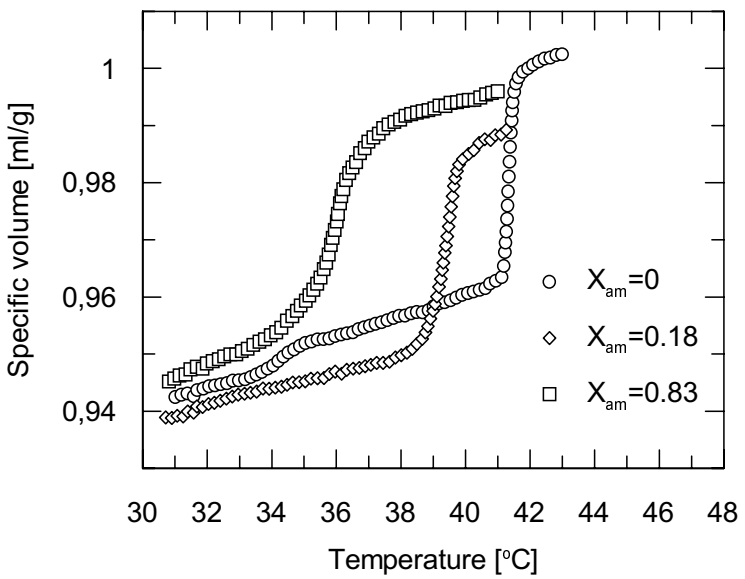

Figure 3. Examples of measured specific volume as a function of temperature at different concentrations of heptacaine in the sample. X_{am} is the molar ratio between the anesthetic and lipid in the whole sample. The scanning rate was 4 mK/min, the number of moles of DPPC is $N_p = 3.4$ mmol/l for each sample. Only non-overlapping experimental points are shown.

described by Nagle and Wilkinson (1978). The temperatures of phase transitions presented above are in good agreement with the results obtained by other groups (Wilkinson and Nagle 1978; Nagle and Wilkinson 1978; Melchior et al. 1980). It should be pointed out that the phase transitions observed in the present work are sharper than those observed in most DSC experiments. This is likely due to the higher scanning rate used in DSC which causes a broadening of phase transition (Biltonen 1990).

The specific volume of the lipid phase can be exactly computed only in the case when the concentration of the additive is zero. In this case, in agreement with earlier works (Wilkinson and Nagle 1978; Nagle and Wilkinson 1978; Melchior et al. 1980) it is supposed that the specific volume of water was not changed in the neighborhood of the lipid. That is why the correct term for such a result is apparent specific volume. Nevertheless, for simplicity we will use the term specific volume, as do other authors.

The situation is more complicated when the additive incorporating into the membrane is also present in the sample. In the case of heptacaine and DPPC, the difference between the specific volume of heptacaine in water and its volume in DPPC membrane is negligible (Bánó and Pajdalová 1999), and it allows a simple calculation of the specific volume of the lipid according to the formula

$$v_p = \frac{M_p + \delta F_{p,a}/g}{\rho'_a M_p} \tag{7}$$

where M_p is the mass of DPPC in the buoy; $\Delta F_{p,a}$ is the difference in equilibrating force between the sample containing DPPC + heptacaine + water and that containing the heptacaine solution only; g is the gravitation constant; and ρ'_a is the density of the anesthetic solution. Formula (7) is incorrect if there is a significant difference in the specific volume of the additive between water and lipid environment. Nevertheless, the formula can also be used in this case to detect of the begin and the end of phase transition.

The addition of the local anesthetic causes a decrease of the temperatures of both phase transitions and a broadening of their temperature spans (Fig. 3). Both phase transitions are detectable in the presented temperature range at heptacaine concentrations $X_{am} \leq 0.18$. At higher concentrations of heptacaine, the pretransition is not detectable and the main phase transition is shifted toward lower temperatures.

In the following section, we will use relation (6) to estimate of the beginning and the end of phase transition. In Fig. 4, we illustrate our method of data evaluation for $X_{am} > 0$.The value of the specific volume is presented as a function of $1/(T_m - T)$, where the temperature T_m of the main phase transition for DPPC in pure water was determined according to Nagle and Wilkinson (1978). The specific volume is approximated by a linear function in the region of the phase transition as well as below and above it. These linear functions intersect at points B and E indicating the estimated temperatures T_{B} and T_{E} of the beginning (B) and the end (E) of phase transition, respectively.

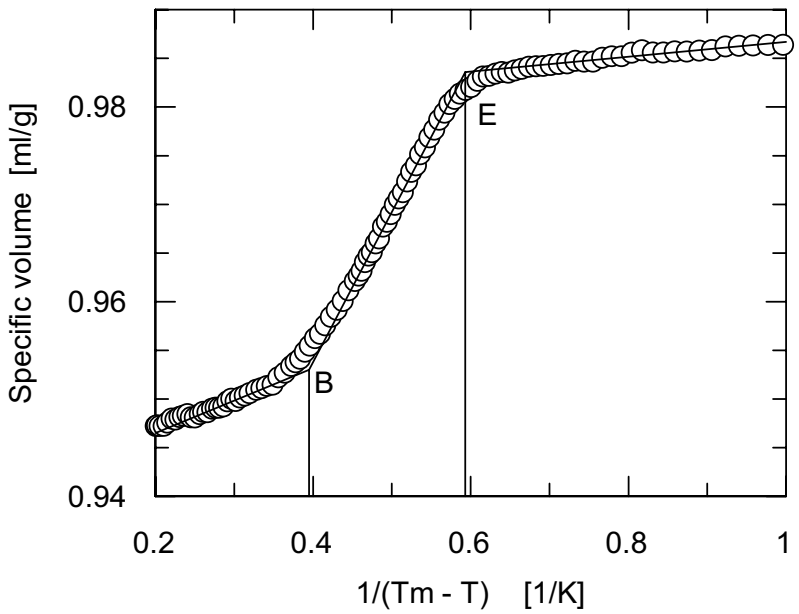

Figure 4. An illustration of the method used for the estimation of the beginning B and the end E of phase transition (for DPPC/heptacaine/water, $X_{am} = 0.18$).

It can be seen that in the neighborhood of B and E the simple model of Suezaki et al. (1990) cannot describe the real course of data, and that, to a certain degree, the position of points B and E is arbitrary. This behavior can be understood from computer simulation of anesthetic/lipid interactions (Jørgensen et al. 1991) which shows that the crossing of solidus and liquidus curves in the phase diagram is not accompanied with any sharp change of the direction of the trajectory as assumed in the model used. Nevertheless, the presented procedure allows to obtain the parameters of phase transition from experiment with relatively small uncertainty.

Using the described method, the shift of temperatures $\Delta T_{\mathrm{B}} = T_m - T_{\mathrm{B}}$ and $\Delta T_{\mathrm{E}} = T_m - T_{\mathrm{E}}$ of the beginning and the end of phase transition was found for each concentration of the anesthetic (see Tab. 1). To obtain the partition coefficients, we further analysed our data in combination with the phase diagram published by Dörfler et al. (1990). This phase diagram is based on DSC measurements of DPPC/heptacaine dispersion in 50 wt % water. It follows from the theoretical analysis based on the paper of Suezaki et al. (1990) that in samples with so small amounts of water nearly all anesthetic molecules are located in the lipid bilayer. Moreover, the analysis performed by Chernik (1995) shows that the phase properties of DPPC bilayer do not depend on the water content if it is higher than 25 wt %. It can be reasonably supposed that neither the structure of the DPPC/heptacaine system depends on the water content if exceeding 50 wt %, and that the Dörfler's

Table 1. Basic experimental results for different concentrations of the local anesthetic. X_{am} is the molar ratio between heptacaine and DPPC in the whole sample, $T_{m,a}$ is the temperature of phase transition, $\Delta T_{\mathrm{B}} = T_m - T_{\mathrm{B}}$ and $\Delta T_{\mathrm{E}} = T_m - T_{\mathrm{E}}$ are the temperature shifts of the beginning and the end of main phase transition, respectively, $K_{p,fw}$ is the partition coefficient of heptacaine distribution between DPPC in fluid state and water, $K_{p,gw}$ is the partition coefficient of heptacaine distribution between DPPC in gel state and water, $K_{p,gf}$ is the partition coefficient of heptacaine distribution between the gel and fluid phases of DPPC.

X_{am}	$T_{m,a}$ [℃]	ΔT_{B} [℃]	ΔT_{E} [℃]	$K_{p,fw}$	$K_{p,gw}$	$K_{p,gf}$
0.18	39.25	2.60	1.69	(1.72±0.74) 10^4	(2.42±0.60) 10^3	0.141±0.061
0.44	37.75	4.23	3.07	(7.29±1.08) 10^3	(1.51±0.22) 10^3	0.207±0.043
0.74	36.75	5.14	4.16	(4.50±0.48) 10^3	(1.06±0.13) 10^3	0.236±0.037
0.83	35.87	6.06	5.00	(4.53±0.45) 10^3	(1.12±0.13) 10^3	0.246±0.037
1.38	34.46	7.30	6.57	(2.96±0.19) 10^3	(7.91±0.54) 10^2	0.267±0.025

phase diagram describes the real content of the anesthetic in the membrane also at a high water excess.

To illustrate our approach, a portion of this phase diagram is presented in Fig. 5 for the temperature region $T > T_m - 7.5$℃. On this phase diagram, we found the points of the beginning (B_i) and the end (E_i) of phase transitions for all anesthetic concentrations used in the present work. The points for the beginning of phase transition are located on the solidus, and they were found according to their temperature T_{B} . The temperature T_{E} allows to find the end of phase transition on liquidus. The temperature of phase transition $T_{m,a}$ (Tab. 1) was calculated according to the formula $T_{m,a} = (T_{\mathrm{B}} + T_{\mathrm{E}})/2$. The trajectories of sample heating in the phase diagram were obtained as suggested by Suezaki et al. (1990). Finally, the concentrations of the anesthetic in the gel and fluid phases were directly obtained from X_a co-ordinates of the beginning and the end of phase transition. Using a simple calculation, also the concentrations of the anesthetic in water as well as the molar fractions were obtained. Partition coefficients are assumed to remain constant over the phase transition. Thus, the value of $K_{p,fw}$ can be simply computed according to (1) at points E_i on the liquidus curve where all the lipid is in the fluid state. Similarly, $K_{p,gw}$ can be computed at points B_i on solidus.

The obtained results (Tab. 1) are in agreement with the theoretical requirement of Kaminoh et al.(1988) and Inoue et al. (1990): if $T_{m,a}$ decreases with the increasing additive concentration, then $K_{p,gw} < K_{p,fw}$.

Discussion

The obtained partition coefficients $K_{p,fw}$were compared with the data published by Balgavý et al. (1992) who studied the interaction of heptacaine and its alky-

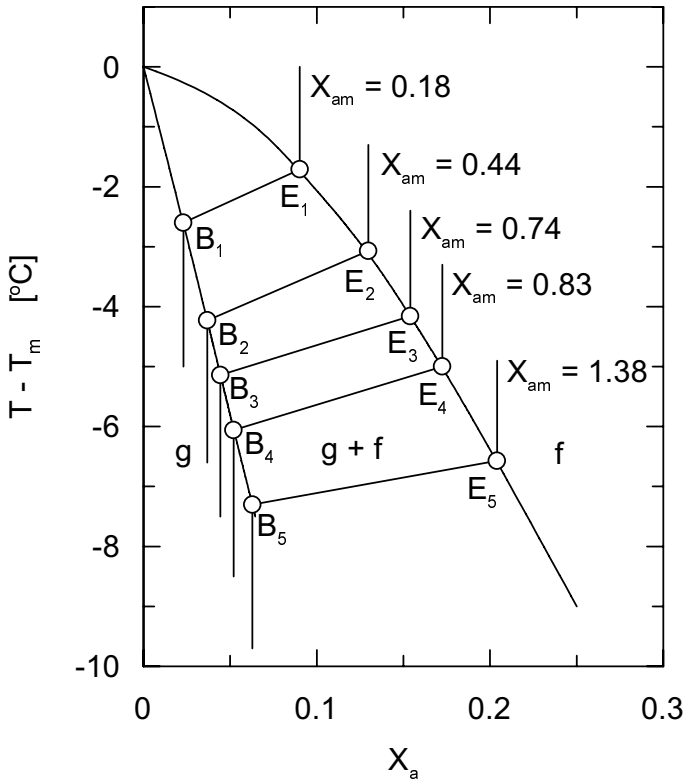

Figure 5. Phase diagram for the pseudo-binary system DPPC/heptacaine in water with the routes of main phase transition for samples with different contents of the anesthetic. X_a is the molar fraction of the anesthetic in the lipid membrane, X_{am} is the molar ratio between the anesthetic and lipid in the whole sample, B_i and E_i are the begin and the end of phase transitions of i-th sample. The liquidus and solidus curves were drawn according to Dörfler et al. (1990).

loxy homologues with unilamellar egg yolk phosphatidylcholine liposomes in fluid state by means of ultraviolet differential spectroscopy. As can be seen in Fig. 6, the value of the partition coefficient published for egg yolk phosphatidylcholine is in satisfactory agreement with our results. The partition coefficients shown in Fig. 6 display a significant decreasing tendency with the increasing anesthetic concentration demonstrating the saturation effect. A similar tendency has been observed by Uhríková et al. (1995) for egg yolk phosphatidylcholine – tetracaine system. These authors suggested that the observed saturation is caused by the influence of the surface potential on the tetracaine binding to the lipid bilayer.

The presented tendency was studied in detail in our earlier paper (Bánó and Pajdalová 1999). We could show that, at low concentrations, the local anesthetic

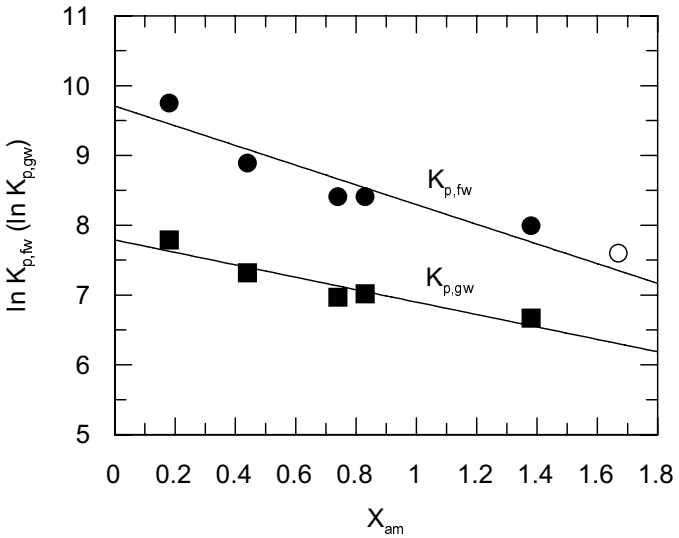

Figure 6. The logarithm of partition coefficients of heptacaine between the fluid ($K_{p,fw}$) or gel ($K_{p,gw}$) phases of DPPC and water as a function of the anesthetic content. X_{am} is the molar ratio between the anesthetic and lipid in the whole sample. The empty circle indicates the result obtained by Balgavý et al. (1992) for the system egg yolk phosphatidylcholine/water in fluid state.

molecules file-in the voids in the structure of the lipid. In this first step, the anesthetic molecules are missing in the defects of the membrane. At higher concentrations, the anesthetic molecules are squeezed into the structure of the membrane. It is accompanied with an expansion of the membrane containing the lipid and anesthetic molecules. However, the volume of the additive molecules in the membrane is equal to that in water.

The observed saturation effect indicates that the absolute value of the binding energy of heptacaine molecule in the membrane decreases with the increasing concentration of the anesthetic in the membrane. The surface potential plays an important role in this effect (Uhríková et al. 1995). Nevertheless, in the light of the above mentioned results also van der Waals potential and the effect of membrane reconstruction should be taken into account.

Finally, the different methods used to estimate partition coefficient were compared with each other. They were divided into three categories. The first one contained methods using separation of water and lipid phases. As already mentioned they are very laborious and the results can be affected by the separation (Lissi et al. 1980, 1990; Welti et al. 1984; Inoue et al. 1990; Babincová and Hianik 1994).

The second group comprised methods measuring additive concentration in water environment. The method of surfactant ion selective electrodes described by Uhríková et al. (1995) is very powerful in cases when the concentration of the

additive in water environment is detectable by the above mentioned method. This approach is simple, it needs no theoretical assumptions, and can also be used in natural biological membranes.

Finally, there are methods that detect the perturbation effect of the additive on the membrane. Our method can be included into this group of methods. Its advantage is that phase transition can be detected by different experimental techniques. They are usually less expensive and less sophisticated than the spectroscopic methods (Šeršeň et al. 1989 or Lissi et al. 1990). Our estimation of partition coefficients using the pseudobinary lipid/additive phase diagram needs no model approach. Only one assumption is necessary: the pseudobinary phase diagram used is independent on lipid concentration in water. This is an important advantage of our method in comparison with the approach of Kaminoh et al. (1988). Moreover, the method of Kaminoh is based on the assumption that the partition coefficient is independent on the concentration of the additive.

We conclude that the present method is more convenient than the very flexible method of Uhríková et al. (1995) in cases when phase transition measurements are made with the aim to obtain further information or when surfactant ion selective electrodes cannot be used.

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