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Lipid Bilayer Thickness and Surface Area in Lamellar Phases of Hydrated Mixtures of Dipalmitoylphosphatidylcholine and Homologs of Local Anesthetic Heptacaine

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The lipid bilayer thickness d_L and the surface area A_L in fluid lyotropic lamellar L_α phases of dipalmitoylphosphatidylcholine (DPPC) containing amphiphilic monohydrochiorides of [2-(alkyloxy)phenyl]-2-(1-piperidinyl)ethyl esters of carbamic acid (C*n*A, n = number of alkyloxy chain carbons) at DPPC:C*n*A = 1:l and H_2O:DPPC· 20:1 molar ratios have been estimated from small angle synchrotron X-ray diffractograms. C*n*As intercalate between DPPC molecules, resulting in a lateral bilayer expansion and in a decrease of d_L. At the reduced temperature $T_r = (T - T_c)/T_c = 0.035$, where T_c is the gel-fluid $L_\beta - L_\alpha$ phase transition temperature, the values of d_L (A_L) equal to 3.289 ± 0.012 nm ($1.060 \pm 0.012\,nm^2$), 3.391 ± 0.011 nm ($1.055 \pm 0.013\,nm^2$), 3.403 ± 0.013 nm ($1.080 \pm 0.012\,nm^2$), 3.418 ± 0.012 nm ($1.090 \pm 0.013\,nm^2$), and 3.730 ± 0.012 nm ($1.025 \pm 0.012\,nm^2$) were obtained for C5A, C7A, C9A, Cl0A, and C12A containing L_α phases, respectively. The increase of d_L with increasing n is caused by the decrease in the difference in DPPC acyl and C*n*A alkyloxy chains lengths. The values of $d_L = 3.733 \pm 0.011$ nm and $A_L = 0.902 \pm 0.012\,nm^2$ obtained for C3A indicate that its location in the L_α phase is different from that of the other C*n*As studied. The nonlinear dependencies of d_L and A_L on n can contribute to C*n*A's biological (local anesthetic, antimicrobial, antiphotosynthetic) effects that display similar dependencies on n.

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Keywords Bilayer thickness, surface area, dipalmitoylphosphatidylcholine, anesthetic, X-ray diffraction

INTRODUCTION

Amphiphilic homologs of heptacaine, monohydrochlorides of tertiary amines [2-(alkyloxy)phenyl]-2-(1-piperidinyl)ethyl esters of carbamic acid (C*n*A, *n* is the number of carbons in the alkyloxy chain) are local anesthetics [1, 2], antimicrobial [3], and antiphotosynthetic [4] agents and inhibitors of the sarcoplasmic reticulum Ca(II)-transporting ATPase [5, 6]. In this homologous series, the biological potencies increase with increasing alkyloxy chain length up to $n = 6$–8, beyond which the potencies decrease (''cut off'' effect). It is believed that these substances act at the biological membrane level. It has been suggested [7–9] that the lateral expansion of phospholipid bilayer of biological membranes caused by the intercalation of amphiphilic heptacaine homologs between the phospholipid molecules and the mismatch between their hydrocarbon chain lengths results in the creation of voids (''free volume'') in the bilayer hydrophobic region. The elimination of the free volume via the hydrocarbon chain *trans-gauche* isomerization and/or interdigitation should result in a change in the bilayer thickness. This change might be responsible for some biological effects of C*n*As. At a constant C*n*A concentration *in the bilayer*, one can expect that the lateral expansion should be the same because the amphiphile polar group is the same in this homologous series. On the other hand, the bilayer thickness change should be smaller when the difference between the C*n*A alkyloxy chain length and lipid acyl chain length is smaller. At a constant C*n*A concentration *in the sample* and very large aqueous phase:lipid volume ratio, the bilayer C*n*A concentration increases exponentially [10–13]. The combination of the partition equilibria and bilayer thickness change should therefore display a cutoff type dependence on the C*n*A alkyloxy chain length.

It has been found that hydrated heptacaine (C7A) and dipalmitoylphosphatidylcholine (DPPC) mixtures form various lyotropic lamellar phases, depending on C7A:DPPC and H_2O:DPPC molar ratios and temperature [14, 15]. The aim of the present paper is to estimate the lipid bilayer thickness and the surface area in fluid lamellar C*n*A + DPPC + H_2O L_a phases as a function of alkyloxy chain length at fixed C*n*A:DPPC = 1:1 and H_2O:DPPC $\approx$ 20:1 molar ratios.

MATERIAL AND METHODS

C*n*As (prepared as described in CižMárik and Borovanský [l6]) were a kind gift of Professor J. Čižmárik, DPPC was purchased from Avanti Polar Lipids (Alabaster, Alabama, USA), and the organic solvents were purchased from Mikrochem (Bratislava, Slovakia). The solvents were redistilled before use. C*n*A + DPPC (molar ratio $n_{CnA} = C_nA{:}DPPC = 1{:}1$) were mixed in chloroform/methanol. Solvent was evaporated under nitrogen gas and evacuated at about 10^{-1} Pa in the presence of P_2O_5 for several hours. Redistilled water was added at the molar ratio of $n_W = H_2O{:}DPPC\cdot$ 20:1, and the precise value of n_W was determined gravimetrically. The C*n*A + DPPC mixtures are fully hydrated at these n_W and n_{CnA} molar ratios [14, 17, 18], but the formation of multilamellar vesicles and the volume of structural defects where water is located [19] and which cause artefacts in the diffraction data evaluation [20–22] is negligible [18]. The C*n*A + DPPC + H_2O mixtures were homogenized in flame-sealed glass tubes by several cycles of freeze-thawing and back-and forth centrifugation at about 50°C. The homogenized mixtures were placed between 25 μm thick mica windows in the 5 mm hole in the center of a 2 cm × 2 cm × 0.8 mm steel plates (sandwich samples). The mica windows were glued to the steel plate by a high vacuum silicon grease (Wacker, Munich, Germany) to prevent the evaporation of water. Before measurements, the sandwich samples were equilibrated at room temperature for several days and then stored at 5–6°C. During measurements, the sandwich was held in a thermostatically controlled sample holder and heated at a scan rate 1°C/min from 5°C up to 50°C. The diffraction data were obtained during this scan every 60 s using the X13 double focusing monochromator-mirror camera of the EMBL Outstation at the Deutsches Elektronen Synchrotron (DESY) in Hamburg on the storage ring DORIS. At this beam line the wavelength selected by a Ge(111) crystal is $\lambda = 0.15$ nm. The experimental setup, data acquisition system, and calibration of reciprocal spacing were described in detail earlier (see [23–25] and references therein). Data were analyzed using the evaluation program OTOKO [26].

RESULTS AND DISCUSSION

In agreement with Dörfler et al. [l4, l5], the small angle (SAX) diffraction patterns of all samples containing C*n*A consisted of Bragg diffraction peaks characteristic of one-dimensional lamellar phases wherein lipid bilayers are separated by water layers. Depending on the C*n*A alkyloxy chain length and

sample history, positions and integral intensities of lamellar peaks displayed 1–3 sudden changes on heating, indicating phase transitions between these lamellar phases as in Dörfler et al. [15]. The sudden change in the positions and integral intensities of SAX peaks observed at the highest temperature was accompanied by a distinct change in the wide-angle region (WAX) diffraction peak. The symmetric peak typical of diffraction on the ordered hexagonal lattice of hydrocarbon chains, mainly in the all-trans conformation and oriented parallel to the lamellar phase director (lamellar solid-like gel phase L_β [15, 27]), transformed into a broad diffraction band characteristic of a lamellar phase wherein the hydrocarbon chains are "melted" and arranged in a quasi-hexagonal disordered lattice (lamellar fluid phase L_α [15, 27]). The L_β -L_α phase transition temperature T_c was determined as a midpoint for the observed changes in the SAX and WAX diffraction patterns in the process of chains melting. The values difference $\Delta T_c = T_c - T_c(DPPC)$, where T_c is the transition temperature in presence of C*n*A and the $T_c(DPPC)$ in its absence, are presented in Figure 1. In agreement with the results of calorimetric experiments [28], the maximum decrease of T_c is observed in the case of C7A.

Using the Bragg equation, the repeat period d of lamellar L_β phase was determined from the reciprocal spacings of SAX diffraction peaks maxima. Since the molecular volumes of H_2O and C*n*A located in the lipid bilayer are within experimental errors that are the same as in the bulk aqueous phase [11, 21, 29, 30], the surface area A_L of DPPC + C*n*A on the lipid bilayer-aqueous phase interface can be simply calculated from the repeat period as

$$A_L = \frac{2(V_{DPPC} + n_{CnA} V_{CnA} + n_W V_W)}{d}, \tag{1}$$

where V_{DPPC}, V_{CnA}, and V_W are the molecular volumes of DPPC, C*n*A, and H_2O, respectively. We have used in the evaluation of our data the molecular volumes calculated from absolute specific volumes or mass densities obtained in the aqueous phase and published in [11, 31–34]. The difference $\Delta A_L = A_L - A_L$(DPPC), where A_L is the surface area in the presence of C*n*A and $A_L(DPPC)$ is the surface area in its absence, was calculated at all temperatures between T_c and 50°C, where the diffractograms were taken in $\sim$1 K intervals. As an example, Figure 1 shows ΔA_L as a function of C*n*A alkyloxy chain length at the reduced absolute temperature $T_r = (T - T_c)/T_c = 0.035$. It is seen that ΔA_L is, within experimental error, approximately the same for C5A, C7A, C9A, and C10A but that it slightly decreases for C12A. The same course of $\Delta A_L = f(n)$ was observed at other reduced

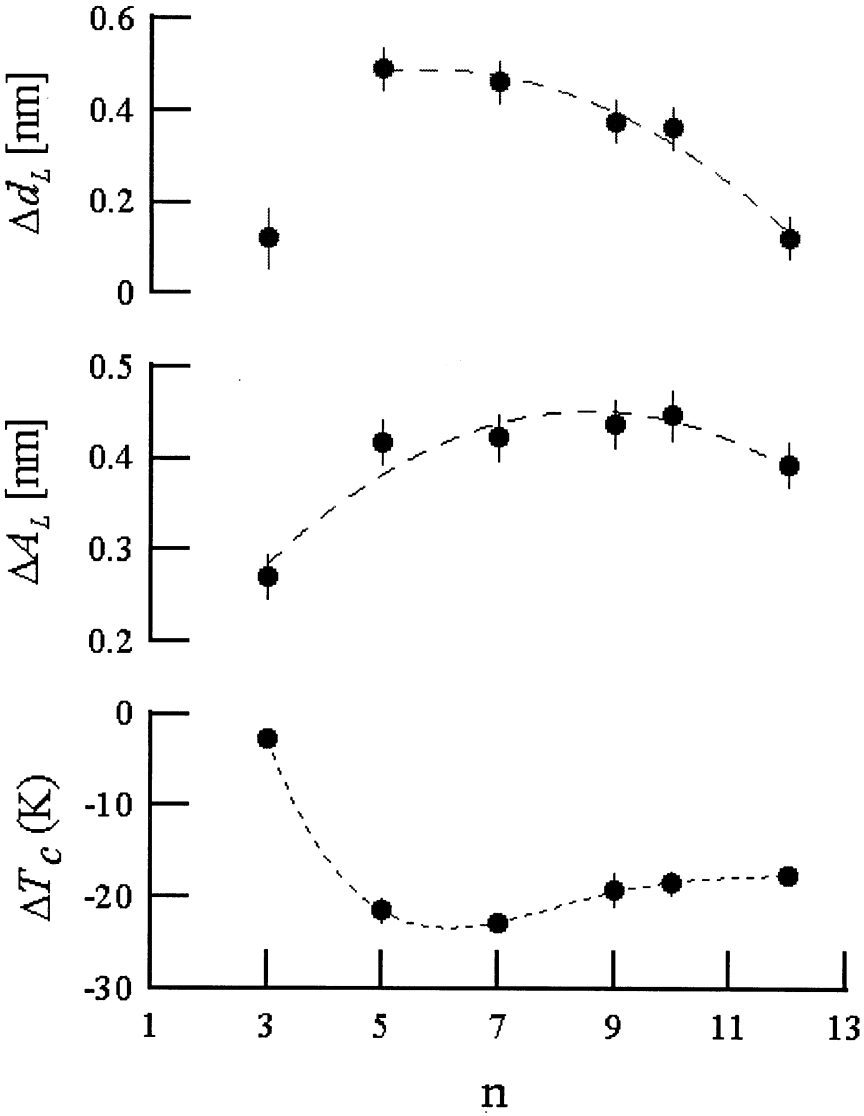

FIGURE 1 Effect of C*n*A on the *L.*-*L.* phase transition temperature, surface area, and bilayer thickness in the DPPC *L.* phase.

absolute temperatures, and the decrease for C12A was even more pronounced when comparing ΔA_L values at equal absolute temperatures T. The small decrease for C12A can be explained by an increased van der Waals attraction between hydrophobic chains in the lipid bilayer, when the

difference between lengths of C*n*A alkyloxy chains and DPPC acyl chain decreases. Notable is the decrease of ΔA_L for C3A that correlates with the small effect of C3A on T_c (see Figure 1). In comparison to the other C*n*A homologs studied in the present paper, the partition coefficient of C3A between aqueous phase and the lipid phase is rather small [10–13], so that one can argue that the C3A concentration in DPPC bilayers could be smaller than in case of other C*n*A homologs, resulting in the observed deviation. However, the volume of the aqueous phase at $n_W \approx 20$ molar ratio is rather small to account for the observed effect. Furthermore, the drop in ΔA_L observed in the present paper correlates with the drop in the probability p_g of *gauche* conformers formation in phosphatidylcholine acyl chains hydrated from the gaseous phase at 81% humidity [9], i.e., when all C3A molecules in the sample are located in the lipid bilayers. The surface area and the concentration of *gauche* conformers in the bilayer are interconnected. For example, a lateral expansion of the phosphatidylcholine bilayers induced by an increased hydration of the polar region results in an increase in p_g in the bilayer hydrophobic core [35]. We would therefore prefer to exclude the low C3A concentration in the bilayer as a possible cause of the observed ΔA_L deviation. More probable is a changed location of short alkyloxy chain C*n*A homologs in the DPPC bilayer in comparison with long alkyloxy chain homologs. While the long chain homologs are anchored in the hydrophobic bilayer core due to their strong attraction with DPPC acyl chains, the short chain homologs are most probably more mobile and can exchange between multiple binding sites in the bilayer, thus minimizing the free energy not only by *trans-gauche* isomerization (and/or interdigitation) of hydrocarbon acyl chains, but also by minimalization of the surface area. Neutron diffraction experiments have shown that the deuterated C1A methyloxy substituent is located at two different positions—1.28 nm and 2.18 nm from the bilayer center in oriented solid-like L_β phase C1A:DPPC = 1:1 bilayers at 97% humidity and 20°C [36]. Two different tertiary amine local anesthetic binding sites in fluid phosphatidylcholine bilayers of the L_α phase at high hydration have been observed by deuterium NMR spectroscopy of specifically deuterated anesthetics tetracaine (4 carbon atoms in linear hydrocarbon substituent on aromate) and procaine (no hydrocarbon substituent on aromate) [37, 38]. It is possible that the second binding site located closer to the bilayer-water interface is populated more frequently by short chain anesthetics and that the surface area is significantly smaller in this case.

The second aim of the present paper is the bilayer thickness estimation. In a model wherein the lipid and water form separate layers, i.e., the water

does not penetrate into the lipid bilayer [27], the distance between bilayers is equal to the aqueous layer, whose thickness is given by

$$d_W = \frac{2(n_W V_W)}{A_L}. \tag{2}$$

The bilayer thickness is given by

$$d_L = d - d_W = \frac{2(n_{CnA} V_{CnA} + V_{DPPC})}{A_L}. \tag{3}$$

The difference $\Delta d_L = d_L(DPPC) - d_L$, where $d_L(DPPC)$ is the bilayer thickness in absence of C*n*A and d_L is the bilayer thickness in its presence, was calculated at all temperatures between T_c and 50°C, where the diffractograms were measured. As an example, Figure 1 shows Δd_L as a function of C*n*A alkyloxy chain length n at the reduced absolute temperature $T_r = (T - T_c)/T_c = 0.035$. It is seen that $\Delta d_L = f(n)$ is a decreasing function for $5 \leqslant n \leqslant 12$. A similar course of $\Delta d_L = f(n)$ function was observed at other reduced temperatures and also when comparing the values of Δd_L at the same absolute temperature. As expected, the value of Δd_L for C3A displays a pronounced deviation from this course. The model used in calculation of d_L by Equation 3 is a simplification of the real situation because some water molecules are located in the bilayer polar region [22]; thus the d_L values obtained are smaller than the real bilayer thickness. However, the dependence on n should give the correct trend of bilayer thickness change if the number of water molecules located in the bilayer polar region is the same in the presence of all C*n*A homologs studied. We found that the DPPC hydration in presence of C4A – C12A was approximately the same within experimental error when the lipid was hydrated from the gaseous phase, while the C2A and C3A homologs displayed a small drop in hydration [9]. When taken into account, this small drop in hydration would slightly increase the Δd_L value for C3A. The free volume model predicts a linear dependence of $\Delta d_L = f(n)$ for a constant number of water molecules located in the bilayer polar region and a constant surface area. A slight deviation from linearity observed in the present paper for $5 \leqslant n \leqslant 12$ is thus caused by the fact that these physical parameters are not constant.

Local anesthetics block the nerve impulse resulting from an action potential. The initiation and propagation of action potentials in the nerve is the result of the combined action of changes in the permeability of the plasma membrane to sodium and potassium ions. Anesthetic effects on the action potential can be a consequence of direct binding of the anesthetic molecule to a voltage gated ion

channel (sodium or potassium) and/or of indirected effects on the channel resulting from changes in bilayer physical properties. The sequencing of the sodium channel protein and subsequent mutagenesis studies identified amino acid residues in the transmembrane IIIS6 and IVS6 α-helical segments that form binding site(s) for some tertiary amine local anesthetics (see [39] and references therein). If the anesthesia were caused by the anesthetic binding to such site(s), the role of the lipid bilayer would be rather passive. On the other hand, if changes in physical properties contribute to anesthesia, then the different locations of the C*n*A molecules with short and long alkyloxy substituents in the bilayer could influence their diffusion to the binding site(s) and thus contribute to the cut off effect. Changes in membrane thickness may also be of importance in determining the behavior of the voltage gated ion channels. Hendry et al. [40] have found that the short-chain phospholipids diheptanoylphosphatidylcholine and dioctanoylphosphatidylcholine reduced the maximum inward sodium current in voltage-clamped squid giant axons. It is highly improbable that the phosphatidylcholines would bind to some specific site(s) on the sodium channel. As expected, the diheptanoylphosphatidylcholine molecules intercalate between long chain phospholipids in the bilayer like amphiphilic anesthetics, perturb its structure [41], and reduce its thickness (Balgavý, unpublished). Mateu et al. [42] and Luzzati et al. [43] have reported that the changes in conduction velocity and in the maximum amplitude of the compound action potential induced by tertiary amine local anesthetics in toad sciatic nerves correlate with the changes in the nerve myelin membrane thickness observed simultaneously by X-ray diffraction. They have suggested that similar structural changes should occur also in the plasma membrane of the Ranvier nodes. Since the final bilayer thickness change depends on the C*n*A anesthetic location in the bilayer, this could contribute to the C*n*A cut off effect in local anesthesia.

The cut off effect has been observed not only in local anesthesia but also in other biological potencies of the C*n*A homologous series (see the Introduction of this article). For example, at the constant 41 μmol/l C*n*A concentration, the inhibition of the purified sarcoplasmic reticulum Ca(II)-transporting ATPase activity was maximal for C6A homolog and lower for shorter and longer homologs [5]. The Ca(II)-transporting ATPase is a transmembrane protein that couples hydrolysis of one molecule of ATP to the transport of two Ca(II) ions across the membrane. When reconstituted into unilamellar liposomes, its activity depends on the lipid bilayer phase state, lipid bilayer thickness, and charge of polar headgroups of annular lipids surrounding the protein. For highest activity, a fluid liquid crystalline lipid bilayer of the appropriate thickness with lipid zwitterionic headgroups

is required [44]. The activity in diacylphosphatidylcholine liposomes is highest in the fluid bilayer of dioleoylphosphatidylcholine, but it is lower in fluid bilayers with shorter or longer monounsaturated acyl chains, i.e., in thinner and thicker bilayers [44–47]. This protein has been reconstituted in fluid egg yolk phosphatidylcholine (EYPC) bilayers with the mean number of acyl chain carbons 17.8 and acyl chain double bonds 1.2. In this reconstituted protein, the 50% inhibition of activity occured at C6A:EYPC $\approx$ 0.4:1 molar ratio, and the 100% inhibition at C6A:EYPC $\approx$ 1.2:1 molar ratio [5, 6]. Similar to DPPC, C*n*A anesthetics decreased the thickness of EYPC bilayers [7] and the bilayer thickness and other bilayer physical changes occured at the same molar ratios as in the reconstituted protein activity tests [18, 48]. The seemingly large difference between the low C*n*A concentration needed to inhibit the purified protein (41 μmol/l) and the high C6A:EYPC molar ratio inhibiting the reconstituted protein (1.2:1) at the same protein concentration could be simply explained by taking into account partition equilibria of C*n*A molecules between lipid bilayers and aqueous phase—in the reconstituted protein the volume of the lipid phase was about 1000 times larger than in the purified protein [6, 10, 12, 13]. However, the inhibition of the protein activity was caused not only by the bilayer thickness change but also by the direct interaction of C*n*A molecules with the protein annular binding sites at the bilayer-protein interface, where the phospholipid molecules were displaced by C*n*A [5, 6]. In this interaction, the different locations of the C*n*A molecules with short and long alkyloxy substituents in the bilayer could contribute to the cut off effect too.

The cut off effect in biological potencies of amphiphiles with linear hydrocarbon substituents can be caused by several different mechanisms—e.g., by diffusion to the site of action through a series of hydrophilic and hydrophobic compartments, by structural perturbation of target biological membrane constituents (lipids and/or proteins and/or their interfaces), by limited aqueous solubilities of long chain homologs, etc. (see [8] for references). The results obtained in the present paper suggest that the physical interaction of amphiphiles with biological membrane could contribute to the cut off effect via a modulation of membrane thickness and lateral packing of its constituents, and by the amphiphile location in the bilayer and in the bilayer-protein interface.

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REFERENCES

1. J. Čižmárik, A. Borovanský, and P. Švec, *Acta Facult. Pharm. Univ Comenianae*, 29, 53–99 (1916).
2. E. Račanská, P. Švec, and V. Račanský, *Pharmazie*, 45, 684–685 (1990).
3. D. Mlynarčík, J. Bittererová, J. Čižmárik, and L'. Masárová, *Československ. Farm.*, 40, 25–28 (1991).
4. K. Kráľová, F. Šeršeň, and J. Čižmárik, *Gen. Physical Biophys.*, 11, 261–267 (1992).
5. F. Andriamainty, J. Filípek, P. Kovács, and P. Balgavý, *Pharmazie*, 51, 242–245 (1996).
6. F. Andriamainty, Effect of hydrophobic and amphiphilic molecules on the properties of sarcoplasmic reticulum (Ca-Mg)ATPase, *PhD. Thesis* (J. A. Comenius University, Bratislava, l996).
7. D. Uhríková, V. Cherezov, S. Yaradaikin, and P. Balgavý, *Pharmazie*, 48, 446–450 (1993).
8. P. Balgavý and F. Devínsky, *Adv. Colloid Interface Sci.*, 66, 23–63 (1996).
9. J. Gallová, F. Andriamainty, D. Uhríková, and P. Balgavý, *Biochim. Biophys. Acta*, 1325, l89–196 (1997).
10. P. Balgavý, I. Benedikovič, B. Kopecká, and J Gallová, *Gen. Physiol Biophys.*, 11, 269–272 (1992).
11. O. Pajdalová, *Magister Thesis*, (P. J. Šafárik University, Košice, Poland, 1994).
12. M. Hammel, Spectrophotometric estimation of partition coefficients of local anesthetics between phospholipid liposomes and aqueous phase, *Magister Thesis*, (J. A. Comenius University, Bratislava, l998).
13. F. Andriamainty, J. Čižmárik, D. Uhríková, and P. Balgavý, in *Advances in Medical Physics, Biophysics and Biomaterials*, E. Kukurová, ed. (Malé Centrum, Bratislava, 1997) pp. 32–35.
14. H. D. Dörfler, G. Brezesinski, and H. Jantschke, *Liq. Cryst.*, 8, 263–277 (1990).
15. H. D. Dörfler, G. Förster, and H. Jantschke, *Liq. Cryst.*, 8, 279–297 (1990).
16. J. Čižmárik and A. Borovanský, *Chem. Zvesti*, 29, 119–123 (1975).
17. J. Národa, P. Balgavý, K. Gawrisch, and J. Čižmárik, *Gen. Physiol Biophys.*, 2, 457–471 (1983)
18. D. Uhríková, Structural changes in lipid bilayers – effects of hydration, temperature and amphiphilic impurities, *PhD. Thesis*, (J. A. Comenius University, Bratislava, l993).
19. K. Gawrisch, W. Richter, A. Möpps, P. Balgavý, K. Arnold, and G. Klose, *Stud. Biophys.*, 108, 5–16 (1985).
20. G. Klose, B. König, H. W. Meyer, G. Schulze, and G. Degovics, *Chem. Phys. Lipids*, 47, 225–234 (1988).
21. P. Balgavý, M. Dubničková, D. Uhrčková, S. Yaradaikin, M. Kiselev, and V. Gordeliy, *Acta Phys. Slov.*, 48, 509–533 (1998).
22. J. F. Nagle and S. Tristram-Nagle, *Biochim. Biophys. Acta*, 1469, 159–195 (2000).
23. M. Rappolt and G. Rapp, *Eur. Biophys. J.*, 24, 381–386 (1996).
24. F. Richter, L. Finegold, and G. Rapp, *Phys. Rev. E*, 59, 3483–3491 (1999).
25. J. M. Holopainen, J. Lemmich, F. Richter, O. G. Mouritsen, G. Rapp, and P. K. J. Kinnunen, *Biophys. J.*, 78, 2459–2469 (2000).
26. C. Boulin, R. Kempf, M. H. J. Koch, and S. M. McLaughlin, *Nucl. Instrum. Methods Phys. Res., Sect. A*, 249, 399–407 (1986).
27. V. Luzzati, in *Biological Membranes*, D. Chapman ed. (Academic Press, London, 1968) pp. 71–124.
28. J. Gallová, J. Bágeľová, J. Čižmárik, and P. Balgavý, *Collect. Czech. Chem. Commun.*, 60, 763–780 (1995).

29. M. C. Wiener, S. Tristram-Nagle, D. A. Wilkinson, L. E. Campbell, and J. F. Nagle, *Biochim. Biophys. Acta*, 938, 135–142 (1988).
30. M. Bánó and O. Pajdalová, *Biophys. Chem*., 80, 53–66 (1999).
31. Weast, R.C., ed. *Handbook of Chemistry and Physics*, 50th ed., (The Chemical Rubber Co., Cleveland, 1969),
32. J. F. Nagle and D. A. Wilkinson, *Biophys. J*., 23, 159–175 (1978).
33. M. Bánó and P. Balgavý, *Pharmazie*, 51, 512–513 (1996).
34. S. C. Costigan, P. J. Booth, and R. H. Templer, *Biochim. Biophys. Acta*, 1468, 41–54 (2000).
35. J. Gallová, F. Andriamainty, and P. Balgavý, *Acta Phys. Slov*., 45, 193–204 (1995).
36. P. Balgavý, V. I. Gordeliy, and A. G. Syrykh, *JINR Communication*, 14-91-387, 1–14 (1991).
37. Y. Boulanger, S. Schreier, L. C. Leitch, and I. C. Smith, *Can. J. Biochem*, 58, 986–995 (1980).
38. E. de Paula and S. Schreier, *Braz. J. Med. Biol. Res*., 29, 877–894 (1996).
39. V. Yarov-Yarovoy, J. Brown, E. Sharp, J. J. Clare, T. Scheuer, and W. A. Catterall, *J. Biol. Chem*., 276, 20–27 (2001).
40. B. M. Hendry, J. R. Elliott, and D. A. Haydon, *Biophys. J*., 47, 841–845 (1985).
41. K. Ondriaš and A. Staško, *Chem.-Biol. Interact*., 84, 143–151 (1992).
42. L. Mateu, O. Moran, R. Padron, M. Borgo, E. Vonasek, G. Marquez, and V. Luzzati, *Biophys. J*., 72, 2581–2587 (1997).
43. V. Luzzati, L. Mateu, G. Marquez, and M. Borgo, *J. Mol. Biol*., 286, 1389–1402 (1999).
44. A. G. Lee, *Biochim. Biophys. Acta*, 1376, 381–390 (1998).
45. A. Johannsson, C. A. Keightley, G. A. Smith, C. D. Richards, T. R. Hesketh, and J. C. Metcalfe, *J. Biol. Chem*., 256, 1643–1650 (1981).
46. M. Caffrey and G. W. Feigenson, *Biochemistry*, 20, 1949–1961 (1981).
47. A. G. Lee, J. M. East, and P. Balgavý, *Pesticide Sci*., 32, 317–327 (1991).
48. J. Gallovâ, *PhD. Thesis*, (J. A. Comenius University, Bratislava, 1993).