

## EXCIMER FLUORESCENCE PROBE AND X-RAY DIFFRACTION STUDY OF DECANE INTERACTION WITH EGG YOLK PHOSPHATIDYLCHOLINE BILAYERS

<sup>1</sup>Balgavý, P. – <sup>1</sup>Uhriková, D., – <sup>1</sup>Gallová, J., – <sup>2</sup>Kučerka, N. – <sup>3</sup>Madarászová, I. –  
<sup>4</sup>Islamov, A. – <sup>4,5,6</sup>Gordeliy, V.

<sup>1</sup>Department of Physical Chemistry of Drugs, Faculty of Pharmacy, Comenius  
University, Bratislava, Slovakia

<sup>2</sup>Department of Chemical Theory of Drugs, Faculty of Pharmacy, Comenius University,  
Bratislava, Slovakia

<sup>3</sup>Petrafarm Pharmacy, Pezinok, Slovakia

<sup>4</sup>Frank Laboratory of Neutron Physics, JINR, Dubna, Russia

<sup>5</sup>Institute of Structural Biology, Research Center Jülich, Jülich, Germany

<sup>6</sup>Center of Biophysics and Physical Chemistry of Supramolecular Structures, Moscow  
Institute for Physics and Technology, Dolgoprudny, Russia

The influence of *n*-decane on the lipid bilayer thickness and on the frequency of lateral collisions of the excimer fluorescence probe 1-pyrenedodecanoic acid was studied in unilamellar and multilamellar egg yolk phosphatidylcholine (EYPC) model membrane systems. In multilamellar systems, the *n*-decane molecules are located predominantly in the bilayer center between apposing monolayers. Up to *n*-decane:EYPC=1:1 molar ratio studied, this location brings about a significant increase in the bilayer thickness, but the frequency of the excimer probe collisions is not influenced. The collision frequency decreases significantly in unilamellar liposomes up to *n*-decane:EYPC=0.4:1 molar ratio. The bilayer thickness does not change up to this molar ratio, the *n*-decane molecules are located thus in the bilayer parallel to the lipid acyl chains. At higher molar ratios, *n*-decane slightly increases the bilayer thickness in unilamellar liposomes without having any effect on the excimer probe collision frequency. This suggests location in the center of bilayer like in the multilamellar systems. The different curvature radii can cause differences in the *n*-decane location in multi- and unilamellar systems. Possible consequences for *n*-decane biological effects are discussed.

## INTRODUCTION

Normal alkanes have several pharmacological effects, which are believed to result from their interactions with hydrophobic target sites in biological membranes. The textbook example is their general anesthetic effect. The traditional view has been that the primary target sites of *n*-alkanes are hydrophobic regions of lipid bilayers in nerve membranes. It is well known that a sudden drop in the anesthetic potency of *n*-alkanes occurs at chain length of 11-12 carbon atoms („cut-off effect“ [1]. It has been suggested that short chain *n*-alkanes (6-10 carbon atoms) given rise to thicker lipid bilayers than long chain *n*-alkanes (12-16 carbon atoms), and this has been correlated with their anesthetic potency and the cut-off effect [2-5]. However, the anesthetic potency and the cut-off effect could result from binding of *n*-alkanes at hydrophobic sites in target proteins in central nervous system, e.g. in fast neurotransmitter-gated receptor channels present at central synapses [6]. Similarly, a modulation of activity of transmembrane sarcoplasmic reticulum Ca-transporting ATPase protein by *n*-alkanes, when reconstituted in phospholipids of defined acyl chain lengths, has been ascribed to their effect on the bilayer thickness  $d_L$  [7]. However, the direct experiments have shown that *n*-decane and its brominated derivatives bind to the ATPase hydrophobic sites [8].

The estimation of the lipid bilayer thickness  $d_L$  in the presence of *n*-alkanes is thus of primary importance for the molecular interpretation of their pharmacological properties and of their effects on membrane proteins. Are these effects direct or indirect, i.e. do the *n*-alkanes interact directly with proteins without affecting the bilayer thickness, or do the *n*-alkanes change the bilayer thickness of the bilayer and secondarily the protein structure? The experiments aimed at solving these questions have been done by several groups of authors. Using X-ray diffraction, McIntosh et al. [9] have observed that short chain *n*-alkanes (6-8 carbons) increased the repeat period  $d$  and the phosphate-phosphate transbilayer distance  $d_{pp}$  in multilamellar phases of dipalmitoylphosphatidylcholine (DPPC), dilauroyl-phosphatidylcholine (DLPC) and egg yolk phosphatidylcholine (EYPC), while the long chain homologues (14-16 carbons) had a very small or no effect upon  $d$  and  $d_{pp}$ . These results imply that long alkanes are primarily located between adjacent lipid hydrocarbon chains in each monolayer of the bilayer (no effect on the bilayer thickness), while short alkanes can partition into the geometric center of the bilayer between apposing monolayers (an increase in the bilayer thickness). On the other hand, Pope et al. [10] have seen practically no effect of *n*-hexane, *n*-octane and *n*-dodecane upon the repeat period  $d$  of the dimyristoylphosphatidylcholine (DMPC) multilamellar phase. In oriented dioleoylphosphatidylcholine (DOPC) lamellar phase at low hydration, the bilayer thickness  $d_L$  remained unchanged up to 0.32 molar ratio of *n*-hexane:DOPC in the lipid phase while an increase of  $d_L$  occurred at higher molar ratios [11]. Finally, we have found in our recent paper by using small-angle neutron scattering on unilamellar DOPC liposomes, that the bilayer thickness parameter  $d_g$  is within the experimental error constant up to the *n*-decane:DOPC=0.4 molar ratio, and then increases by  $0.24 \pm 0.13$  nm up to *n*-decane: DOPC=1.2 molar ratio [12]. It is evident from this short review of relevant experimental results that the effect of *n*-alkanes on the lipid bilayer thickness

depends not only on the *n*-alkane length and *n*-alkane:lipid molar ratio, but most probably also on the model system used. It is not excluded that the location of *n*-alkanes in the lipid bilayer could be different in multilamellar systems and in unilamellar liposomes at full lipid hydration. In the present paper we study the location of *n*-decane in lipid bilayers using excimer fluorescence probe both in multilamellar and unilamellar EYPC liposomes and X-ray diffraction on the lamellar fluid EYPC phase.

## MATERIALS AND METHODS

### Chemicals

Phosphatidylcholine from hen egg yolks (EYPC) was prepared, purified and analyzed by a thin layer chromatography according to Singleton et al. [13]. Its molecular weight of 779.7 g/mol was calculated from the chemical composition of its acyl chains [14]. The Silufol chromatographic plates were from Kavalier (Sázava, Czech Republic), *n*-decane (C10) from Merck (Darmstadt, Germany), sodium cholate from Sigma (St. Louis, USA), 1-pyrenedodecanoic acid (12PY) from Molecular Probes (Leiden, Netherlands), and the organic solvents from Mikrochem (Bratislava, Slovakia). The other chemicals were purchased from Lachema (Brno, Czech Republic). The commercial chemicals were of analytical purity. Water and the organic solvents were redistilled before use.

### Sample preparation

**Lamellar phase:** To the dry EYPC in a glass tube, liquid *n*-decane was added carefully using a Hamilton microsyringe. The tube was flame-sealed and the content was homogenized by several cycles of forth-and-back centrifugation. The tube was opened and a part of homogenized mixture transferred to another glass tube. The redistilled water was then added to this mixture, the tube was sealed and its content homogenized as above. The composition of the mixture was controlled gravimetrically.

**Multilamellar liposomes:** EYPC, *n*-decane and 12PY were mixed in chloroform-methanol (1:1) at molar ratios given below. The solvents were evaporated to dryness under a stream of pure gaseous nitrogen. To the dry mixture, redistilled water was added. The glass tube with this EYPC+*n*-decane dispersion was purged with pure gaseous nitrogen and sealed with Parafilm M (American National Can, Greenwich, USA). Multilamellar liposomes were prepared from this dispersion by vortexing and sonication in the Tesla UC 405 BJ-1 bath sonicator (Vráble, Slovakia).

**Unilamellar liposomes:** The mixture of EYPC, *n*-decane and 12PY in chloroform-methanol was evaporated and dried as above. This dry mixture was solubilized by the aqueous micellar solution of sodium cholate (47.1 mmol/l) at the cholate:EYPC=4.37:1 molar ratio. This micellar solution was then diluted by redistilled water in two steps to reach a final cholate concentration of 0.2524 mmol/l. Upon such

dilution, the molecules of cholic acid partition into the aqueous phase and unilamellar liposomes are formed [15].

### Fluorescence measurements

The fluorescence spectra were measured using the Luminiscence LS 50B spectrofluorimeter (Perkin Elmer, Boston, USA) and quartz 1 cm spectrofluorometric cuvettes (Hellma, Müllheim, Germany). If not otherwise stated, the excitation wavelength was set to 342 nm, the 12PY monomer emission was measured at 396 nm, and the excimer emission at 480 nm. The excitation and emission slits were 2.5 nm. The cuvettes were kept in a thermostated holder and the temperature was controlled to an accuracy of  $25.0 \pm 0.2$  °C.

### X-ray diffraction

The small-angle X-ray diffraction (SAXD) data on lamellar phase and/or multilamellar liposomes were obtained using the small angle X-ray diffraction camera of the DRON-4-07 diffractometer (LNPO Burevestnik, St. Peterbourg, Russia) with Ni filtered  $\text{CuK}\alpha$  radiation (0.154 nm) from a conventional X-ray generator, Bragg-Brentano focusing, SDN.03-02 NaI scintillation detector, sample to detector distance of 20 cm, and  $\theta$ - $2\theta$  scan with a  $2\theta$  step of  $0.01^\circ$ , as described in detail in [16]. The glass tube with the sample was opened, and the sample quickly transferred on the glass slide. Then the sample was pressed between the glass slide and a Mylar foil and mounted directly in the diffraction camera. The diffractograms were measured at  $20^\circ\text{C}$ . The reciprocal spacing was calibrated using the silver behenate standard [17]. From the reciprocal spacings of diffraction maxima, the repeat period  $d$  was calculated using the Bragg equation. From the repeat period, the surface area per one EYPC molecule at the bilayer – aqueous phase interface was calculated using equations

$$A_L = 2(V_L + n'V_D + nV_W) / d \quad (1)$$

$$V_i = M_i / (\rho_i N_A) \quad (2)$$

where  $n$  and  $n'$  are the molar ratios of  $\text{H}_2\text{O}:\text{EYPC}$  and  $n\text{-decane}:\text{EYPC}$ , respectively,  $M_i$  are the molar weights,  $V_i$  are the molecular volumes,  $N_A$  is the Avogadro number, and indices  $i=L, D$  and  $W$  denote EYPC,  $n$ -decane and water, respectively. The mass densities of EYPC  $\rho_L=1,018 \text{ g/cm}^3$  [18], of  $n$ -decane  $\rho_D=0.73 \text{ g/cm}^3$  and of  $\text{H}_2\text{O}=0.998 \text{ g/cm}^3$  [19] were used. It is supposed that these molecular volumes are additive in the lamellar phase. From the surface area, the bilayer thickness  $d_L$  was obtained using the model of separated aqueous and lipid layers [20]

$$d_L = d - (nV_W / A_L) \quad (3)$$

It is supposed that water molecules do not penetrate into the phospholipid bilayer in this model.

## RESULTS AND DISCUSSION

The fluorescence spectrum of the 12PY probe incorporated into EYPC bilayers of unilamellar liposomes is shown in Fig. 1. The structured monomer emission with maxima at 376.9 nm, 396.5 nm and 415.4 nm is seen in the region of 360-430 nm and the broad excimer emission in the region from 430 nm to 580 nm. The ratio of the excimer to monomer quantum yield  $\Phi_{exc}/\Phi_{mon}$  is given by

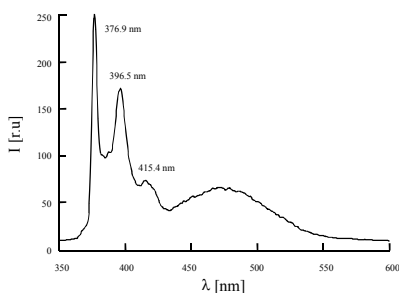
$$\Phi_{exc}/\Phi_{mon} = ck_a \tau k_{exc}/k_{mon} \quad (4)$$

where  $c$  is the 12PY probe concentration,  $ck_a$  is the association constant of the ground state and excited state 12PY monomers,  $\tau$  is the excimer lifetime, and  $k_{mon}/k_{exc}=0.14$  is the constant ratio of the transition probabilities for the radiative decay of the excited excimer and monomer [21]. When the ratio of excimer and monomer fluorescence intensities is used, the eqn. 4 can be rewritten as

$$I_{exc}/I_{mon} \sim k v_{coll} \tau \quad (5)$$

where  $v_{coll}=ck_a$  is the collision frequency of the 12PY probes and  $k$  is a constant [21]. Since the excimer lifetime  $\tau$  is independent (within experimental error) of the unsaturation and length of acyl chains in phosphatidylcholine bilayers [21], changes in the ratio  $I_{exc}/I_{mon}$  indicate changes in the 12PY probe collision frequency.

We have studied the  $I_{exc}/I_{mon}$  ratio as a function of the 12PY:EYPC molar ratio in the range of 12PY:EYPC=0.0034-0.034 at different EYPC concentrations in the sample both for unilamellar and multilamellar liposomes. We have found that the  $I_{exc}/I_{mon}$  ratio



**Fig. 1. Fluorescence spectrum of the 12PY probe (2.45  $\mu\text{mol/l}$ ) incorporated into EYPC unilamellar liposomes at the molar ratio of 12PY:EYPC=0.034:1. The scan rate was 120 nm/min.**

is a linear function of the 12PY:EYPC molar ratio as expected from eqns. 4 and 5 and that there is no deviation from this linear function for different EYPC concentrations (0.15 mmol/l – 1.5 mmol/l) in the sample (not shown). These data indicate that there is no significant effect of the partition equilibrium of the 12PY probe between aqueous and lipid phases on the  $I_{exc}/I_{mon}$  ratio, i.e. that practically all probe molecules are incorporated into the bilayers. In the bilayer, the polar 12PY fragment is anchored in the EYPC polar headgroups region and the 12PY hydrocarbon chain with the fluorescent fragment extends into the bilayer hydrophobic core. The  $I_{exc}/I_{mon}$  ratio provides thus information on the 12PY probes collisions due to their lateral motion parallel to the bilayer surface [21].

The solubility of *n*-decane in the aqueous phase is very low. There are different possibilities how to deliver it into the lipid phase, e.g. a) by adding it to the preformed liposomes in methanol solution, b) by adding it to the dried lipid directly, or c) by mixing the lipid and *n*-decane in the chloroform-methanol solution and removing the organic solvents. In the procedures b) and c) the dry lipid+*n*-decane mixture is hydrated to prepare the lamellar phase or multilamellar liposomes, or this mixture is solubilized in the cholate micellar solution to prepare unilamellar liposomes by dilution of the micellar phase.

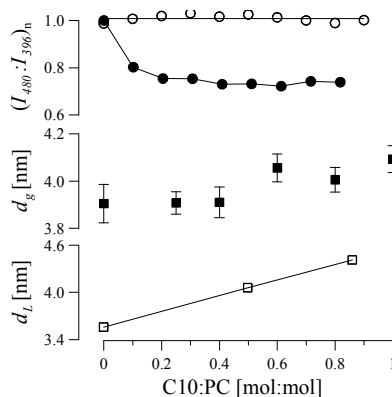
The results of experiments where the procedure a) was used for sample preparation are summarized in the Table 1. In these experiments, the  $I_{exc}/I_{mon}$  ratio for the 12PY probe in unilamellar EYPC liposomes (at 12PY:EYPC=0.034 molar ratio) without

**Table 1. Effect of *n*-decane on the  $I_{exc}/I_{mon}$  ratio in unilamellar EYPC liposomes. Column EYPC – the data measured immediately before the *n*-decane addition into the sample, column EYPC+C10 - the data measured immediately after the addition of *n*-decane in methanol solution into the sample at the *n*-decane:EYPC molar ratio indicated in the column C10:EYPC. The concentration of the 12PY probe in samples was 1.2  $\mu$ mol/l at 12PY:EYPC=0.034 molar ratio.**

Sample	EYPC $I_{480}:I_{396}$	EYPC+C10 $I_{480}:I_{396}$	C10:EYPC [mol:mol]
0	0.21	0.31	0
1	0.21	0.30	0.1
2	0.22	0.29	0.2
3	0.24	0.30	0.3
4	0.22	0.29	0.4
5	0.20	0.26	0.5
6	0.19	0.25	0.6
7	0.19	0.22	0.7
8	0.19	0.21	0.8
9	0.20	0.20	0.9
10	0.21	0.20	1.0

*n*-decane added was measured first (column EYPC). The mean value of  $I_{exc}/I_{mon}$  ratio and the standard deviation of it were  $0.207 \pm 0.016$ . To the control sample 0, a small amount of methanol without *n*-decane was added at the final 1.65% methanol concentration in the sample. When comparing the data obtained (second row in the Table 1), it is seen that the  $I_{exc}/I_{mon}$  ratio was increased by more than 47%, i.e. methanol increased the collision frequency of the 12PY probes in EYPC bilayers. When keeping the methanol concentration in all samples constant and increasing the *n*-decane concentration (samples 1-10), two different *n*-decane:EYPC molar ratio regions were seen wherein the effect of *n*-decane was different (see column EYPC+C10 in Table 1). In the region of  $0.1 \leq n\text{-decane:EYPC} \leq 0.4$ , the values of  $I_{exc}/I_{mon}$  ratio were changing within the experimental error only. At  $n\text{-decane:EYPC} > 0.4$ , the value of  $I_{exc}/I_{mon}$  ratio decreased continuously with the increase of *n*-decane, reaching the value in the control samples without methanol at highest *n*-decane concentrations. The complicating factor in the experiments described was the presence of methanol in samples. During the methanol evaporation, *n*-decane microemulsion may form in the aqueous phase. In such a case, *n*-decane would not be dissolved totally in the lipid bilayer. We have also observed a continuous change in the 12PY fluorescence intensity within 15 min after methanol solution of *n*-decane to the sample (not shown) indicating that the samples were not equilibrated fully during this time interval. The data shown in Table 1 (column EYPC+C10) were obtained immediately after the *n*-decane addition to mimic the experimental conditions under which the *n*-decane effect on the activity of transmembrane sarcoplasmic reticulum Ca-transporting ATPase reconstituted in phosphatidylcholine liposomes was studied [8]. When supposing that all *n*-decane molecules are incorporated into lipid bilayers, one can conclude from the results in Table 1 that up to  $n\text{-decane:EYPC} = 0.4$  molar ratio the *n*-decane is located primarily in the bilayer center between apposing monolayers. At this location, the *n*-decane would increase the bilayer thickness, while its effect on the 12PY probe collision frequency should be rather small. The decrease of  $I_{exc}/I_{mon}$  ratio at molar ratios  $n\text{-decane:EYPC} > 0.4$  could indicate a threshold value for the insertion of *n*-decane molecules between the EYPC acyl chains; in case of parallel orientation of *n*-decane molecules with acyl chains, the distance between the probe molecules should increase resulting in the decreased probe collision frequency. However, the samples measured were clearly in a nonequilibrium state and the microemulsion formation could not be excluded. The threshold value of  $n\text{-decane:EYPC} = 0.4$  molar ratio could thus also indicate beginning of the penetration of the *n*-decane into the bilayer.

We have studied also the effect of *n*-decane addition in the methanol solution on the  $I_{exc}/I_{mon}$  ratio in multilamellar liposomes. We have observed no effect of *n*-decane in this case up to  $n\text{-decane:EYPC} = 1:1$  molar ratio (not shown). Beside the possibility of microemulsion formation, important could be also a slow diffusion of *n*-decane through series of lipid bilayers in this case. To avoid possible problems with microemulsion formation and/or slow diffusion through bilayers in multilamellar systems, we have studied the samples prepared by other methods. First, multilamellar liposomes were prepared by using the procedure b) -mixing of EYPC, *n*-decane and 12PY in organic solvents and hydrating the waxy dry mixture after removing of organic solvents was



**Fig. 2.** Effect of *n*-decane on the normalized  $(I_{exc}/I_{mon})_n$  ratio in multilamellar ( $\circ$ ) and unilamellar ( $\bullet$ ) liposomes, and on the lipid bilayer thickness in the lamellar EYPC phase ( $d_L$ ,  $\square$ ) and in unilamellar DOPC liposomes ( $d_g$ ,  $\blacksquare$ ). The 12PY probe (1.965  $\mu\text{mol/l}$ ) was incorporated into EYPC liposomes at the molar ratio of 12PY:EYPC=0.034:1. The  $I_{exc}/I_{mon}$  values were normalized using the values for control samples without *n*-decane.

hydrated. In these samples, the  $I_{exc}/I_{mon}$  ratio of 12PY probe was practically constant up to C10:EYPC=1:1 molar ratio (Fig. 2, open circles). These data indicate that the *n*-decane addition has no effect on the 12PY probe collision frequency in the bilayers in multilamellar liposomes, i.e. that *n*-decane is not located in the bilayers parallel with EYPC acyl chains. To test if the *n*-decane were located between apposing monolayers in the center of bilayer, we have performed X-ray diffraction experiment with the multilamellar EYPC phase. The results of this experiment have unequivocally shown that the bilayer thickness increases in the presence of *n*-decane (Fig. 2, open squares). One can conclude thus that *n*-decane is located in the center of bilayer between apposing phospholipid acyl chains in multilamellar systems.

Finally, we prepared unilamellar liposomes containing *n*-decane by the procedure c) - the dry mixture of EYPC, *n*-decane and 12PY was solubilized by the aqueous micellar solution of sodium cholate and the resulting solution of mixed micelles was diluted to obtain unilamellar liposomes. In these samples, the  $I_{exc}/I_{mon}$  ratio of 12PY probe decreased up to about C10:EYPC=0.4:1 molar ratio and then remained constant (Fig. 2, full circles). From these data one can conclude that the *n*-decane addition decreases the 12PY probe collision frequency in the bilayers in unilamellar liposomes up to C10:EYPC=0.4:1 molar ratio, i.e. that *n*-decane is located in the bilayers parallel with EYPC acyl chains in unilamellar liposomes. At higher C10:EYPC molar ratios, the constant value of  $I_{exc}/I_{mon}$  could be caused by an inclusion of additional *n*-decane between apposing monolayers in the bilayer. This mechanism of *n*-decane interaction with unilamellar liposomes is supported by results of small-angle neutron scattering (SANS) on unilamellar DOPC liposomes [12]. In these experiments, we have evaluated



the bilayer thickness parameter  $d_g$  which changes parallel the changes in the lipid bilayer thickness [22]. The data obtained up to C10:DOPC=1:1 molar ratio are shown in Fig. 2 (full squares). It is seen that the thickness parameter  $d_g$  is within the experimental error constant up to the *n*-decane:DOPC=0.4 molar ratio, and then increases by 0.14 nm up to *n*-decane:DOPC=1:1 molar ratio. Noteworthy is a relatively small change in thickness when comparing the results obtained with multilamellar system (open squares). This could be caused by a decreased adsorption of *n*-decane in curved bilayers in unilamellar liposomes [23].

In conclusion, we have found that the location of *n*-decane in the lipid bilayer and its influence on the lipid bilayer thickness are different in unilamellar and multilamellar model membrane systems. In multilamellar liposomes, the *n*-decane molecules are located predominantly in the bilayer center between apposing monolayers. This location brings about a significant increase in the bilayer thickness, but the frequency of the excimer fluorescence probe collisions is not influenced in this case. On the other hand, the collision frequency decreases significantly in unilamellar liposomes. However, this increase is observed at lower molar ratios only, up to *n*-decane:EYPC=0.4:1. The bilayer thickness does not change up to this molar ratio, and one can conclude thus that the *n*-decane molecules are located in the bilayer parallel to the lipid acyl chains. At higher molar ratios, *n*-decane slightly increases the bilayer thickness without having any effect on the probe collision frequency. This suggests location in the center of bilayer like in the multilamellar systems.

Experimental findings in the present work are important for understanding of molecular mechanisms of biological action of *n*-decane. Gruen a Haydon [23] suppose that most biological membranes have average radii of curvature sufficiently large, so that their adsorption of *n*-decane into bilayers should be similar to that in planar bilayers. Consequently, the changes in the bilayer thickness must be taken into account in explaining the *n*-decane pharmacological effects. On the other hand, when the bilayer is curved like in unilamellar liposomes, other effects can be more important. For example, the method of sarcoplasmic reticulum Ca-transporting ATPase reconstitution in synthetic phosphatidylcholine bilayers is very similar to the methods of a) and c) of unilamellar liposome preparation and *n*-decane addition used in the present study. In this case the effects of *n*-decane on the activity of enzyme cannot be explained by the bilayer thickness changes, but rather by the direct *n*-decane interactions with protein hydrophobic binding sites as suggested by results of ATPase fluorescence study [8].

## ACKNOWLEDGEMENTS

This study was supported by the Slovak Ministry of Education grants 1/7704/2000 to P.Balgavý and 1/9313/2002 to J. Gallová. The experiments in Dubna were supported within the JINR project 07-4-1031-99/03.

## REFERENCES

1. BALGAVÝ, P. - DEVÍNSKY, F.: Cut-off effects in biological activities of surfactants. *Adv. Colloid Interface Sci.* 66, 1996, 23-63.
2. HAYDON, D. A. - HENDRY, B. M. - LEVINSON, R. - REQUENA, J.: Anaesthesia by the n-alkanes. A comparative study of nerve impulse blockage and the properties of black lipid bilayer membranes. *Biochim. Biophys. Acta* 470, 1977, 17-34.
3. HAYDON, D. A. - HENDRY, B. M. - LEVINSON, R. - REQUENA, J.: The molecular mechanisms of anaesthesia. *Nature* 268, 1977, 356-358.
4. HAYDON, D. A. - REQUENA, J. - URBAN, B. W.: Some effects of aliphatic hydrocarbons on the electrical capacity and ionic currents of the squid giant axon membrane. *J. Physiol. (Lond)* 309, 1980, 229-245.
5. HAYDON, D. A. - KIMURA, J. E.: Some effects of n-pentane on the sodium and potassium currents of the squid giant axon. *J. Physiol. (Lond)* 312, 1981, 57-70.
6. FRANKS, N. P. - LIEB, W. R.: Which molecular targets are most relevant to general anaesthesia? *Toxicol. Lett.* 100-101, 1998, 1-8.
7. JOHANNSSON, A. - KEIGHTLEY, C. A. - SMITH, G. A. - RICHARDS, C. D. - HESKETH, T. R. - METCALFE, J. C.: The effect of bilayer thickness and n-alkanes on the activity of the (Ca<sup>2+</sup>-Mg<sup>2+</sup>)-dependent ATPase of sarcoplasmic reticulum. *J. Biol. Chem.* 256, 1981, 1643-1650.
8. LEE, A. G. - EAST, J. M. - BALGAVÝ, P.: Interaction of insecticides with biological membranes. *Pesticide Sci.* 32, 1991, 317-327.
9. MCINTOSH, T. J. - SIMON, A. - MACDONALD, R. C.: The organization of n-alkanes in lipid bilayers. *Biochim. Biophys. Acta* 597, 1980, 445-463.
10. POPE, J. M. - WALKER, L. W. - DUBRO, D.: On the ordering of N-alkane and N-alcohol solutes in phospholipid bilayer model membrane systems. *Chem. Phys. Lipids* 35, 1984, 259-277.
11. KING, G. I. - JACOBS, R. E. - WHITE, H.: Hexane dissolved in dioleoyllecithin bilayers has a partial molar volume of approximately zero. *Biochemistry* 24, 1985, 4637-4645.
12. UHRÍKOVÁ, D. - BALGAVÝ, P. - KUČERKA, N. - ISLAMOV, A. - GORDELIY, V. - KUKLIN, A.: Small-angle neutron scattering study of the n-decane effect on the bilayer thickness in extruded unilamellar dioleoylphosphatidylcholine liposomes. *Biophys. Chem.* 88, 2000, 165-170.
13. SINGLETON, W. - GRAY, M. - BROWN, M. L. - WHITE, J. L.: Chromatographically homogenous lecithin from egg phospholipids. *J. Amer. Oil Chem. Soc.* 42, 1965, 53-56.
14. FILÍPEK, J. - GELIENOVÁ, K. - KOVÁCS, P. - BALGAVÝ, P.: Effect of lipid autooxidation on the activity of the sarcoplasmic reticulum (Ca<sup>2+</sup>-Mg<sup>2+</sup>)ATPase reconstituted into egg yolk phosphatidylcholine bilayers. *Gen. Physiol. Biophys.* 12, 1993, 55-68.

15. PEDERSEN, J. - EGELHAAF, U. - SCHURTENBERGER, P.: Formation of polymer-like mixed micelles and vesicles in lecithin - bile salt solutions: A small-angle neutron-scattering study. *J. Phys. Chem.* 99, 1995, 1299-1305.
16. GORDELIY, V. I. - LUCHKIKOV, V. I. - SYRYKH, A. G. - CHEREZOV, V. G.: Application of the X-ray diffractometer DRON to the study of long period structures. *JINR Communication* 14-91-B-388, 1991.
17. HUANG, T. C. - TORAYA, H. - BLANTON, T. N. - WU, Y.: X-ray powder diffraction analysis of silver behenate, a possible low-angle diffraction standard. *J. Appl Cryst.* 26, 1993, 180-184.
18. WHITE, H. - JACOBS, R. E. - KING, G. I.: Partial specific volumes of lipid and water in mixtures of egg lecithin and water. *Biophys. J.* 52, 1987, 663-665.
19. WEAST, R. C.: *Handbook of Chemistry and Physics*, The Chemical Rubber Co., Cleveland 1969.
20. LUZZATI, V.: X-Ray Diffraction Studies of Lipid-Water Systems. In: *Biological Membranes* (D. Chapman, Ed.), Academic Press, London, New York 1968, 71-124.
21. GALLA, H.-J. - HARTMANN, W.: Excimer-forming lipids in membrane research. *Chem. Phys. Lipids* 27, 1980, 219-
22. BALGAVÝ, P. - DUBNIČKOVÁ, M. - KUČERKA, N. - KISELEV, M. A.: Bilayer thickness and lipid interface area in unilamellar extruded 1,2-diacylphosphatidylcholine liposomes: A small-angle neutron scattering study. *Biochim. Biophys. Acta* 1512, 2001, 40-52.
23. GRUEN, D. W. - HAYDON, D. A.: The adsorption of nonpolar molecules into lipid bilayer membranes. *Biophys. J.* 30, 1980, 129-136.