

# Bimodal Effect of Amphiphilic Biocide Concentrations on Fluidity of Lipid Membranes

Marian Podolak<sup>a</sup>, Dariusz Man<sup>a</sup>, Stanisław Waga<sup>a</sup> and Stanisław Przestalski<sup>b</sup>

<sup>a</sup> Institute of Physics, Opole University, Oleska 48, 45–052 Opole, Poland

<sup>b</sup> Department of Physics and Biophysics, Agricultural University, Norwida 25, 50–375 Wrocław, Poland

Z. Naturforsch. **51c**, 853–858 (1996); received April 2/August 16, 1996

Lecithin Liposomes, Fluidity, Quaternary Ammonium Salts, ESR

Using the spin label method (ESR) it has been shown that biologically active, amphiphilic compounds (quaternary ammonium salts – AS) containing polar heads with single and double positive charge caused, at low concentrations, decrease fluidity of liposome membranes formed with egg yolk lecithin (EYL). At higher concentrations an increase in fluidity was observed. With compounds having a single positive charge minimum fluidity of membrane structure occurs in the range of 1 to 3%, with compounds containing double positive charge – in the range of 4–6%. That effect does not depend on polar head size and length of alkyl chains of the AS used. Analysis of the electrostatic interaction between positive charges and dipole system suggest that at low ion concentrations the binding energy of the system increases, while it decreases at high concentrations. For the model presented, maximum of binding energy of the system occurs at 3% of positive monovalent ions and at 6% of positive divalent ions admixed.

## Introduction

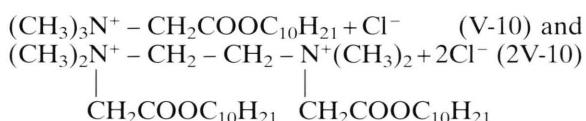
It has been found that biologically active, amphiphilic compounds, e.g. quaternary ammonium salts, caused increased lipid membrane fluidity (Podolak *et al.*, 1987; Subczyński *et al.*, 1988; Podolak *et al.*, 1988) and exerted destructive effects on biological membranes (Cubkova *et al.*, 1981; Kleszczyńska *et al.*, 1986; Ancelin and Vial, 1986; Lindstedt *et al.*, 1990; Gallova *et al.*, 1990; Przestalski and Kuczera, 1992; Balgavy and Devinsky, 1994). In the presence of these compounds the rate of ion permeation across lipid membranes increases (Kuczera *et al.*, 1985; Kuczera *et al.*, 1987; Kuczera *et al.*, 1989). Those effects occur at rather high concentrations of the compounds studied (above 10% mol compound/mol lecithin). Isomaa *et al.*, (1989) could show that biologically active, amphiphilic compounds (ionic, among others) inhibited the process of erythrocyte hemolysis at small concentrations and accelerated it at higher concentrations.

The objective of the present work was to investigate the biological effect of selected ammonium

salts (AS) on fluidity of egg yolk lecithin liposome membranes, with emphasis on low-concentration range, and molecular structure of the compounds.

## Materials and Methods

The lipid membranes used in this study were in the form of liposomes obtained from fresh hen egg yolks lecithin (EYL) in the process of sonication. Four compounds which differ in alkyl chain length, polarity and size of their polar heads were chosen for the investigation, which was performed using the ESR spin labels. EYL concentration in a sample was 0.04 M, and the spin label to EYL molar ratio 0.01. Admixtures of the compounds studied were introduced to the samples after liposome formation, and their concentration was varied within the range of 0–15% with respect to EYL. The amphiphilic ammonium salts (admixtures) used in the study can be divided into two groups as far as their molecular structure is concerned. Salts of the first group are of the structural formula:



---

Reprint requests to Dr. M. Podolak.

0939–5075/96/1100–0853 \$ 06.00 © 1996 Verlag der Zeitschrift für Naturforschung. All rights reserved.

D



Dieses Werk wurde im Jahr 2013 vom Verlag Zeitschrift für Naturforschung in Zusammenarbeit mit der Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. digitalisiert und unter folgender Lizenz veröffentlicht: Creative Commons Namensnennung-Keine Bearbeitung 3.0 Deutschland Lizenz.

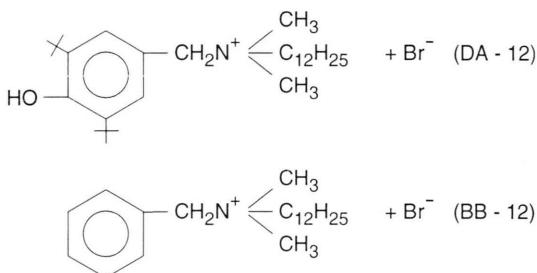
Zum 01.01.2015 ist eine Anpassung der Lizenzbedingungen (Entfall der Creative Commons Lizenzbedingung „Keine Bearbeitung“) beabsichtigt, um eine Nachnutzung auch im Rahmen zukünftiger wissenschaftlicher Nutzungsformen zu ermöglichen.

This work has been digitized and published in 2013 by Verlag Zeitschrift für Naturforschung in cooperation with the Max Planck Society for the Advancement of Science under a Creative Commons Attribution-NoDerivs 3.0 Germany License.

On 01.01.2015 it is planned to change the License Conditions (the removal of the Creative Commons License condition "no derivative works"). This is to allow reuse in the area of future scientific usage.

These salts have alkyl chains (hydrophobic parts) of the same length but different polar heads (hydrophilic parts). Compound 2V-10 has two hydrophobic chains and a head with double positive charge. The molecule of compound V-10 is a fragment of the 2V-10 molecule. It is composed of a single hydrocarbon chain and a univalent positive polar head.

The second group include salts of the structural formula:



Compounds of this group have identical 12-carbon single hydrophobic chain and univalent positive polar heads of different size. DA-12 has a phenyl substituent with antioxidant properties, containing two t-butyl groups, and thus its polar head is bigger than that of the BB-12 compound.

Three spin labels were used in the investigation: 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), 4-(2-*n*-undecyl-3-oxyl-4,4-dimethyloxazolidyn-2-yl) butyric acid – FA(10,3) and 2-(14-carboxytetradecyl)-2-ethyl-4,4-dimethyl-3-oxazolidinyl oxyl – FA(1,14), that penetrate different regions of the liposome membranes. The TEMPO spin label dissolves in both the hydrophobic part of the membrane and aqueous medium. On the basis of ESR spectra the partition parameter  $F$  of the label between the membrane and water medium was determined. Parameter  $F$  is defined as ratio of the high-field line amplitude of the ESR spectrum of a label placed in lipid medium ( $H$ ) to the sum of  $H$  and amplitude of the high-field line of label placed in aqueous medium ( $P$ ) –  $F = H/H+P$  (Fig. 1). (For the control sample, without admixture of the compounds studied, the partition parameter is denoted by  $F_k$  and its value is constant). The value of parameter  $F$  is connected with membrane fluidity. Increase of membrane fluidity caused increase of the parameter  $F$  (Shimshick and McConnell, 1973).

Spin label FA(10,3) is localized in the hydrophobic part of the membrane, near the lipid bilayer surface. Based on the ESR spectra of the label, the parameter  $2T$  was determined. Its value is proportional to the order parameter (Scheier *et al.*, 1978). The order parameter of the spin label gives the information on the ordering degree of its molecules and molecules of lipid medium. In this study increase of the membrane fluidity caused a decrease of both the order parameter and parameter  $2T$ . The measure of the  $2T$  parameter is the distance between utmost lines of the FA(10,3) spin label spectrum (Fig. 2). The value of the parameter for the control sample, without admixture of the compounds studied, is denoted by  $2T_k$  (constant value).

Spin label FA(1,14), like FA(10,3), is also localized in the hydrophobic part of the membrane, but in the middle of the lipid bilayer. On the basis of the ESR spectra of this label the  $\tau$  parameter – a quantity inversely proportional to the rotational velocity of the label was determined. This parameter was calculated from ESR spectra using the formula for rotational correlation time (Hemminga, 1983) (Fig. 3). The value of parameter  $\tau$  for the control sample is denoted by  $\tau_k$  (constant value).

For to underline the changes occurring in the samples due to the admixtures of investigated compounds, the relative values of experimentally determined spectroscopic parameters ( $F/F_k$ ,  $2T/2T_k$ ,  $\tau/\tau_k$ ) are discussed.

## Results and Discussion

Fig. 1 shows the concentration dependence of the relative spectroscopic parameter  $F$  ( $F/F_k$ ) of the TEMPO label dissolved in water suspension of EYL liposomes containing admixtures of compounds of the first group. As seen in the figure, both the compounds cause a decrease in the value of the parameter  $F/F_k$  (lowered fluidity) at concentrations between 1% and 2%, whereas for concentrations above this range the parameter increased (fluidity increased). For concentrations higher than 2% liposome membranes admixed with 2V-10 had greater fluidity than those with V-10.

Fig. 2 shows the concentration dependence of the relative spectroscopic parameter  $2T(2T/2T_k)$

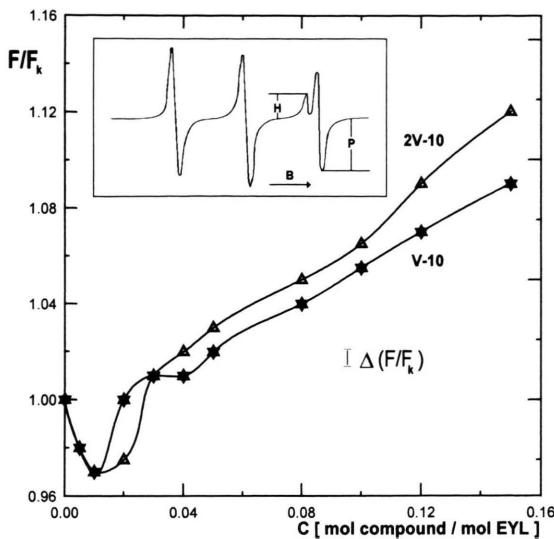


Fig. 1. Dependence of relative value of the partition parameter  $F$  ( $F/F_k$ ) of TEMPO spin label dissolved in water suspension of egg yolk lecithin (EYL) liposomes on concentration  $c$  of admixtures V-10 and 2V-10 ammonium salts (AS). The figure presents the mean error of a measurement series and an ESR spectrum of TEMPO label in the samples on inset. Partition parameter  $F = H/H+P$ , were  $H$  and  $P$  denote high field line amplitudes of TEMPO label placed in the lipid medium (membrane) ( $H$ ) and in the aqueous medium ( $P$ ), respectively (inset). The arrow in the inset denotes direction of magnetic field, ( $B$ ) increase. Symbol  $F_k$  denotes partition parameter  $F$  for control sample (without admixture of the ammonium salts studied). The value of parameter  $F_k$  is 0.4 and constant. Measurement temperature was 22 °C.

of label FA(10,3) embedded in EYL liposome membranes containing admixtures from the first group. From the figure it follows that compounds of that group caused an increase in the value of parameter  $2T/2T_k$  at concentrations between 2% and 4%, thus indicating lowered membrane fluidity. Above 4% concentration the parameter decreased (fluidity increased). For AS concentrations higher than 4% fluidity of liposomes containing 2V-10 admixtures was higher than that for liposomes containing of V-10. These results are in qualitative agreement with those obtained using the TEMPO spin label.

Fig. 3 shows the concentration dependence of the relative spectroscopic parameter  $\tau$  ( $\tau/\tau_k$ ) of label FA(1,14) embedded in EYL liposomes containing admixtures of compounds of the first group.

It follows from Fig. 2, in the internal of small concentrations ranging from 2% to 6%, the  $\tau/\tau_k$  parameter reaches its largest value for both the compounds of this group (the membrane fluidity reaches a minimum value). For concentrations higher than 6% the value of  $\tau/\tau_k$  parameter decreases (the membrane fluidity increases). Similarly as for TEMPO and FA(10,3) spin labels (Figs. 1 and 2) in the range of AS concentrations higher than 6%, the FA(1,14) spin label indicated that the fluidity of liposome membranes containing of the 2V-10 compound is greater than that for liposome membranes doped with the V-10 compound.

The FA(10,3) and FA(1,14) spin labels built into the liposome membranes indicate that the minimum of fluidity for membranes doped with 2V-10 compound (double positive polar head) is shifted towards higher concentrations (4% – 6%) with respect to the fluidity minimum observed in the case of V-10 admixtures (single-charged polar heads),

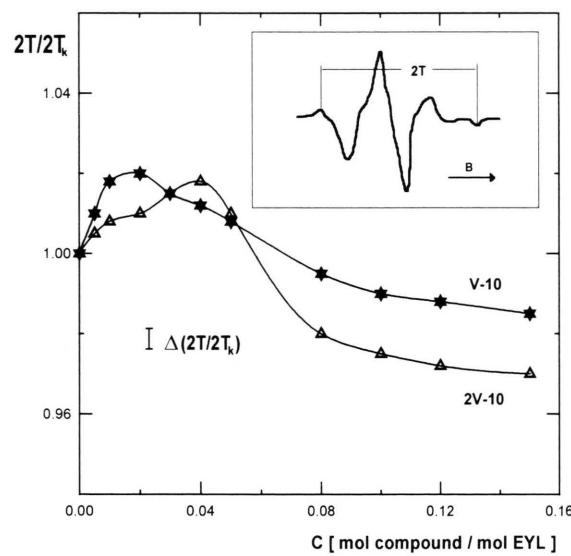


Fig. 2. Dependence of relative value of parameter  $2T$  ( $2T/2T_k$ ) of FA(10,3) spin label embedded in EYL liposome membranes on concentration  $c$  of V-10 and 2V-10 admixtures. The figure presents mean error of a measurement series and an ESR spectrum of FA(10,3) label in the samples, on inset. The arrow in the inset means direction of magnetic field ( $B$ ) increase. Parameter  $2T$  denotes the distance between outermost lines of the FA(10,3) spin label spectrum (inset). Symbol  $2T_k$  denotes parameter  $2T$  for control sample. The value of parameter  $2T_k$  is constant and equivalent to 24.9 gauss. Measurement temperature was 22 °C.

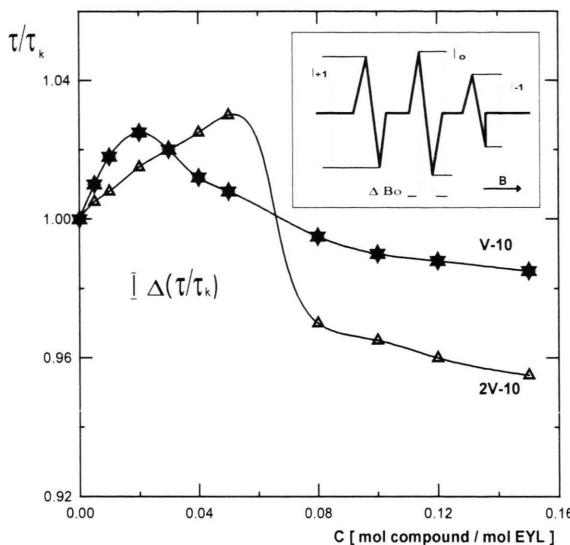


Fig. 3. Dependence of relative value of parameter  $\tau$  ( $\tau/\tau_k$ ) of FA(1,14) spin label embedded in EYL liposome membranes on concentration  $c$  of admixtures V-10 and 2V-10. The figure presents mean error of a measurement series and an ESR spectrum of FA(1,14) label in the samples, on inset. Parameter  $\tau = 5.59 \Delta B_o (\sqrt{I_0/I_{+1}} + \sqrt{I_0/I_{-1}} - 2)^{-10} s$  (Hemminga, 1983), where  $\Delta B_o$  means the peak-to-peak linewidth of the center line of ESR spectrum (in gauss);  $I_{+1}$ ,  $I_0$ ,  $I_{-1}$  – amplitudes of the low, center and high magnetic field lines respectively (inset). The arrow on the inset denotes direction of magnetic field (B) increase. Symbol  $\tau_k$  denotes parameter  $\tau$  for control sample. The value of parameter  $\tau_k$  is constant and equivalent to 4.2 ns. Measurement temperature was 22 °C.

occurring within the concentration interval 1% – 2%. In the case of liposome membranes studied using the TEMPO label this effect has not been observed.

Fig. 4 shows how the spectroscopic parameter  $F/F_k$  of the TEMPO label dissolved in water suspension of EYL liposomes depends on the concentration of admixtures of the second group (DA-12 and BB-12). For both compounds DA-12 and BB-12, the greatest decrease of the parameter  $F/F_k$  (decreased liposome membrane fluidity) occurs in the concentration interval ranging from 1% to 2%; above that concentration range the  $F/F_k$  parameter increases (membrane fluidity increases). In the low concentration range compound DA-12 has been proved to be the more active, while for BB-12 the same is true for higher concentrations.

Fig. 5 presents the dependence of the spectroscopic parameter  $\tau/\tau_k$  of label FA(1,14) embedded in liposome membranes on the concentration of admixtures of the second group (DA-12 and BB-12). For both AS added to liposomes the value of parameter  $\tau/\tau_k$  reached its maximum values (minimum membrane fluidity) at a concentration between 1% and 2%. Above 2% the parameter value decreased (membrane fluidity increased).

The occurrence of minimum fluidity of liposome membranes, studied, at low concentrations of the AS may be due to the electrostatic interaction between the polar heads of the hydrophobic membrane layer. EYL molecules introduce into the layer the large, positively charged choline groups and negatively charged phosphate groups that make the polar heads to become electric dipoles. These dipoles are located at very small angles (4°) to the membrane surface (Raudino and Mauzerall, 1986), with a distance of 0.6 nm between the choline and phosphate groups (Barratt and Lagger, 1974). The polar heads of the AS studied introduce a single charge into the hydrophilic part of the membrane (V-10, BB-12 and DA-12) or double positive charge (2V-10).

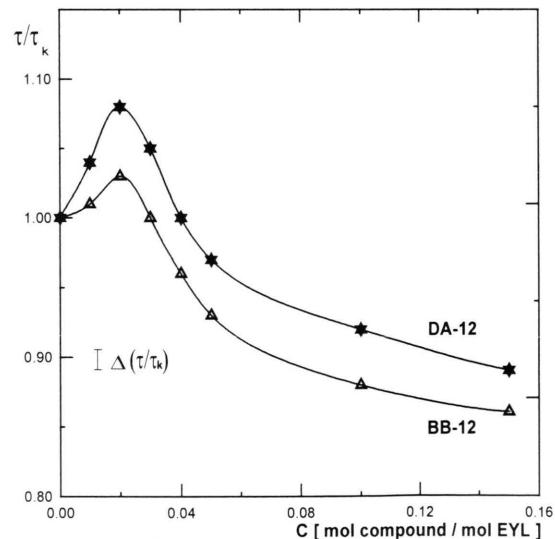


Fig. 4. Dependence of relative value of parameter  $\tau$  ( $\tau/\tau_k$ ) of FA(1,14) label embedded in EYL liposome membranes on concentration  $c$  of admixtures DA-12 and BB-12 ( $\tau_k = 4.2$  ns). The figure indicates mean error of a measurement series. Measurement temperature was 22 °C.

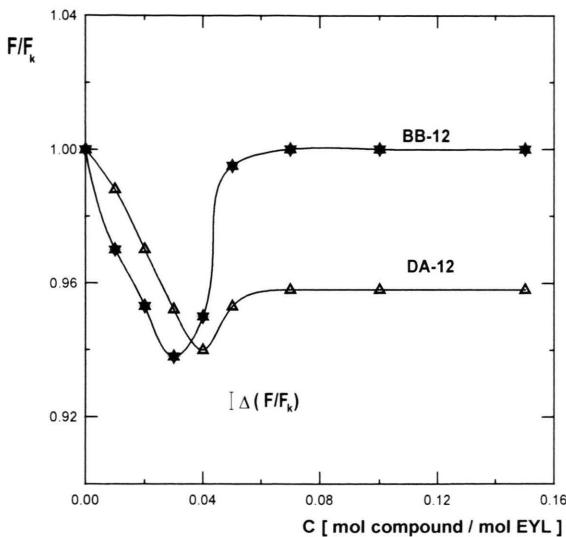


Fig. 5. Dependence of relative value of the partition parameter  $F/F_k$  of TEMPO label dissolved in water suspension of EYL liposomes on concentration  $c$  of admixtures DA-12 and BB-12 ( $F_k = 0.5$ ). The figure indicates mean error of a measurement series. Measurement temperature was 22 °C.

In the present work an analysis of electrostatic interaction in a system of dipoles admixed with positive single and double electric charges has been performed. A very simple model was applied in the analysis used earlier (Podolak *et al.*, 1994). That model contains  $N=100$  electric dipoles, 0.6 nm long, aligned on a straight line. It was arbitrarily assumed that the distance between them is 1.2 nm.

Using a computer programme of our own written in TURBO-PASCAL, we have calculated the binding energy per one dipole as dependent on admixture of single and double positive charges. The idea of the programme was to calculate the electrostatic interaction energy between a dipole and the positive and negative charges of the remaining ( $N-1$ ) dipoles. The energy of elementary interaction between any two charges can be expressed as  $E = +/- kq_1q_2/r$ , with “+” for charges of the same sign, “-” for charges of different sign, where  $k$  – coefficient,  $q_1$  and  $q_2$  - charges value,  $r$  – distance between charges. Total energy of the linear alignment of dipoles is equal to the sum of interaction energy between  $N$  dipoles.

The algorithm used in the programme assumes single mutual interaction between the dipoles. The programme spreads the admixed ions uniformly along the linear chain of dipoles and calculates the energy of the dipole system with admixtures, and the mean energy per dipole using the iteration method. The programme needs to be fed with the number of dipoles ( $N$ ), length of a dipole, distance between dipoles in the chain, number and charge of admixed ions. The results of calculations are presented in Table I. The calculations show that the binding energy of the dipole system increases with increasing admixture concentration and reaches a maximum at 3% concentration of positive univalent charges and 6% concentration of positive divalent charges. For concentrations

Table I. Binding energy per dipole.

Admixture concentration [%]	Single positive charge		Double positive charge	
	$-E$ [eV]	$E/E_k$	$-E$ [eV]	$E/E_k$
0	0.91512	1.0000	0.91512	1.0000
1	0.91603	1.0010	0.92621	1.0121
2	0.91689	1.0019	0.93721	1.0241
3	0.91724	1.0023	0.94623	1.0340
4	0.91700	1.0020	0.95301	1.0414
5	0.91612	1.0011	0.95732	1.0461
6	0.91446	0.9993	0.95876	1.0477
7	0.91209	0.9967	0.95737	1.0462
8	0.90903	0.9934	0.95357	1.0420
9	0.90497	0.9889	0.94580	1.0335
10	0.90016	0.9837	0.93541	1.0222
12	0.88767	0.9700	0.90324	0.9870
15	0.86120	0.9411	0.82787	0.9047

$E$ -binding energy per dipole.  $E_k$ -binding energy per dipole for a system without uncompensated charge.

higher than the above mentioned, the binding energy decreases with increasing concentration of the charges. The results of calculations (Table I) are in good agreement with experimental results (Figs. 1–5). Thus, one can expect that the observed changes in fluidity of EYL liposome mem-

branes, in the presence of the AS studied, are mainly due to the changes in the energy of electrostatic interactions in the hydrophilic layer of the membranes. Apparently differences in dimensions and chemical structure of the AS molecules have no significant effect.

Ancelin M.L. and Vial H.J. (1986), Quaternary ammonium compounds efficiently inhibit *Plasmodium falciparum* growth in vitro by impairment of choline transport, *Antimicrob. Agents Chemother.* **29**, 814–820.

Balgavy P. and Devinsky F. (1994), Cut-off effects in biological activities of surfactants. In Proc. 12th School on Biophysics of Membrane, Wrocław, Poland, part I, 21–68.

Barratt M.D. and Laggner P. (1974), The pH – dependence of ESR spectra from nitroxide probes in lecithin dispersions. *Biochim. Biophys. Acta* **363**, 127–133.

Cubkova V., Mlynarcik D., Devinsky F. and Lacko I. (1981), The effect of quaternary ammonium compounds and amine oxides on spores of *Bacillus cereus*. *Folia Microbiol.* **26**, 189–195.

Gallova J., Devinsky F. and Balgavy P. (1990), Interaction of surfactants with model and biological membranes II. Effect of N-alkyl-N,N,N-trimethylammonium ions on phosphatidylcholine bilayers as studied by spin probe ESR. *Chem. Phys. Lipids* **53**, 231–241.

Hemminga M.A. (1983), Interpretation of ESR and saturation transfer ESR spectra of spin labeled lipids and membranes. *Chem. Phys. Lipids* **32**, 323–383.

Isomaa B., Hagerstrand H. and Paatero G. (1989), Interaction of surfactants with biological membranes. In *Surfactants in Solution* (K.L. Mittal, ed.) Plenum Publishing Corporation **8**, 223–233.

Kleszczyńska H., Sarapuk J., Przestalski S. and Witek S. (1986), The role of the alkyl chain in the interaction of glycine esters with erythrocyte and model erythrocyte lipid membranes. *Stud. Biophys.* **116**, 115–122.

Kuczera J., Fogt A., Kubica K., Witek S., Pluciński J. and Przestalski S. (1989), Effect of surface active anions on the activity of dodecyloxy-methylenemethylmorpholinium chloride as modifier of sulphate ion transport across lecithin liposome membranes. *Stud. Biophys.* **133**, 209–230.

Kuczera J., Janas T., Przestalski S., Witek S. and Oświecimska M. (1985), Influence of some amphiphilic derivatives of glycine esters on sulphate ion transport across lecithin liposome membranes. *Stud. Biophys.* **105**, 167–176.

Kuczera J., Sarapuk J., Janas T., Witek S. and Grobelny D. (1987), Effect of some cyclic elements containing amphiphilic compounds on stability and transport properties of model lipid membranes. *Gen. Physiol. Biophys.* **6**, 645–654.

Lindstedt C.A., Allenmark S., Thompson R.A. and Edebo L. (1990), Antimicrobial activity of betaine esters, quaternary ammonium amphiphiles with spontaneously hydrolyze into nontoxic components, *Antimicrob. Agents Chemother.* **34**, 1949–1954.

Podolak M., Lassmann G., Witek S. and Przestalski S. (1987), Changes in fluidity of liposome membranes caused by admixtures of selected amphiphile derivatives of glycine esters. *Stud. Biophys.* **118**, 3, 197–204.

Podolak M., Lassmann G., Witek S. and Przestalski S. (1989), The effect of double-chain amphiphilic glycine ester derivatives on lecithin liposome fluidity. *Stud. Biophys.* **131**, 1–2, 45–53.

Podolak M., Waga S. and Przestalski S. (1994), The effect of amphiphilic biocides contained in a medium on liposome membranes fluidity. *Polish J. Environ. Stud.* **3**, 2, 23–27.

Przestalski S. and Kuczera J. (1992), Molecular mechanisms of interaction between amphiphilic ammonium salts and membranes. *Polish J. Environ. Stud.* **1**, 2, 13–22.

Raudino A. and Mauzerall D. (1986), Dielectric properties of the polar head group region of zwitterionic lipid bilayers. *Biophys. J.* **50**, 441–443.

Schreier S., Polnasek C.F. and Smith I.C.P. (1978), Spin labels in membranes. Problems in practice. *Biochim. Biophys. Acta* **515**, 357–434.

Shimshick E.J. and McConnell H.M. (1973), Lateral phase separation in phospholipid membranes. *Biochemistry* **12**, 2351–2360.

Subczyński W.K., Przestalski S., Kuczera J., Podolak M. and Hyde J.S. (1988), Effect of some amphiphilic ammonium salts on phase transition and fluidity of phosphatidylcholine membranes. A spin label study. *Stud. Biophys.* **125**, 2, 155–163.