Organometallics and Quaternary Ammonium Salts Affect Calcium Ion Desorption from Lecithin Liposome Membranes

Teresa E. Kral*, Janina Kuczera and Stanisław Przestalski

Department of Physics and Biophysics, Agricultural University, Norwida 25, 50–375 Wrocław, Poland. Fax: (+48)-71-32-05-172. E-mail: tek@ozi.ar.wroc.pl

* Author for correspondence and reprint requests.
Present address: Teresa E. Kral, Department of Physics and Biophysics, Agricultural University, Norwida 25, 50–375 Wrocław, Poland.
Fax: (+48)-71-32-05-172. E-mail: tek@ozi.ar.wroc.pl

Z. Naturforsch. **56 c,** 395–401 (2001); received September 7, 2000/January 26, 2001

Organometallics, Quaternary Ammonium Salts, Cooperative Effect

The objective of the present work was to compare the effects of groups of tin and lead organometallic compounds and their mixtures with amphiphilic quaternary ammonium salts (QAS) on the process of calcium ion desorption from lecithin liposome membranes, as dependent on the properties of the hydrophilic and hydrophobic parts of QAS. In the investigations the method of radioactive labels was applied. Synergism and antagonism in the action of both groups of compounds were found. The effectiveness of the cooperation depended more on chain length of QAS compounds than on the size and polarity of their hydrophobic parts. The most effective of all compounds studied was a the mixture of benzyldimethylammonium chloride in a mixture with tripropyltin. Since the rate of calcium desorption proved to be a good measure of efficacy of biologically active surfactants, it seems that the conclusions reached in this paper may be useful for choosing compounds which are able to decontaminate the environment polluted with heavy metals.

Introduction

This study is concerned with the effect of some organometallic compounds on liposomes a model biological membranes, or a mixture of these compounds and cationic surfactants, amphiphilic quaternary ammonium salts (QAS). Both groups of compounds have a wide range of industrial application (Blunden *et al.*, 1985; Crowe, 1987; Lindstedt *et al.*, 1990; Rubingh and Holland, 1991). Their biological effects have attracted much attention since both groups of compounds may contribute to a environmental pollution (Craig, 1982; Davis and Jordan, 1989; Fent, 1996).

The molecular nature of the organometallic – membrane interactions is not yet fully clear; not clear is also the nature of the joint action of a mixture of both above mentioned groups of compounds, so we thought it useful to carry out an investigation on this topic.

Our preliminary investigations of some organotin compounds and only one mixture of tripropyltin with the cationic surfactant trimethyldodecylammonium bromide showed that the organometallic compounds studied had an effect on calcium ion desorption from lecithin liposomes, and the cationic surfactant inhibited the effectivity of tripropyltin (Kuczera *et al.*, 1997). This effect seemed interesting enough to undertake further studies with a greater group of organotins and also with organoleads, and with mixtures of both groups of organometallics with a group of cationic surfactants with different properties of their hydrophilic and hydrophobic parts.

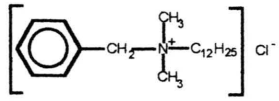
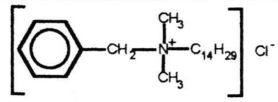
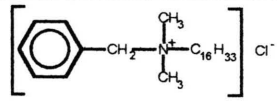
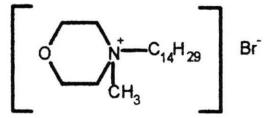
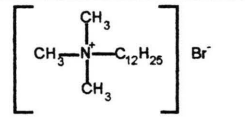
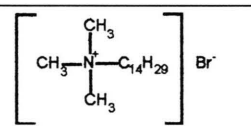
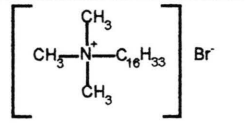
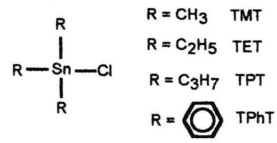
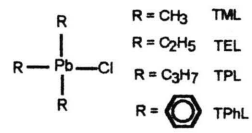
Materials and Methods

Materials. Egg lecithin (PC) was prepared according to Singleton *et al.* (Singleton *et al.* (1965). The compounds used, i.e. organometallic compounds and amphiphilic quaternary ammonium chlorides are presented in Table I. All chemicals were of analytical grade.

Radioactive tracer experiments. Small unilamellar liposomes (SUV) were prepared from yolk lecithin by the sodium cholate method in Liposomat (DIANORM) (Weder and Zumbuhl, 1984). The solution used to form vesicles contained veronal-acetate buffer of pH 7.5 and 0.3 mM $CaCl_2$ labelled with radioactive Ca-45. During vesicle for-

0939–5075/2001/0500–0395 $ 06.00

Table I. Compounds studied.

Code	Chemical structure	Chemical name	Company
BDDA		N-benzyl-N,N-dimethyl-N-dodecylammonium chloride	FLUKA
BDTA		N-benzyl-N,N-dimethyl-N-tetradecylammonium chloride	ALDRICH
BDHA		N-benzyl-N,N-dimethyl-N-hexadecylammonium chloride	FLUKA
MTM		N-methyl-N-tetradecylmorpholinium bromide	*
TMDA		N,N,N-trimethyl-N-dodecylammonium bromide	SERVA
TMTA		N,N,N-trimethyl-N-tetradecylammonium bromide	FLUKA
TMHA		N,N,N-trimethyl-N-hexadecylammonium bromide	FLUKA
TAT		trimethyltin chloride triethyltin chloride tripropyltin chloride triphenyltin chloride	MERCK FLUKA
TAL		trimethyllead chloride triethyllead chloride tripropyllead chloride triphenyllead chloride	ACRC FLUKA

* – Compound synthesised in the Institute of Organic Chemistry and Polymer Technology of the Technical University of Wrocław.

mation calcium cations were adsorbed at the outer and inner liposome membranes (Kuczera and Żyłka, 1979). The radioactive tracer was removed from the external medium during liposome preparation.

The measuring set-up was composed of 16 vessels, each containing an outer chamber with a coaxially mounted inner cylindrical chamber with cellophane side walls. The chambers were kept at 25 °C. The inner chamber was filled with the lipo-

some suspension (with Ca^{2+} to lecithin concentration 1:10), and the outer one with the solution alone. Defined amounts of the organotin compounds studied were added to both compartments to give identical concentrations on both sides of the cellophane wall. The final concentrations ranged between 0.5 and 6.0 mM.

In experiments with liposome membrane modification by QAS surfactants, defined amounts of stock solution of those compounds were added at first of both chambers. After one hour incubation, to the liposomes with surfactants proper amounts of organometallic compounds were added to both compartments in equimolar concentrations of the surfactants studied. Aliquots were taken at chosen time intervals and their radioactivity was measured. The experiments were repeated 4–6 times for each compound studied. Standard error was below 10%.

The theoretical work-out of the transport and desorption measurements as previously described (Mazgis and Kuczera, 1981) was used with minor modifications. Briefly, in order to determine the rate constant of the ion desorption process, a three-compartment analysis was used. Calcium ions released from the liposome membrane (first compartment) were present in the inner chamber (second chamber) and from there they passed through a cellophane membrane to the outer chamber (third compartment). The ion flux observed results from the desorption process and permeation from the interior of the liposomes. However, the latter flux is negligibly small because of the very low concentration of Ca^{2+} in the bulk inner medium and its very low permeability through the lipid bilayer (Kuczera and Żyłka, 1979).

Solving a system of kinetic equations of balance for the amount of radiotracer present in each compartment, the following solution for relative radioactivity, U is obtained:

$$U = (A_\infty - A)/A_\infty = [\beta/(\beta-\alpha)]\, e^{-\alpha t} - [\alpha/(\beta-\alpha)]\, e^{-\beta t} \qquad (1)$$

with:

A_∞ – equilibrium radioactivity (in cpm), determined as $A^\infty = [V_0/(V_0+V_i)]\, A + [V_i/(V_0+V_i)]\, A_i$; A_i and A – radioactivity of samples taken from the inner and outer chamber, respectively; V_i and V_0 – volumes of the inner and outer chamber; t – time, α – rate constant of calcium ion desorption process from the liposome membrane, β – rate constant of calcium ion transport through the cellophane membrane (β was determined in a separate experiment).

Plots of logarithm of the relative radioactivity, $\ln U$, against time were constructed from experimental points. Theoretically calculated curves from equation (1) were fitted to them using a computer-programmed Newton iteration method that allows to determine the optimal value of the rate constant α (Kubica *et al.*, 1994).

Experimental conditions

In order to determine the effect of hydrophobic groups in the organometallic compounds on the calcium ion desorption process, the organotin and organolead compounds presented in Table I were used. The organometallic compounds and one of the cationic surfactants, namely TMDA, in equimolar mixtures were used to determine the effect on the calcium ion desorption process of mixtures of organometallic compounds and cationic surfactants as dependent on the number of hydrophobic groups in organometallics. The influence of chain length of surfactants was determined with two groups of cationic surfactants: TMDA, TMTA, TMHA and BDDA, BDTA, BDHA in equimolar mixtures with tripropyltin or tripropyllead. The effect of electric and steric properties of the polar parts of surfactants was determined with three compounds with the same alkyl chain and different polar parts (BDTA, MTM and TMTA) in equimolar mixtures with tripropyltin and tripropyllead.

Results and Discussion

The results of kinetic studies on the calcium ion desorption process are presented in Figs 1 and 2, where the relative rate constants are plotted against concentration of the compounds when present in the solutions alone and in mixtures with cationic surfactants. In Tables II and III, the values of the relative rate constant are presented for chosen concentrations. The relative rate constant α/α_0, being a measure of compound's efficiency, is defined as the ratio of the rate constant of calcium ion desorption in the presence of the compounds studied to that measured in the absence of modifiers. As follows from Fig. 1a, the coefficient α/α_0

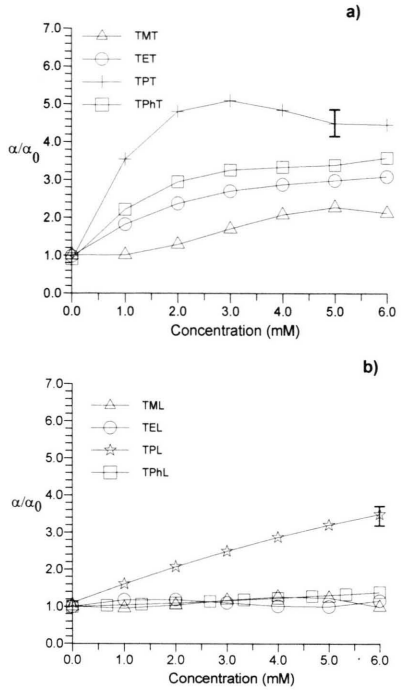


Fig. 1. Relative rate constant, α/α_o, of calcium ion desorption process from liposome membranes against concentration of an organometallic compound: a) organotins – TMT, TET, TPT, TPhT and b) organoleads – TML, TEL, TPL, TPhL. α and α_o are kinetic constants for the modified and unmodified membrane, respectively.

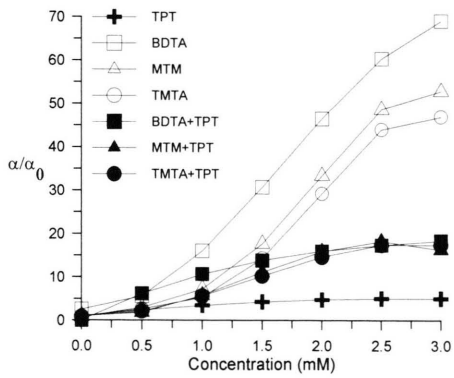


Fig. 2. Relative rate constant, α/α_o, of calcium ion desorption from liposome membranes against concentration of the BDTA, MTM and TMTA compounds and their mixtures with tripropyltin. α and α_o are kinetic constants for the modified and unmodified membrane, respectively.

Table II. Relative rate constant, α/α_o, of calcium ion desorption from liposome membranes for using a 2 mM concentration of the TMDA compound and its mixtures with organotins.

Compounds and mixtures	α/α_o
TMDA	1.5 ± 0.2
TMT	1.33 ± 0.3
TMT+TMDA	1.22 ± 0.4
TET	2.2 ± 0.3
TET+TMDA	1.1 ± 0.4
TPT	4.7 ± 0.3
TPT+TMDA	2.2 ± 0.4
TPhT	2.8 ± 0.3
TPhT+TMDA	1.2 ± 0.4

Table III. Relative rate constant, α/α_o, of calcium ion desorption from liposome membranes for a chosen concentration of tripropyltin and tripropyllead compounds and their mixtures with alkylbenzylammonium chlorides and alkyltrimethylammonium chlorides.

	α/α_o		
Compounds and mixtures	Concentration [mM] 1.0	1.5	2.0
TPT	3.5 ± 0.3	4.0 ± 0.3	4.7 ± 0.3
TPL	1.5 ± 0.2	1.5 ± 0.2	2.0 ± 0.2
BDDA	5.0 ± 0.5	12 ± 0.8	27 ± 1.0
BDTA	16.0 ± 1.0	30.0 ± 1.0	46 ± 1.0
BDHA	12.5 ± 1.0	17.0 ± 1.0	16.0 ± 1.0
BDDA+TPT	4.0 ± 0.6	7.5 ± 0.6	12.5 ± 0.6
BDTA+TPT	10.0 ± 1.0	14.0 ± 1.0	15.5 ± 1.0
BDHA+TPT	12.0 ± 1.0	14.5 ± 1.0	16.0 ± 1.0
BDDA+TPL	6.5 ± 0.7	9.5 ± 0.7	13.0 ± 0.7
BDTA+TPL	8.0 ± 0.8	14.0 ± 0.8	17.0 ± 0.8
BDHA+TPL	3.5 ± 0.6	8.5 ± 0.6	14.0 ± 0.6
TMDA	1.0 ± 0.3	1.0 ± 0.3	1.5 ± 0.3
TMTA	7.0 ± 0.8	16.0 ± 0.8	28.0 ± 0.8
TMHA	6.0 ± 0.9	13.0 ± 0.9	22.0 ± 0.9
TMDA+TPT	2.0 ± 0.4	2.0 ± 0.4	2.2 ± 0.4
TMTA+TPT	5.0 ± 0.7	11.0 ± 0.7	16.0 ± 0.7
TMHA+TPT	6.5 ± 0.8	14.0 ± 0.8	20.0 ± 0.8
TMDA+TPL	1.5 ± 0.4	2.0 ± 0.4	2.5 ± 0.4
TMTA+TPL	3.5 ± 0.6	8.5 ± 0.6	14.5 ± 0.6
TMHA+TPL	5.5 ± 0.7	10.0 ± 0.7	14.5 ± 0.7

increased with increasing concentration of alkyl organotins (TAT) until a concentration of about 3 mM, the increase became stronger with longer alkyl chains. For the most effective compound TPT the increase was 5-fold. Above 3 mM concentration α/α_0 did not change, even decreased for TPT. The curve describing the action of TPhT runs between the curves for TET and TPT. In the case of organolead compounds (TAL) as follows from

Fig. 1b, the relative rate constant increased with concentration only for TPL, and the effect was weaker than for TPT.

The differences in effectiveness between organotins and organoleads in their action on calcium ion desorption from lecithin liposome membranes seem to result from properties of both the groups of compounds and properties of the medium and membrane. Within each group of trialkyl compounds an increase in chain length results in increased hydrophobicity, and hence increased partition coefficient between the membrane and medium. Though the molecular size increases also, imparting lower mobility and partition coefficient, but surely the increased hydrophobicity is the dominant factor. However, in the case of phenyl compounds the dominant factor is their size, which is substantially greater than that of other compounds. The same sequence of effectiveness of organometallics has been observed for processes and objects other than those studied here (Gray *et al.*, 1987; Boyer, 1989).

Under our experimental conditions, pH 7.5, organotins are present in non-ionic forms as $R_3SnOH \times H_2O$, while organoleads exist almost exclusively as $R_3Pb(H_2O)_2^+$ (Tobias, 1978). The positively charged membrane, due to calcium ion adsorption, is easier accessed by the non-ionic molecules than their positively charged counterparts. The unionic molecules locate inside membrane, inducing structural changes, weaken the ionic bounds between Ca^{2+} and membrane with resulting desorption. The ionic molecules locate in the hydrophilic part of the membrane, or in the adjacent hydrophobic layer, so that Ca^{2+} ions are released as a result of competition for binding sites. Among the organolead ions only TPL with the longest alkyl chain can anchor in the membrane and effectively compete with calcium ions for binding sites.

The following Figures and Tables present the dependence of α/α_0 on concentration not only for the tested mixtures of the organometallic compounds with QAS compounds but also, for comparison, include curves and data for QAS compounds acting alone (results published previously by Kuczera *et al.*, 1996). Among the QAS compounds chosen for the study of the cooperate effect only TMDA does not exert any action at concentrations up to 3.5 mM. Selection of this compound allowed to differentiate the cooperation of the respective organometallic compounds with long-chain cationic compounds.

In Table II are presented values of the relative rate constant α/α_0 for a chosen concentration, C = 2 mM, of organotin compounds, TMDA and their mixture. As documented, a long-chain cationic compound present in the membrane caused similar results for almost all organotins, namely a decreased efficacy of the compounds on the desorption process. The decrease for TMT, TET and TPhT was found at almost the same level, which is slightly lower than the efficacy of TMDA alone. Only for TPT the effectiveness is a little greater than for TMDA alone. From the results obtained for the organoleads it can be concluded that the compounds TML, TEL and TPhL in mixtures with TMDA in liposome dispersion act almost the same as TMDA alone, while TPL was found to be markedly more effective when in mixture with TMDA.

It is evident that for the weaker acting organometallic compounds of tin and lead, i.e. TMT, TET, TPhT and TML, TEL, TPhL, the interaction is dominant between the membrane and TMDA. As a result of incorporation of the cationic compound into the membrane its positive potential on the surface increases, and for the weakly acting cationic compounds the membrane is even more difficult to access. For the stronger acting propyltin, TPT, the cooperation of these molecules with TMDA molecules is sufficient to overcome the calcium-membrane binding forces and increased desorption results for both TPT and TMDA when applied separately. This corresponds to the „threshold effect" observed earlier for TMDA in its separate action (Kuczera *et al.*, 1988), indicating that its effect begins above a certain concentration. The observed lowering of the effectiveness of the TMDA+TPT mixture relative to TPT may result from a more difficult incorporation into the membrane of TPT molecules in ionic and non-ionic forms due to competition with TMDA molecules present in the membrane.

For the most effective of the organometallic compounds, i.e. TPT and TPL, their cooperation with cationic compounds that differ in their hydrophobic and hydrophilic parts has been investigated.

Fig. 2 presents the results obtained for mixtures of TPT with three amphiphilic cationic compounds

that differ in their polar heads. For comparison the dependence of α/α_0 on concentration of the compounds studied is shown when applied separately. As follows from Fig. 2, all mixtures operate weaker than the ammonium salts acting separately, but several times stronger than the organometallic compounds. The action of all the mixtures is almost the same. For mixtures of TPL with a cationic compound the courses of the relationships are very similar to those for mixtures with tripropyltin. The substantial weakening of the effectiveness of QAS+TPT mixtures, seen in Fig. 2, compared with the single action of the compounds, may result from structural changes in the membrane after incorporation of organometallic compounds. Due to their large cross-section the organometallic molecules may displace QAS molecules from the membrane binding centres, and thus weaken the interaction between the centres and QAS molecules.

The considerable differences in the effects of QAS compounds with different polar parts were reduced when present in mixtures with compounds TPT and TPL. This seems to be due to the dominating role of molecular size of the organometallics compared with the structural differences between the respective QAS molecules.

At variance to the results presented in Table II for TMDA, TPT compounds and their mixture TMDA+TPT, for the strongly acting QAS a stronger mixture effect was observed than with the organometallic compounds alone. It is thus apparent that the efficacy of QAS compounds is the dominant factor.

Table II presents data for mixtures of compounds TPT and TPL with two groups of compounds: alkylbenzylammonium chlorides and alkyltrimethylammonium chlorides, which differ in their alkyl chains lengths. Values of the relative rate constant α/α_0 for three chosen concentrations of the compounds studied are documented. Analysing the results, it can be stated that for mixtures of the homologous series of quaternary ammonium salts with the two organometallic compounds the dependence on chain length of QAS gives a clearer differentiation of the results than the dependence on polar head differences. In all the cases, aside of the TMDA+TPT and TMDA+TPL mixtures previously analysed, a lowering in the efficacy of QAS compounds in the presence of organometallic compounds is observed, or increased efficacy of organometallic compounds in the presence of QAS. The cut-off effect (Gabrielska *et al.* 1981; Balgavy and Devinsky, 1996), observed for separately acting QAS compounds, (with a maximum of effectiveness observed mostly for 14-carbon-atom chains), is disturbed by the addition of organometallic compounds. For the mixture BDTA+TPL this effect is still observed, although for the other mixtures a steady increase with QAS alkyl chain length is observed. As in the cases presented in Fig. 2, the greatest changes in efficacy of mixtures in comparison with the compounds alone are observed for BDTA, which is the most effective compound for both groups. Apparently the location of the compound molecules, whose polar heads are nearest to the membrane active centres and thus to Ca^{2+} ions, apparently is caused by disturbances of the organometallic compounds that are incorporated into the membrane.

The results presented in this paper indicate both synergism and antagonism in the action of compounds of the two groups. By proper choice of an organometallic compound and an amphiphilic quaternary ammonium salt one can obtain either an increase or decrease in the rate constant of calcium ion desorption from phospholipid membranes. In view of our previous studies, showing a far-reaching coincidence between standard tests on fungicidal compounds and the effect of biologically active compounds on ion desorption process from liposome membranes (Gabrielska *et al.* 1979; Rucka *et al.*, 1980), one can expect that synergism of the groups of compounds studied may be of importance for problems of environment protection. Thus, if we want to weaken the action of organometallic compounds, very weakly acting cationic compounds should be applied in addition. If, instead, increased efficacy is needed, cationic amphiphilic compounds of strong action should be used. In case biological tests confirm the mutual influence of the groups of compounds studied, the method may be applied for decontamination of soils polluted with heavy metals, by cultivating proper plants (Wierzbicka and Panufnik, 1988).

Acknowledgements

This work was supported by the Polish Research Committee (KBN) Grant No. 6P04G4313.

Balgavy P. and Devinsky F. (1996), Cut-off effects in biological activities of surfactants, Adv. Colloid Interface Sci. **66**, 23–63.

Blunden S. J. Cusak P. A. and Hill R. (1985), The Industrial Uses of Tin Compounds, The Royal Society of Chemistry, London.

Boyer I. J. (1989), Toxicity of dibutyltin, tributyltin and other organotin compounds to humans and experimental animals. Toxicology **55**, 253–298.

Craig P. I. (1982), Environmental aspects of organometallic chemistry. In: Comprehensive Organometallic Chemistry (Wilkinson G., Stone F. G. A., Abel E. W., eds.). Pergamon Press, Oxford, 979–1019.

Crowe A. J. (1987), Organotin compounds in agriculture since 1980. Part I. Fungicidal, bactericidal and herbicidal properties. Appl. Organomet. Chem. **1**, 143–155.

Davis B., and Jordan P. (1989), Industrial application of surfactants II, In: Karsa, D. R. (Ed.), Cationic Surfactants, Royal Society of Chemistry, Cambridge, Special publication, No. **77**, 195–210.

Fent K. (1996), Ecotoxicology of organotin compounds. Crit. Rev. Toxicol. **26**, 1–117.

Gabrielska J., Grobelny D., Kuczera J., Przestalski S., Witek S. and Żyłka R. (1979), Effect of some benzylammonium chlorides on sulphate and calcium ion transport across liposome membrane. Stud. Biophys. **77**, 193–200.

Gabrielska J., Kuczera J., Oswięcimska M., Przestalski S. and Witek S. (1981), Effect of alkyl chain length in alkoxymethylene trimethylammonium chlorides on ion transport across liposome membranes. Stud. Biophys. **82**, 149–155.

Gray B., Porvaznik M., Flemming C. and Lee L. (1987), Organotin-induced cell hemolysis, shape transformations and intramembranous aggregates in human erythrocytes. Cell Biol. Toxicol. **3**, 23–38.

Kuczera J. and Żyłka R. (1979), Calcium ion binding to lecithin vesicles. Stud. Biophys., **75**, 25–33.

Kuczera J., Fogt A. and Witek S. (1988), Calcium ion desorption from lecithin membrane influenced by amphiphilic mono- and bis-ammonium chlorides. Stud. Biophys. **128**, 169–177.

Kuczera J., Chojnacki H., Kral T. E. and Przestalski S. (1996), Effect of amphiphilic cationic compounds on calcium ion desorption from lecithin liposome membranes. Kinetic studies and quantum chemical calculations, Z. Naturforsch. **51c**, 219–225.

Kubica K., Fogt A., Kuczera J., (1994), Influence of amphiphilic anionic and cationic mixture on calcium ion desorption from lecithin liposomes. II Computer simulation, Polish Journal of Environ Studies, **Vol. 3**, 4, 37–41.

Kuczera J., Gabrielska J., Kral T. E. and Przestalski S. (1997), A synergistic effect of selected organotin compounds and ionic surfactants on liposome membranes. Appl. Organomet. Chem. **11**, 591–600.

Lindstedt M., Allenmark R., Thompson R. A. and Edebo L. (1990), Antimicrobial activity of betaine esters, quanternary ammonium amphiphiles which spontaneously hydrolyze into nontoxic components. Antimicrob. Agents Chemiother. **34**, 1949–1954.

Mazgis B., and Kuczera J. (1981), A graphical method of evaluation of the ionic permeability constant of the single bilayer liposome membrane based on the compartmental analysis. Stud. Biophys. **82**, 35–46.

Rubingh D. N. and Holland P. M. (1991), Cationic Surfactants. Physical Chemistry, **Vol 37**, Surfactant science series, Marcel Dekker Inc. New York, 325–436.

Rucka M., Oswięcimska M., Pawlaczyk-Szpilowa M. and Witek S.(1980), New biocides for cooling water treatment. I. Selected quaternary ammonium salts. Environ. Protection Eng. **6**, 455–463.

Singleton W. S., Gray M. S., Brown M. L. and White J. L. (1965), Chromatographically homogeneous lecithin from egg phospholipids. J. Am. Oil Chem. Soc. **42**, 53–56.

Tobias S. R. (1978), The chemistry of organometallic cations in aqueous media. Am. Chem. Soc. 130–147.

Weder H. D. and Zumbuhl O. (1984), In: Liposome Technology, Preparation of Liposomes (Gregoriadis G., ed.). CRC Press, Boca Raton, Florida, **Vol. I**, 79–107.

Wierzbicka M. and Panufnik D. (1988), The adaptation of *Silene vulgaris* to growth on a calamine waste heap (S. Poland). Environ. Pollution **101**, 415–426.