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## MODIFICATION OF MECHANICAL PROPERTIES OF MODEL MEMBRANES BY SOME BIFUNCTIONAL SURFACTANTS

HALINA KLESZCZYŃSKA, JANUSZ SARAPUK  
and BOŻENNA RÓŻYCKA-ROSZAK

Agricultural University, Department of Physics and Biophysics, Norwida 25,

**Abstract:** Interaction of two series of new surfactants with an incorporated anti-oxidant functional group with erythrocytes and planar lipid membranes was studied. Surfactants were synthesized in order to be potentially used as common bio-cides or as agents protecting biological and/or model membranes against lipid peroxidation. Both applications need the use of such bifunctional surfactants in significantly different concentrations. The aim of this work was to find the concentration range in which surfactants studied could be used as biocides. Two different models were chosen in order to do it; pig erythrocyte and asolectin planar membranes. The studied parameters of these models were hemolysis of red blood cells and stability of BLM in the presence of the compounds studied, i. e., the parameters describing mechanical properties of model membranes used. Additionally, the role of the counterions in the interaction of bifunctional surfactants with model membranes was studied. It was found that both homologous series of the surfactants influence model membranes to different degree depending on the length of their hydrophobic part and the kind of counterion. In the latter case it seems that the differences in the hydrated radii of bromide and chloride ions, and hence the differences in their ability to modify electrostatic interaction between the lipid polar heads and compounds studied, are responsible for the effects observed.

**Key Words:** Bifunctional Surfactants, Erythrocytes, Planar Lipid Membranes, Counterions

## INTRODUCTION

The bifunctional surfactants studied can be used as biocides or as antioxidants preventing peroxidation of biological and /or model membranes. It has been found that practical concentrations of this type compounds depends on the application. When a bifunctional surfactant is to be used as an agent that kills microorganisms then its concentration usually is about one to two orders of magnitude higher in comparison with those used in the second of the mentioned applications, i. e., when it is to be used as antioxidant protecting effectively biological or model membranes against peroxidation and its consequences [1-5]. The act of destroying a microorganism by amphiphilic surfactants begins at the cell membrane as a place of first contact and depends on many various factors. Those connected with surfactants are polarity, which in turn depends on steric effects of the polar part of surfactant, its net charge and charge density and on the hydrophobicity which depends on the number and length of alkyl chains of a surfactant. Both, hydrophilicity and hydrophobicity can be modified by presence of different intermediate or interspacial groups introduced between polar and hydrophobic part of an amphiphilic surfactant. The significance of all these factors in the interaction between surfactants and model membranes was thoroughly studied [6-12] and conclusions drawn apply to biological activity of surfactants. It was also found that the type of the counterion is an important factor in the interaction of surfactants with model and biological membranes [2, 13]. The aim of this work was twofold: to determine the concentrations of surfactants studied that cause irreversible breakdown of model membranes studied, i. e., concentrations at which surfactants can be used as effective biocides and to find and explain possible differences in the interaction of chloride and bromide types of surfactants with the membranes.

## MATERIALS AND METHODS

Analytically pure compounds were studied. They were N-(3,5-di-*t*-butyl-4-hydroxy) phenylcarboxyethyl-N-alkyl-piperidinium bromides (PPPA-n) and N-(3,5-di-*t*-butyl-4-hydroxy)phenylethylcarboxyethyl-N-alkoxymethylmorpholinium chlorides (PPME-n) of structures shown in Fig. 1. They were synthesized

purity and structure of bifunctional surfactants were checked by NMR spectra and elemental analysis.

Fresh heparinized pig blood was used in hemolytic experiments. Blood was centrifuged for 3 min at 1000×g, the plasma removed and the cells washed twice with isotonic phosphate buffer solution (131 mM NaCl, 1.79 mM KCl, 0.86 mM MgCl<sub>2</sub>, 11.79 mM Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O, 1.80 mM NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O) of pH 7.4. The erythrocytes were then incubated for half an hour at 37 °C in the same solution containing different concentrations of compounds studied. Four

different hemato-crites were used (2%, 4%, 6% and 8%). To bring about the same hemolysis (100%) different concentrations of the compounds were needed at different hema-tocrites. The linear dependence of hematocrit on the concentration enabled us, by extrapolation, to calculate the unit hematocrit (1%). After modification samples were taken, centrifuged and the supernatant was assayed for hemoglobin content using Spekol 11 (Carl Zeiss, Jena) spectrophotometer at 540 nm. The hemoglobin concentration in the supernatant of totally hemolyzed erythrocytes was a measure of the extent of hemolysis. Good mixing of the suspension during all procedure stages was insured.

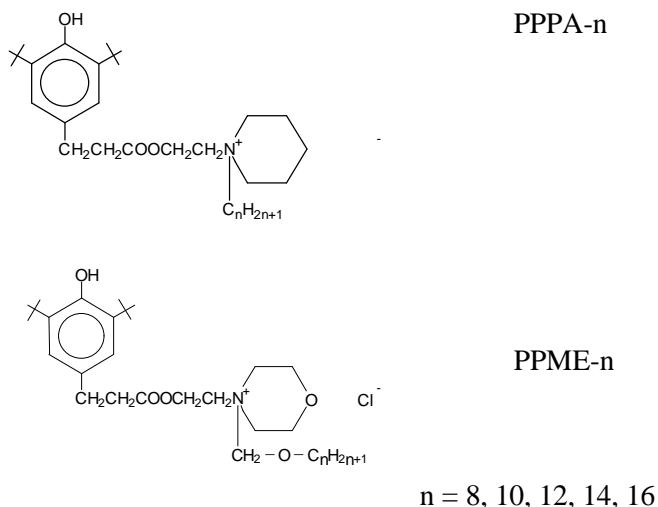


Fig. 1. General formulas of the bifunctional surfactants studied.

Planar lipid membranes (BLM) were formed from a solution of 1.5% (w/v) azolectin (Sigma Chem. Co.) in n-butanol:n-decane (1:1) on a 1.7 mm hole in the partition of two-compartmental measurement cell filled with 0.9% NaCl bath solution. Bifunctional surfactants were pipetted into bath solution until their concentrations reached values that caused breakdown of BLMs in no more than 3 min. These concentrations are further on referred as critical concentrations (CC).

Measurements were performed at room temperature (about 22 °C) and BLM were monitored optically and electrically. The details of the experimental set-up were given earlier [5].

## RESULTS AND DISCUSSION

The results of hemolytic experiments are shown in Fig. 2, which presents the values of the concentrations of both homologous series of bifunctional

surfactants that cause 100% hemolysis of red blood cell ( $C_{100}$ ) per unit hematocrit. The  $C_{100}$  parameter was chosen because of its qualitative similarity to the CC parameter in BLM experiments. Both used parameters describe total membrane disruption. The values of  $C_{100}$  for all hematocrites are tabulated in Tab. 1.

Tab. 1. The values of the concentration of bifunctional surfactants causing 100% hemolysis of red blood cells ( $C_{100}$ ).

Compound	Concentration $C_{100}$ [mM]			
	Hematocrit [%]			
	2	4	6	8
PPPA-8	4.00	4.60	5.45	6.20
PPPA-10	2.70	3.20	3.80	4.25
PPPA-12	0.85	1.15	1.60	2.00
PPPA-14	0.70	1.00	1.30	1.75
PPPA-16	0.60	0.85	1.20	1.65
PPME-8	2.65	3.00	3.45	4.00
PPME-10	1.90	2.35	2.80	3.35
PPME-12	1.00	1.40	1.65	2.00
PPME-14	0.65	1.00	1.35	1.60
PPME-16	0.40	0.70	1.05	1.50

Results of BLM experiments are shown in Fig.3. Planar azolectin membranes were highly stable in the absence of the surfactants studied. This stability diminished gradually as concentration of surfactant in the measurement chamber increased.

The results show that both PPPA-n (bromides) and PPME-n (chlorides) surfactants destabilize used model membranes. When their concentrations were high enough the surfactants caused total destruction of the membranes. The CC and  $C_{100}$  values depended on the length of the hydrophobic part of compounds. This dependence was not so sharp as that observed for other surfactants [6-8]. It is rather similar to that found for another homologous series of bifunctional surfactants [3] and is probably due to the fact that these homologous series of surfactants studied had very large polar parts. Apparently this large polar head does not allow a surfactant molecule to incorporate into the bilayer structure of model membranes as deeply as in the case of surfactants of smaller polar heads. It may result in weaker hydrophobic interaction between surfactant alkyl chain and acyl chains of lipid molecules of model membranes. The elongation of the surfactant hydrophobic part only slightly influences the localization of surfactant molecule in the model membranes and its efficiency in the destabilization of these membranes. However, the observed increase in the

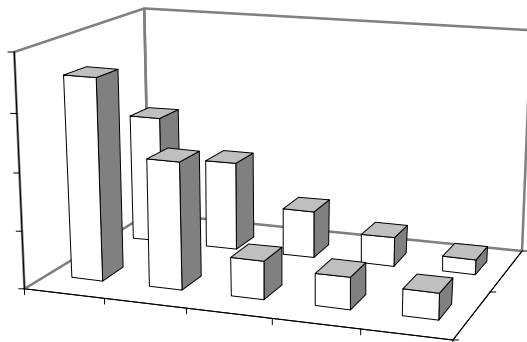


Fig. 2. The values of the concentration of bifunctional surfactants causing 100% hemolysis of red blood cells per unit hematocrit.

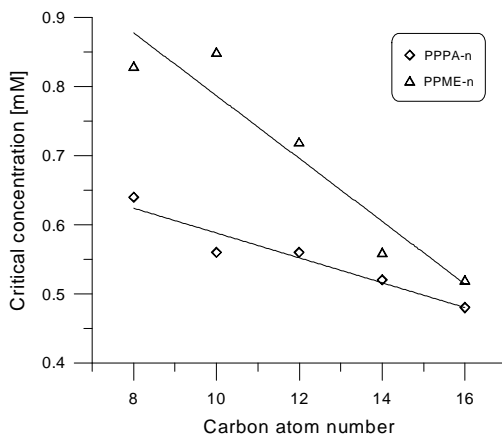


Fig. 3. The dependence of critical concentrations (CC) of bifunctional surfactants on the length of hydrocarbon chain in BLM experiments. Standard deviation was 0.02.

efficiency of model membrane destabilization with the elongation of hydrophobic part of bifunctional surfactants is apparently also influenced by the partition coefficient that favours long-chain amphiphiles. Under the same concentration conditions more molecules of a long-chain compound may incorporate into the membranes than molecules of a short-chain compound. It must be noted here that PPME-n compounds have inbuilt oxymethylene group between their alkyl chain and the polar head. It results in an elongation of their hydrophobic part because oxymethylene group is equivalent to two methylene group as it was shown earlier [7]. No intermediate group was incorporated into PPPA-n compounds. In such a case one would expect slightly stronger

interaction of PPME-n surfactants with model membrane than PPPA-n surfactants. The results of hemolysis experiments show that the interaction is similar for both series of bifunctional surfactants, especially for the compounds possessing  $C_{12}H_{25}$  and longer alkyl chains. It seems that the hydrophobic chain elongation effect is compensated by the fact that PPPA-n compounds are bromides. This is especially evident in the case of BLM experiments where bromides were shown to be more effective in destabilizing planar membranes than corresponding chlorides. Similar results were obtained earlier for another chloride and bromide type of surfactants [13].

The reason for such differentiation between chlorides and bromides may be smaller hydrated radius of bromide ion and resulting greater mobility of this ion [14] and thus a greater ability to weaken electrostatic interactions between polar heads of lipids and compounds. This may facilitate incorporation of cationic bifunctional surfactants into model membranes used. The conclusion finds support in results of experiments on cationic-anionic films adsorbed from equimolar solutions of these ions pointing to stronger modifying possibilities of bromides in comparison with chlorides [15, 16]. Additionally, one must take into account that morpholinium group of PPME-n compounds is more hydrophilic than piperidinium one of PPPA-n surfactants which should result in a deeper incorporation of the latter compounds into the bilayer structure of the model membranes used. This is confirmed by the rough qualitative estimation of polarity of both types of surfactants studied done by comparison of the obtained linear dependencies between critical concentration and hydrophobic chain length of the surfactants. It is seen, especially in the short-chain part of the plot, that much greater concentration of PPME-n compound than that of PPPA-n is needed to destroy model membranes. It may mean that PPME-n compounds do not intercalate membranes so deeply as PPPA-n and destabilize them less efficiently. The reason may be the greater polarity of PPME-n compounds.

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