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*Original Research*

# The Role of Counterions in the Interaction of Some Cationic Surfactants with Model Membranes

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**Abstract**

This paper studies the interaction of benzyltrimethyl- and dodecyltrimethylammonium bromides and chlorides with red blood cells and planar lipid membranes. The aim of the work was to find whether counterion type influences that interaction. The model membranes were pig erythrocytes and planar lipid membranes (BLM). A change of mechanical properties of these membranes was observed in the presence of the compounds studied. The measured parameters were a degree of hemolysis of erythrocytes and stability of BLM's.

The critical concentration of a compound was the measure of BLM stability .The lifetime of BLMs under such concentrations of a compound did not exceed 3 min. It was found in each case that bromides influenced model membranes slightly stronger than the corresponding chlorides. A possible explanation of the results obtained is presented. Namely, it seems that the greater efficiency of bromides to destabilize model membranes in comparison with chlorides can be attributed to greater mobility and smaller radius of the hydrated bromide ion. This can be the reason why bromide anions modify surface potential of model membranes more efficiently, which in turn leads to more intensive interaction of bromides with such modified bilayer in comparison to their analog chlorides.

**Keywords:** erythrocytes, hemolysis, planar membranes, stability, surfactants, counterions.

## Introduction

It was shown that the efficiency of amphiphilic substances to modify model membranes depends on various factors. These are: polarity, which depends on steric properties of the polar head of a compound and its net charge or charge distribution, and lipophilicity, which depends on the number and length of alkyl chains of the compound [1, 2]. Lipophilicity of a compound can be changed by incorporating an intermediate group between the polar head of the compound and its lipophilic alkyl chain. The same applies to so-called gemini compounds built from two molecules bridged by an interspatial group (a spacer) incorporated between their polar heads [3, 4].

The above formulated conclusions drawn from model experiments also apply to biological activity of amphiphilic compounds [5-8]. However, it was found in both biological and model experiments that the interaction of amphiphilic compounds with membranes depends on the type of the salt these compounds constitute. The most often used amphiphiles are chlorides and bromides. It was found that the type of counterion must be included in a group of factors that decide the efficiency of the interaction of a compound with membranes [9-12].

This paper contains some results obtained on the interaction of two pairs of quaternary ammonium salts with model membranes (planar lipid membranes and erythrocytes). Each pair consisted of a chloride and bromide of the same cationic amphiphile. One of the studied pairs had a dodecyl alkyl chain instead of methylene and benzyl groups. Thus, both pairs exhibited significantly different lipophilicities. They were synthesized to be used predominantly as biologically active compounds.

The aim of the work was to determine whether or not replacing a chloride compound with its bromide analog significantly influenced the interaction of the compound with model membrane and, if so, what the possible explanation of the differences in the membrane modyfying efficiencies of the salts studied might be.

## Materials and Methods

Fresh heparinized pig erythrocytes were used in hemolytic experiments. The blood was centrifuged for 3 min at 1000g, the plasma removed and the cells washed four times with an isotonic phosphate (131 mM NaCl, 1.79 mM KCl, 0.86 mM $MgCl_2$, 11.79 mM $Na_2HPO_4$ x $2H_2O$, 1.80 mM $NaH_2PO_4$ x $H_2O$ ) or citrate solution (3.63 mM $Na_2HPO_4$

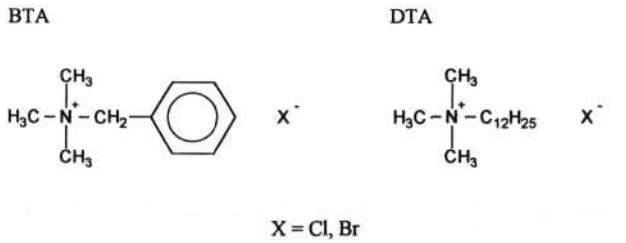


Fig. 1. General formulae of the surfactants studied.

x $2H_2O$). In both cases pH was 7.4. The erythrocytes were then treated for half an hour at 37°C with the same solutions containing various concentrations of the compounds studied. Four different hematocrites were used (2%, 4%, 6% and 8%). After modification samples were taken, centrifugated and the supernatant assayed for hemoglobin content using a spectrophotometer (Spekol 11, Carl Zeiss, Jena) at 540 nm wavelength. The measure of the extent of hemolysis was hemoglobin concentration in the supernatant of totally hemolyzed cells.

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 a partition of a two-compartmental chamber filled with a 0.9% NaCl bath solution. 0.01 M solutions of the compounds studied were prepared and proper volumes of these solutions were pipetted directly into the measurement chamber until compound concentrations reached a value that caused BLM breakdown in no more than 3 min. These concentrations are later on referred to as critical ones (CC).

Dodecyltrimethylammonium (DTA) and benzyltrimethylammonium (BTA) chlorides and bromides (Fig. 1), were obtained from Aldrich Chemical Co. and Kodak.

## Results

Azolectin BLMs used in the experiments were highly stable in the absence of the surfactants studied. This stability, the measure of which was the lifetime of BLM, gradually decreased as surfactant concentration in the bath solution increased. The obtained values of critical concentrations (CC) of the compounds, i. e. concentrations causing destruction of BLMs in less than 3 minutes are tabulated in Tab. 1.

Table 1. Critical concentrations (CC) of benzyltrimethylammonium (BTA) and dodecyltrimethylammonium (DTA) salts.

| CC [mM] | | |
|---|---|---|
| Compound | Chloride | Bromide |
| BTA | 2.80 | 2.40 |
| DTA | 0.12 | 0.11 |

Values represent the mean of 3 experiments. Standard deviation was 0.02

The hemolytic experiments revealed that benzyltrimethylammonium salts did not cause significant hemolysis of erythrocytes up to 0.01 M concentrations of these compounds. The highest hemolysis observed at this concentration was about 6%. Nevertheless, BTA bromide-induced hemolysis was slightly greater than that induced by BTA chlorides. Typical results of the hemolytic experiments are

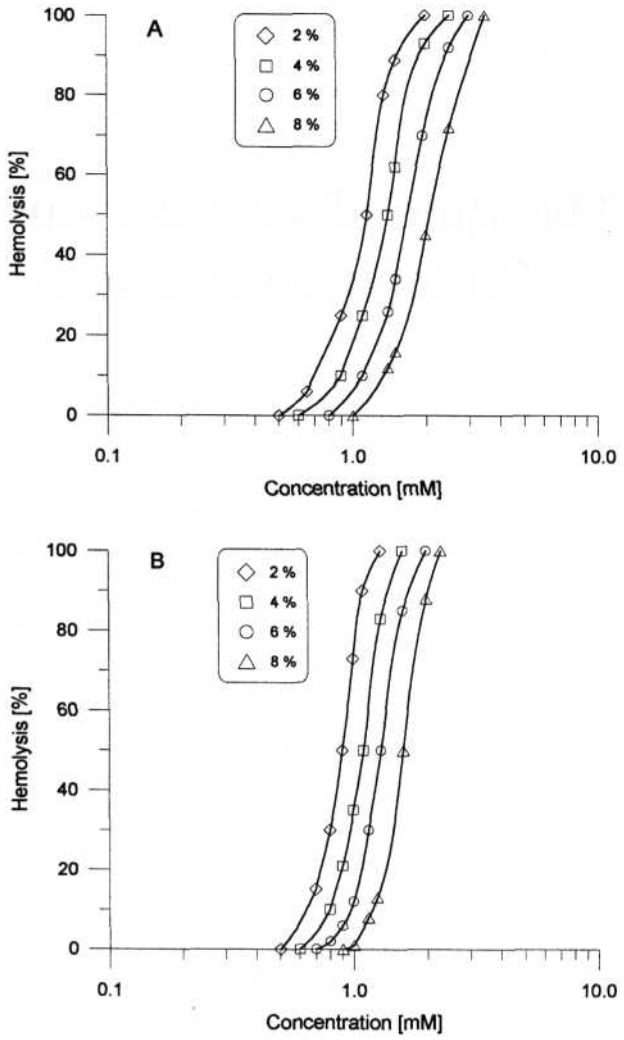


Fig. 2. Dependence of the degree of red blood cell hemolysis on concentrations of DTA chloride (A) and bromide (B).

shown in Fig. 2 for DTA chloride (A) and DTA bromide (B). Citrate buffer was used in these experiments.

Hemolytic curves enabled an estimation of the concentrations of compounds causing 100% hemolysis ($C_{100}$). Such an approach was chosen because the $C_{100}$ is qualitatively similar to the CC parameter determined in BLM experiments. Namely, both parameters describe total membrane disruption. Estimated values of $C_{100}$ are given in Tab. 2.

Tab. 2. Concentrations of the compounds studied causing 100% hemolysis of red blood cells ($C_{100}$).

| $C_{100}$ [mM] | | | | | |
|---|---|---|---|---|---|
| Buffer | Compound | Hematocrit [%] | | | |
| | | 2 | 4 | 6 | 8 |
| Phosphate | DTA bromide | 1.20 | 1.40 | 1.65 | 2.00 |
| | DTA chloride | 1.50 | 1.60 | 1.80 | 2.30 |
| Citrate | DTA bromide | 1.30 | 1.60 | 2.00 | 2.30 |
| | DTA chloride | 2.00 | 2.50 | 3.00 | 3.50 |

Values represent the mean of 3 experiments. Standard deviation was 0.05.

## Discussion

Both the BLM and erythrocyte studies show that the surfactants studied act as destabilizers of model membranes when used in high enough concentrations. It was shown that the destruction of membranes depended mainly on the length of the hydrophobic part of a compound. BTA compounds devoid of long hydrophobic alkyl chains were destabilizing the used model membranes significantly less than DTA compounds. It was also found that the interaction of both types of compounds depended on the kind of their counterion and the aqueous environment they were dissolved in. The hemolytic experiments showed that citrate buffer stabilized suspended erythrocytes and higher concentrations of the compounds were needed to cause the same hemolytic effects as those observed for suspension of erythrocytes in phosphate buffer. However, in both cases bromide compounds were found to be more efficient in destabilizing model membranes than chloride ones. The effect can be connected with greater possibilities of bromide anions to modify a potential barrier at the model membranes surfaces. Namely, one can assume that bromide ions can modify electric properties of the model membrane surface to a greater extent than chloride ions, enabling the surfactants to interact more intensively with those membranes. In monolayer adsorption experiments stronger modifying possibilities of bromide were found [13-15]. It was shown in those experiments that at a constant concentration of inorganic ions there is a greater increase in cationic surfactants adsorption to cationic-anionic monolayers in the presence of NaBr than in the presence of NaCl.

The difference in the effects of ions of the same valency can be explained by their different binding to charged surfaces resulting from different polarizability of various anions or their hydration and mobility [16]. The smaller effective radius of a bromide ion permits it to interact stronger with positively charged groups of membrane surfaces and such screening of positive charges facilitates the incorporation of a cationic surfactant into the membrane. The results of studies on the interactions of some bifunctional surfactant bromides and chlorides with erythrocytes confirm the above conclusions [17].

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