

Short communication

## GEMINI ESTER QUAT SURFACTANTS AND THEIR BIOLOGICAL ACTIVITY

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**Abstract:** Cationic gemini surfactants are an important class of surface-active compounds that exhibit much higher surface activity than their monomeric counterparts. This type of compound architecture lends itself to the compound being easily adsorbed at interfaces and interacting with the cellular membranes of microorganisms. Conventional cationic surfactants have high chemical stability but poor chemical and biological degradability. One of the main approaches to the design of readily biodegradable and environmentally friendly surfactants involves inserting a bond with limited stability into the surfactant molecule to give a cleavable surfactant. The best-known example of such a compound is the family of ester quats, which are cationic surfactants with a labile ester bond inserted into the molecule. As part of this study, a series of gemini ester quat surfactants were synthesized and assayed for their biological activity. Their hemolytic activity and changes in the fluidity and packing order of the lipid polar heads were used as the measures of their biological activity. A clear correlation between the hemolytic activity of the tested compounds and their alkyl chain length was established. It was found that the compounds with a long hydrocarbon chain showed higher activity. Moreover, the compounds with greater spacing between their alkyl chains were more active. This proves that they incorporate more easily into the lipid bilayer of the erythrocyte

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Abbreviations used: DMALM-12 – n-dodecylalaninate N,N,N-trimethyl ammonium bromide; DPH – 1,6-diphenyl-1,3,5-hexatriene; GP – generalized polarization; Laurdan – 6-dodecanoyl-2-dimethylaminonaphthalene; TMEAL-n (Br) – ethylene-bis-alanine-n-alkylesterquats bromides; TMPAL-n (Br) – 1,3-propylene-bis-alanine-n-alkylesterquats bromides

membrane and affect its properties to a greater extent. A better understanding of the process of cell lysis by surfactants and of their biological activity may assist in developing surfactants with enhanced selectivity and in widening their range of application.

**Key words:** Gemini surfactants, Cationic amphiphiles, Hemolytic activity, Membrane fluidity, Generalized polarization, Anisotropy, Erythrocyte membrane, Lipid packing order, Hemolysis, Fluorescent probes

## INTRODUCTION

The designing of novel surfactants with higher efficiency and specific properties for targeted applications is of great fundamental and practical importance. Various surfactant architectures have been described in recent literature [1-3], including a composition featuring two monomeric surfactant molecules chemically bonded together by a spacer group. These so-called cationic gemini or dimeric cationic surfactants constitute an important class of surface-active compounds [4-7]. Cationic surfactants with this architecture exhibit a much higher surface activity than their monomeric counterparts, can easily be adsorbed at interfaces, and can interact with the cellular membranes of microorganisms, giving them high antimicrobial activity. Owing to their special ability to self-assemble, they show great promise in various areas, such as gene transfection [8-11], drug entrapment and release [12-14], and vitamin solubilization [15], and as components of body care products [16] and antibacterial and antifungal formulations [17, 18]. Conventional cationic surfactants are highly chemically stable, but have poor chemical and biological degradability, which limits their use. Furthermore, due to their strong interactions with negatively charged surfaces, including the lipid membranes of cells, cationic surfactants are toxic to aquatic organisms and show hemolytic activity.

An interesting and useful strategy is the synthesis of surfactants with designs that mimic natural compounds, such as lipoaminoacids, phospholipids and glycolipids. Much attention has been directed towards amino acid-based surfactants as an important class of natural surface-active molecules. Such compounds have been the subjects of many studies [19, 20] because of the enormous potential for their use in pharmaceutical, cosmetic, household and food products. They show physicochemical properties that are similar to those of conventional cationic surfactants, but are more environmentally friendly and less toxic.

Another approach in the design of biodegradable surfactants is to insert a bond with limited stability (the so-called labile) between the polar head group and the hydrophobic tail of the surfactant. Such surfactants are often referred to as cleavable, but other terms, such as chemodegradable, destructible, triggerable, temporary, hydrolyzable and acid-sensitive or alkali-sensitive surfactants can also be found [21-24]. The best-known example of cleavable surfactants is the family of ester quats, which are cationic surfactants with a labile ester bond

inserted between the hydrocarbon tail(s) and the quaternary ammonium head group(s).

Cleavable compounds may represent a step forward in surfactant design, because of the growing need for new non-toxic and biodegradable compounds. This study is a continuation of our previous research on the synthesis and properties of both monomeric and dimeric (gemini) ester quats, which are molecular mimics of alanine derivatives. Such compounds have been found to be excellent inhibitors of ATPases [25] and MDR pumps [26] in yeast. They interact with natural and artificial biological membranes [27]. As part of our research, we investigated hemolytic activity and erythrocyte membrane fluidity in the presence of synthesized gemini ester quats. Table 1 shows the formulas of the investigated compounds. The obtained results were compared with those for the corresponding monomeric surfactant.

Table 1. Structural formulas of the investigated gemini ester quats and their linear counterparts.

Structure	Abbreviation
$  \begin{array}{c}  \text{H}_3\text{C}^+ \quad \text{CH}_3 \quad \text{O} \\    \quad   \quad    \\  \text{N}-\text{CH}-\text{C}-\text{O}-\text{C}_n\text{H}_{2n+1} \\    \\  \text{H}_3\text{C} \quad \text{CH}_2 \\    \\  \text{H}_3\text{C} \quad \text{CH}_2 \\    \\  \text{H}_3\text{C}^+ \quad \text{CH}_3 \quad \text{O} \\    \quad   \quad    \\  \text{N}-\text{CH}-\text{C}-\text{O}-\text{C}_n\text{H}_{2n+1}  \end{array}  $	<p>2 Br<sup>-</sup></p> <p>TMEAL-n (Br)</p> <p>n = 6, 8, 10, 12, 14</p>
$  \begin{array}{c}  \text{H}_3\text{C}^+ \quad \text{CH}_3 \quad \text{O} \\    \quad   \quad    \\  \text{N}-\text{CH}-\text{C}-\text{O}-\text{C}_n\text{H}_{2n+1} \\    \\  \text{H}_3\text{C} \quad \text{CH}_2 \\    \\  \text{H}_3\text{C} \quad \text{CH}_2 \\    \\  \text{H}_3\text{C}^+ \quad \text{CH}_3 \quad \text{O} \\    \quad   \quad    \\  \text{N}-\text{CH}-\text{C}-\text{O}-\text{C}_n\text{H}_{2n+1}  \end{array}  $	<p>2 Br<sup>-</sup></p> <p>TMPAL-n (Br)</p> <p>n = 6, 8, 10, 12, 14</p>
$  \begin{array}{c}  \text{H}_3\text{C}^+ \quad \text{CH}_3 \quad \text{O} \\    \quad   \quad    \\  \text{N}-\text{CH}-\text{C}-\text{O}-\text{C}_{12}\text{H}_{25} \\    \\  \text{H}_3\text{C} \quad \text{CH}_3  \end{array}  $	<p>Br<sup>-</sup></p> <p>DMALM-12</p>

## MATERIALS AND METHODS

### Materials

All of the starting materials and solvents were of commercial grade and were not additionally purified before use. 2-bromo-propanoyl bromide, hexanol, octanol, decanol, dodecanol, tetradecanol, *N,N,N',N'*-tetramethyl-ethylene diamine and

*N,N,N',N'*-tetramethyl-propylene diamine were purchased from Aldrich Chemical Co. (Milwaukee, WI).

### Synthesis of gemini ester quat surfactants

The first step in the synthesis of the linear ester quat DMALM-12 was the reaction of 2-bromo-propanoyl bromide with dodecanol. The obtained n-dodecyl 2-bromopropionate was then reacted with dimethylamine and quaternized with methyl bromide to form the final product. The details of the synthesis are described in [25].

The gemini ester quats TMEAL-*n* and TMPAL-*n* were synthesized in two steps using the method presented in Fig. 1. In the first step, intermediates – alkyl 2-bromopropionates with 6, 8, 10, 12 and 14 carbon atoms in their alkyl chain – were obtained by acylation of the appropriate alkanols with 2-bromo-propanoyl bromide. In the second step, the alkyl 2-bromopropionates were reacted with *N,N,N',N'*-tetramethyl-ethylene diamine (for TMEAL-*n* synthesis) or *N,N,N',N'*-tetramethyl-propylene diamine (for TMPAL-*n* synthesis). Bis-quaternization was performed in the acetonitrile solution, with the reaction mixture gently boiling.

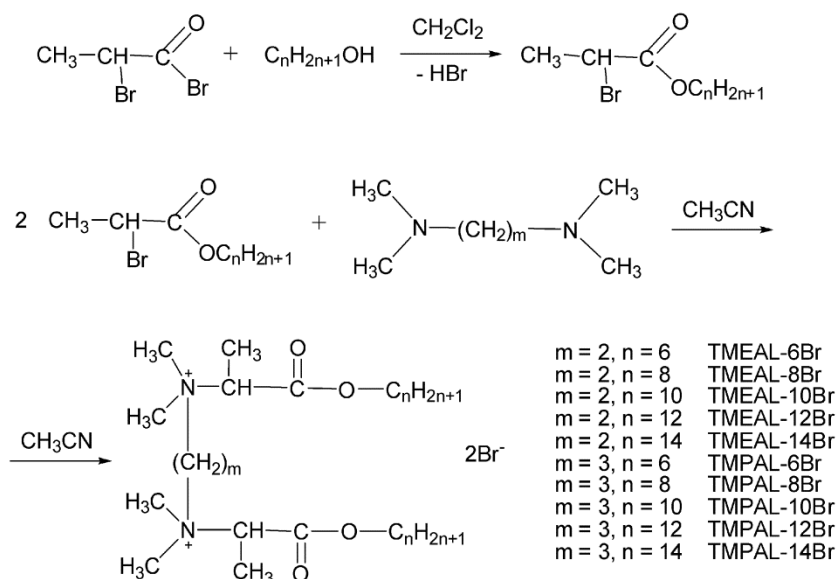


Fig. 1. Synthetic route for the synthesis of gemini ester quat surfactants.

### Synthesis of alkyl 2-bromopropionates

In a 500-cm<sup>3</sup> three-necked round-bottom flask equipped with a condenser, a dropper and a mechanical stirrer, 0.5 mole of an appropriate alkanol was placed in 400 cm<sup>3</sup> of dichloromethane. The condenser was connected to two absorbers, one empty and one filled with sodium hydroxide solution for hydrogen bromide absorption. The reaction mixture was gently heated until simmering point (mild boiling), and 0.7 mole of 2-bromo-propanoyl bromide in

100 cm<sup>3</sup> of dichloromethane was slowly added. The reaction mixture was gently heated under reflux and stirred for 8 h. Finally, the reaction mixture was cooled to room temperature, shaken with 200 cm<sup>3</sup> of saturated NaHCO<sub>3</sub> solution, and washed with 200 cm<sup>3</sup> of water. After separation, the organic layer was dried under anhydrous MgSO<sub>4</sub> for 24 h. Then the mixture was filtered off and the solvent was evaporated. All the propionates were obtained as liquids at a yield of 75-83%.

#### **Quaternization of tetramethyl-diamines with 2-bromopropionates**

In a 250-cm<sup>3</sup> three-necked round-bottom flask, 0.1 mole of *N,N,N',N'*-tetramethyl-ethylene diamine or *N,N,N',N'*-tetramethyl-propane diamine was placed in 100 cm<sup>3</sup> acetonitrile. The mixture was gently boiled and 0.21 mole of the appropriate 2-bromopropionate in 100 cm<sup>3</sup> acetonitrile was dropped in. The reaction mixture was heated with stirring for 30 h and then cooled and filtered off. The yields of the crude products amounted to 35-40%. The crude reaction products were purified by repeated crystallization from organic solvents (selected individually). The structures of the purified ester quats were confirmed by nuclear magnetic resonance <sup>1</sup>H NMR (Bruker Avance 300 MHz, CDCl<sub>3</sub>, TMS, δ ppm). The <sup>1</sup>H NMR spectra of TMEAL-12Br and TMPAL-12Br (shown as examples) are described as follows:

**TMEAL-12Br:** 0.828-0.860 [6H, t, 2(-CH<sub>3</sub>)<sub>2</sub>]; 1.235 [36H, d, 2(-CH<sub>2</sub>-)<sub>9</sub>]; 1.578-1.660 [4H, m, 2{-O-CH<sub>2</sub>-CH<sub>2</sub>-}]; 1.778-1.797 [6H, t, 2(CH-CH<sub>3</sub>)]; 3.500-3.539 [12H, d, 2(N-CH<sub>3</sub>)<sub>2</sub>]; 3.759 [4H, t, N-CH<sub>2</sub>-CH<sub>2</sub>-N]; 4.114-4.226 [4H, d, 2(-O-CH<sub>2</sub>)]; 4.595 [2H, s, 2(-N-CH)].

**TMPAL-12Br:** 0.819-0.864 [6H, t, 2(-CH<sub>3</sub>)<sub>2</sub>]; 1.225 [36H, d, 2(-CH<sub>2</sub>-)<sub>9</sub>]; 1.565-1.664 [4H, m, 2{-O-CH<sub>2</sub>-CH<sub>2</sub>-}]; 1.775-1.800 [6H, t, 2(CH-CH<sub>3</sub>)]; 2.690 [2H, s, N-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-N]; 3.500-3.539 [12H, d, 2(N-CH<sub>3</sub>)<sub>2</sub>]; 3.911 [4H, t, N-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>]; 4.116-4.250 [4H, d, 2(-O-CH<sub>2</sub>)]; 4.663 [2H, s, 2(-N-CH)].

#### **Erythrocytes**

This study used pig erythrocytes and isolated erythrocyte membranes (ghosts) prepared from fresh pig blood by the Dodge method [28]. The pig red blood cell membrane is known to be closest to the human erythrocyte membrane with respect to its lipid composition. Samples of fresh pig blood were taken into a physiological solution with heparin added.

#### **Hemolysis**

The experiments were conducted on fresh heparinized blood. Full blood was centrifuged for 3 min at 2500 rev/min and 4°C to remove plasma and leucocytes. The obtained erythrocytes were washed three times with a cool (ca. 4°C) isotonic phosphate solution at pH 7.4 (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 Na<sub>2</sub>H<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O). Then the washed erythrocytes were suspended in a mixture of buffer solution (pH 7.4) and aqueous solutions of the studied compounds at defined concentrations. The extent of hemolysis was measured at 2% hematocrit. The prepared samples were subjected to modification in the presence of the investigated compounds for 1 h at 37°C. After

modification, 2 ml of an isotonic phosphate solution at pH 7.4 were added, the samples were centrifuged for 15 min at 2500 rev/min, and the supernatant was assayed for hemoglobin content using a spectrophotometer (Specord® 40, AnalytikJena) at a wavelength of 540 nm. Hemoglobin concentration in the supernatant, expressed as a percentage of the hemoglobin concentration in the supernatant of the totally hemolyzed cells, was assumed to be the measure of the extent of hemolysis.

### Fluorimetric method

The fluorimetric method was used to study the effect of the compounds on the packing arrangements of lipids in the erythrocyte membrane ghosts. The ghosts were suspended in an amount of isotonic phosphate solution at pH 7.4 such that the protein concentration in the samples amounted to approximately 100 µg/ml. The control samples contained an erythrocyte ghost suspension and a fluorescent probe, while the investigated samples also contained defined concentrations of the studied compounds. Fluorescence intensity was measured using two fluorescent probes, Laurdan and DPH (purchased from Molecular Probes), at concentrations of 10 µM, while the concentrations of the compounds were in the range of 10-50 µM. The above-mentioned fluorescent probes were used since each of them embeds itself in a different region of the lipid bilayer: DPH in the hydrophobic region and Laurdan in the hydrophilic region. Such differentiated incorporation of the probes gives an insight into the structural changes caused by the incorporation of the studied compounds.

Measurements were taken at a temperature of 37°C with a CARY Eclipse fluorimeter (VARIAN) equipped with a Peltier DBS temperature controller (temp. accuracy ± 0.1°C). The excitation and emission wavelengths for the DPH probe were  $\lambda_{\text{ex}} = 360$  nm and  $\lambda_{\text{em}} = 425$  nm, respectively. For the Laurdan probe the excitation wavelength was 360 nm and the emitted fluorescence was recorded at two wavelengths:  $\lambda_{\text{b}} = 440$  and  $\lambda_{\text{r}} = 490$  nm. Fluorescence anisotropy for the DPH probes was calculated from the formula [29]:

$$A = \frac{(I_{\parallel} - GI_{\perp})}{(I_{\parallel} + 2GI_{\perp})} \quad (1)$$

where  $I_{\parallel}$  and  $I_{\perp}$  are fluorescence intensities observed in directions respectively parallel and perpendicular to the polarization direction of the exciting wave.  $G$  is an apparatus constant dependent on the emission wavelength.

Changes in the polar group packing arrangement of the hydrophilic part of the membrane were investigated using the Laurdan probe on the basis of generalized polarization (GP) and were calculated from the formula [30]:

$$GP = \frac{(I_{\text{b}} - I_{\text{r}})}{(I_{\text{b}} + I_{\text{r}})} \quad (2)$$

where  $I_{\text{b}}$  is fluorescence intensity at  $\lambda_{\text{b}} = 440$  nm and  $I_{\text{r}}$  is fluorescence intensity at  $\lambda_{\text{r}} = 490$  nm.

### Statistics

The results are presented in the form: mean value  $\pm$  standard deviation, calculated at confidence level  $\alpha = 0.05$  ( $p < 0.05$ ) from 5 independent measurements. A two-factor analysis of variance was carried out using ANOVA. The differences between the controls and the samples containing the investigated compounds were found to be significant on the basis of the Dunnett test. The calculations were performed using StartSoft STATISTICA 9.

## RESULTS AND DISCUSSION

### Synthesis

All of the studied gemini amphiphiles (TMEAL-n, TMPAL-n) were prepared by relatively simple reaction of the appropriate alkanols with 2-bromo-propanoyl bromide leading to the formation of alkyl 2-bromopropionates with 6, 8, 10, 12 and 14 carbon atoms in their alkyl chains, followed by quaternization of *N,N,N',N'*-tetramethyl-ethylene diamine or *N,N,N',N'*-tetramethyl-propylene diamine with the obtained alkyl 2-bromopropionates to form the final products. The purity and the structures of the synthesized compounds were assessed by  $^1\text{H}$  NMR spectroscopy. All the salts were water-soluble white solids.

### Hemolysis

Due to their amphiphilic character, the studied compounds incorporate into the lipid phase of the membrane of erythrocytes and at certain concentrations induce hemolysis [31-34]. The alkyl chains of the studied compounds penetrate the hydrophobic lipid bilayer of the erythrocyte membrane due to hydrophobic interaction, while the polar heads of the compounds remain in the hydrophilic part of the membrane. When incorporated into the membrane, the compounds with longer chains weaken the interaction between the lipid molecules, causing a disturbance in erythrocyte membrane structure leading to hemolysis. The dependence between the percentage of hemolysis and the compound concentrations in the solution was determined based on the results (Fig. 2). Using the obtained plots, the compounds concentrations that caused 50% hemolysis ( $C_{50}$ ) were found. The  $C_{50}$  values (Table 2) were adopted as the measure of hemolytic activity.

Table 2. Compound concentrations that caused 50% hemolysis of erythrocytes ( $C_{50}$ ).

Compound	$C_{50}$ (mM)	Compound	$C_{50}$ (mM)
TMEAL-14Br	1.00	TMPAL-14Br	0.10
TMEAL-12Br	0.34	TMPAL-12Br	0.06
TMEAL-10Br	0.57	TMPAL-10Br	0.09
TMEAL-8Br	9.09	TMPAL-8Br	1.30
TMEAL-6Br	> 10	TMPAL-6Br	5.15
DMALM-12	0.62		

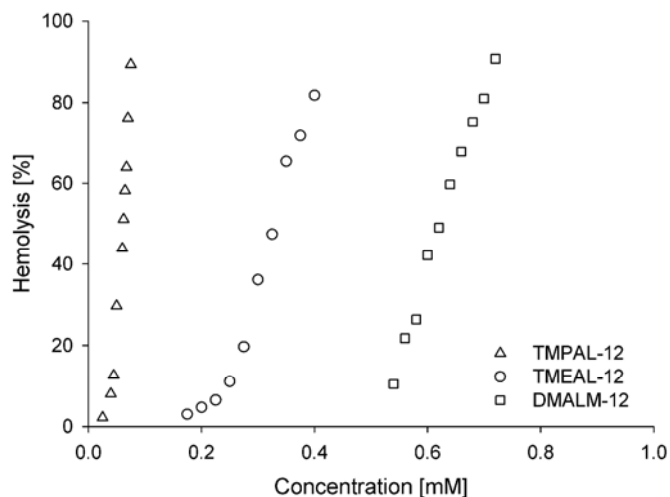


Fig. 2. Percentage of hemolysis as a function of surfactant concentration.

For both of the studied gemini ester quat homologous series (TMEAL-*n* and TMPAL-*n*), the hemolytic activity depends on the alkyl chain length. The amphiphiles with 10 and 12 carbon atoms in their alkyl chains exhibit the highest hemolytic activity, whereas those with shorter chains (TMEAL-6Br and TMEAL-8Br) induce hemolysis only at very high surfactant concentrations (> 9mM). Unlike for the other compounds, for the 14-carbon compound (TMEAL-14Br), the hemolytic activity does not correlate with the alkyl chain length. For comparison, the monomeric counterpart (DMALM-12) of the studied gemini surfactants (TMEAL-12Br and TMPAL-12Br) shows lower hemolytic activity than the dimeric structures.

The gemini derivatives of *N,N,N',N'*-tetramethyl-ethylene diamine (TMEAL-*n*) show lower hemolytic activity (higher  $C_{50}$ ) than the corresponding *N,N,N',N'*-tetramethyl-propylene diamine (TMPAL-*n*) derivatives. This is probably due to the length of the spacer group in the investigated gemini structures. The spacer group in TMPAL-*n* compounds is longer, so the hydrocarbon chains are spaced at a larger distance than in the case of TMEAL-*n*, allowing for easier incorporation of the amphiphiles into the membrane of the erythrocytes, which leads to higher hemolytic activity.

#### Fluorimetric study

The effect of the studied compounds on the fluidity and density of the lipids in the erythrocyte membrane was investigated using the fluorimetric method with different fluorescence probes. The fluidity of erythrocyte lipids in the hydrophobic region was investigated with the DPH probe (Table 3) and the packing order of the membrane lipids was described by the general polarization (GP) of the Laurdan probe (Table 4).



Table 3. Fluorescence anisotropy of the DPH probe for erythrocyte ghosts modified with the studied compounds.

Compound	Concentration ( $\mu\text{M}$ )				
	10	20	30	40	50
Control	0.235 $\pm$ 0.015				
TMEAL-14Br	0.222 $\pm$ 0.008	0.210 $\pm$ 0.011	0.193 $\pm$ 0.014	0.186 $\pm$ 0.017	0.175 $\pm$ 0.016
TMEAL-12Br	0.222 $\pm$ 0.012	0.199 $\pm$ 0.012	0.185 $\pm$ 0.014	0.177 $\pm$ 0.011	0.159 $\pm$ 0.010
TMEAL-10Br	0.236 $\pm$ 0.006	0.219 $\pm$ 0.006	0.212 $\pm$ 0.010	0.199 $\pm$ 0.012	0.198 $\pm$ 0.013
TMEAL-8Br	0.241 $\pm$ 0.008	0.237 $\pm$ 0.005	0.235 $\pm$ 0.005	0.229 $\pm$ 0.008	0.228 $\pm$ 0.009
TMEAL-6Br	0.249 $\pm$ 0.005	0.241 $\pm$ 0.007	0.239 $\pm$ 0.006	0.233 $\pm$ 0.007	0.231 $\pm$ 0.008
TMPAL-14Br	0.192 $\pm$ 0.003	0.164 $\pm$ 0.008	0.144 $\pm$ 0.010	0.132 $\pm$ 0.012	0.125 $\pm$ 0.011
TMPAL-12Br	0.201 $\pm$ 0.001	0.171 $\pm$ 0.008	0.156 $\pm$ 0.006	0.144 $\pm$ 0.007	0.134 $\pm$ 0.011
TMPAL-10Br	0.220 $\pm$ 0.007	0.202 $\pm$ 0.004	0.200 $\pm$ 0.008	0.191 $\pm$ 0.002	0.186 $\pm$ 0.002
TMPAL-8Br	0.236 $\pm$ 0.013	0.227 $\pm$ 0.010	0.220 $\pm$ 0.005	0.213 $\pm$ 0.008	0.205 $\pm$ 0.008
TMPAL-6Br	0.243 $\pm$ 0.007	0.240 $\pm$ 0.006	0.235 $\pm$ 0.003	0.233 $\pm$ 0.003	0.230 $\pm$ 0.006
DMALM-12	0.232 $\pm$ 0.002	0.224 $\pm$ 0.003	0.221 $\pm$ 0.003	0.219 $\pm$ 0.007	0.218 $\pm$ 0.003

Table 4. Values of generalized polarization of the Laurdan probe for erythrocyte membranes modified with the studied compounds.

Compound	Concentration ( $\mu\text{M}$ )				
	10	20	30	40	50
Control	0.329 $\pm$ 0.008				
TMEAL-14Br	0.302 $\pm$ 0.007	0.300 $\pm$ 0.007	0.280 $\pm$ 0.006	0.274 $\pm$ 0.006	0.265 $\pm$ 0.006
TMEAL-12Br	0.229 $\pm$ 0.005	0.209 $\pm$ 0.005	0.160 $\pm$ 0.004	0.140 $\pm$ 0.003	0.110 $\pm$ 0.003
TMEAL-10Br	0.260 $\pm$ 0.006	0.256 $\pm$ 0.006	0.210 $\pm$ 0.005	0.212 $\pm$ 0.005	0.209 $\pm$ 0.005
TMEAL-8Br	0.327 $\pm$ 0.008	0.326 $\pm$ 0.008	0.322 $\pm$ 0.007	0.318 $\pm$ 0.007	0.293 $\pm$ 0.007
TMEAL-6Br	0.338 $\pm$ 0.008	0.343 $\pm$ 0.008	0.343 $\pm$ 0.008	0.342 $\pm$ 0.008	0.328 $\pm$ 0.008
TMPAL-14Br	0.402 $\pm$ 0.009	0.094 $\pm$ 0.002	0.010 $\pm$ 0.001	-0.022 $\pm$ 0.001	-0.068 $\pm$ 0.002
TMPAL-12Br	0.082 $\pm$ 0.002	-0.030 $\pm$ 0.001	-0.091 $\pm$ 0.002	-0.131 $\pm$ 0.003	-0.186 $\pm$ 0.004
TMPAL-10Br	0.242 $\pm$ 0.006	0.221 $\pm$ 0.005	0.209 $\pm$ 0.005	0.192 $\pm$ 0.004	0.108 $\pm$ 0.003
TMPAL-8Br	0.324 $\pm$ 0.007	0.320 $\pm$ 0.008	0.308 $\pm$ 0.007	0.283 $\pm$ 0.007	0.256 $\pm$ 0.006
TMPAL-6Br	0.309 $\pm$ 0.007	0.309 $\pm$ 0.007	0.305 $\pm$ 0.008	0.304 $\pm$ 0.007	0.250 $\pm$ 0.006
DMALM-12	0.252 $\pm$ 0.009	0.188 $\pm$ 0.004	0.155 $\pm$ 0.008	0.133 $\pm$ 0.008	0.117 $\pm$ 0.008

At concentrations significantly lower the  $C_{50}$ , i.e., those that did not cause hemolysis, the studied compounds were found to have structurally determined changes on the membrane fluidity and lipid arrangements in the hydrophilic region of the membrane. The single-chain compound DMALM-12 does not change the fluidity (measured as fluorescence anisotropy) in the hydrophobic

part of the bilayer, but substantially diminishes the packing order (measured as generalized polarization, GP) in the hydrophilic part of the membrane. TMEAL-6Br and TMEAL-8Br do not change the GP or fluidity. The other investigated compounds, especially TMEAL-12Br, diminish the packing order of the membrane in the polar heads region. TMPAL-14Br and TMPAL-12Br caused even greater changes (negative GP values), resulting in membrane destruction.

In general, the changes in the hydrophobic region are markedly smaller than the changes in packing order in the hydrophilic region. TMPAL-6Br and TMPAL-8Br do not alter GP or fluidity, while the other compounds diminish membrane packing in the polar heads region. The changes in the polar heads region are substantially larger than those in the alkyl chain region.

The compounds enter both the hydrophilic and hydrophobic parts of the membrane, as indicated by the changes in the values of anisotropy and GP. The extent of the changes depends on the location of the polar head and the depth of compound incorporation, which is mainly connected with alkyl chain length.

## CONCLUSIONS

Hemolytic activity and changes in the fluidity and packing order of membrane lipid polar heads were adopted as measures of the biological activity of the studied ester quat compounds. The compounds induce hemolysis, which indicates that they incorporate into the membrane, easily causing disturbances in its structure by weakening the interaction between the membrane components. If the number of molecules incorporated is high enough, this leads to membrane destruction and hemolysis. It follows from the biological activity of the two homologous series of gemini ester quats (TMEAL-*n* and TMPAL-*n*) that the distance between the alkyl chains has an effect on the biological activity of the compounds. The results of our investigations indicate that compounds with larger spacing between the alkyl chains are more active, proving that they incorporate more easily into the lipid bilayer of the erythrocyte membrane and affect its properties to a greater extent. A better understanding of surfactant biological activity and the process by which surfactants cause cell lysis may assist in developing surfactants with enhanced selectivity and in widening their range of application.

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