Alkylated 1,4-diazabicyclo[2.2.2]octanes: self-association, catalytic properties, and biological activity*

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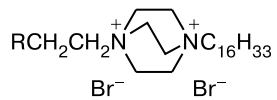
Aggregation of 1-hexadecyl-4-aza-1-azoniabicyclo[2.2.2]octane bromide in the presence of diethyl 4-nitrophenyl phosphate was studied using ^{1}H NMR spectroscopy. The quantitative characteristics of the aggregation were determined. The data obtained were used to explain the catalytic effect of micelles on the hydrolysis of the phosphate. It was found that the aggregation properties and biological activity of alkylated mono- and dicationic 1,4-diazabicyclo-[2.2.2]octanes are correlated.

Key words: alkylated 1,4-diazabicyclo[2.2.2]octanes, association, critical micelle concentration, aggregation number, hydrolysis, biological activity.

Solutions of surfactants are widely used in modern technology, including such areas as the synthesis of nanoparticles and mesoporous materials, dispersion of carbon nanotubes, oil extraction, catalysis, biodiagnostics, *etc*.[1—5] Cationic surfactants are of particular research interest for a number of reasons. Having a hydrophobic fragment and positively charged groups, cationic surfactants can insert themselves into lipid bilayers and efficiently interact with intracellular membranes, the phosphate groups of nucleic acids, and other negatively charged biosubstances. This makes cationic surfactants very attractive for design of nonviral vectors, carriers for drugs and diagnostic agents, and antimicrobial medicines. Cationic surfactants are traditionally employed in micellar catalysis for decomposition of organophosphorus ecotoxicants *via* basic hydrolysis.[6] At the same time, the head groups of cationic surfactants substantially vary in chemical structure, including the charged atom (N, P, or S) and its substituents; the presence of cyclic head groups is also possible. This allows systematic investigations of homologous series to find structure—property correlations. The use of surfactants containing aryl[7] and various functional substituents in the head group,[8] as well as passing from hexadecyl(trimethyl)-ammonium bromide to hexadecylpyridinium bromide,[9] enhances the catalytic activity of micellar systems in the hydrolysis of phosphorus acid esters. Only few cationic surfactants with a bicyclic head group have been documented as micelle-forming catalytic agents.[10,11] At the same time, such surfactants are better ligands to metal ions and thus can enhance the catalytic activity of systems by forming metallomicelles[10] rather than ordinary micelles; in addition, such surfactants can be functionalized and transformed from the mono- to dicationic type.[11]

In the present work, we studied the catalytic and biological activities of some cationic surfactants (**1**—**6**) containing a bicyclic fragment in the head group and analyzed the results obtained with consideration of their micelle-forming and solubilizing properties.

1—4



5, **6**

n = 12 (**1**), 14 (**2**), 16 (**3**), 18 (**4**)
R = H (**5**), OH (**6**)

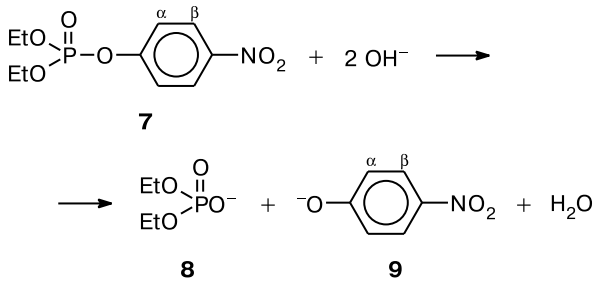
For our catalytic activity studies, we carried out basic hydrolysis of diethyl 4-nitrophenyl phosphate (**7**) in a micellar solution of monocationic 1,4-diazabicyclo[2.2.2]-octane (**3**) (Scheme 1). Prior to this experiment, we examined the aggregation of cationic surfactant **3** in the presence of phosphate **7** by FT-NMR spectroscopy with a pulsed magnetic field gradient (PFG FT-NMR) to esti-

* Dedicated to Academician of the Russian Academy of Sciences V. N. Charushin on the occasion of his 60th birthday.

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 1, pp. 110—118, January, 2012.

mate the influence of the solubilizate on the aggregation properties of the surfactant and quantitatively describe the binding of phosphate **7** by micelles **3**. The hydrolysis kinetics of phosphate **7** was studied by ^{1}H and ^{31}P NMR spectroscopy and UV spectrophotometry. In addition, we tested the biological activity of mono- and dicationic 1,4-diazabicyclo[2.2.2]octane derivatives **1**—**6** and studied its relationship with the aggregation properties of the surfactants under consideration.

Scheme 1



Experimental

Compounds **1**—**4** were prepared from 1,4-diazabicyclo-[2.2.2]octane (DABCO) and appropriate alkyl bromides as described earlier.[12]

1-Ethyl-4-hexadecyl-1,4-diazoniabicyclo[2.2.2]octane dibromide (5). A solution of cationic surfactant **3** (3 g, 7.2 mmol) and ethyl bromide (7.8 g, tenfold excess) in acetonitrile (30 mL) was refluxed for 10 h. After the reaction was completed, the solvent and unreacted ethyl bromide were removed. The precipitate of the salt that formed was dissolved in a small amount of ethanol, reprecipitated from the hot solution with acetone, and dried *in vacuo*. Yield 2.95 g (78%), m.p. 216—218 °C. IR (KBr), ν/cm^{-1}: 2960, 2920, 2850, 1464, 1397, 1113, 1058, 855, 806, 724. ^{1}H NMR (D_2O), δ: 0.85 (t, 3 H, $N^+CH_2CH_2(CH_2)_{13}C\underline{H}_3$, J = 6.6 Hz); 1.28—1.40 (m, 26 H, $N^+CH_2CH_2(C\underline{H}_2)_{13}CH_3$)); 1.44 (t, 3 H, $N^+CH_2\underline{CH}_3$, J = 7.3 Hz); 1.88 (br.s, 2 H, $N^+CH_2C\underline{H}_2(CH_2)_{13}CH_3$); 3.70—3.71 (m, 4 H, $N^+C\underline{H}_2CH_2$-$(CH_2)_{13}CH_3$ + $N^+C\underline{H}_2CH_3$); 4.05—4.15 (m, 12 H, 2 $N^+(C\underline{H}_2)_3$).

4-Hexadecyl-1-hydroxyethyl-1,4-diazoniabicyclo[2.2.2]-octane dibromide (6). A solution of cationic surfactant **3** (1 g, 2.4 mmol) and 2-bromoethanol (0.359 g, 1.2-fold excess) in acetonitrile (20 mL) was refluxed for 20 h. The precipitate of the salt that formed was filtered off, recrystallized from ethanol, and dried *in vacuo*. Yield 0.82 g (63%), m.p. 190—192 °C. IR (KBr), ν/cm^{-1}: 3346, 2958, 2920, 2851, 1468, 1398, 1378, 1123, 1087, 1056, 865, 722. ^{1}H NMR (D_2O), δ: 0.86 (t, 3 H, $N^+CH_2CH_2(CH_2)_{13}C\underline{H}_3$, J = 8.6 Hz); 1.27—1.39 (m, 26 H, $N^+CH_2CH_2(C\underline{H}_2)_{13}CH_3$); 1.86 (br.s, 2 H, $N^+CH_2C\underline{H}_2$-$(CH_2)_{13}CH_3$); 3.79 (s, 2 H, $CH_2C\underline{H}_2OH$); 4.11—4.18 (m, 14 H, 2 $N^+(C\underline{H}_2)_3$ + $N^+C\underline{H}_2CH_2OH$).

Diethyl 4-nitrophenyl phosphate (**7**) (Sigma) was used as purchased.

The dependence of the self-diffusion coefficient D of the surfactant molecules and solubilizate (phosphate **7**) molecules was measured on a Bruker AVANCE 400 FT-NMR spectrometer with a pulsed gradient G of the polarizing magnetic field. The spectrometer was equipped with a Pulsed Field Gradient attachment producing gradient strengths up to 0.53 T m^{-1}. The D values were determined from the decline of the integral intensity of the stimulated spin echo signals for the protons of different groups in the alkyl and cyclic fragments of the surfactant; the decline was caused by a field gradient change in a sequence of three 90°-pulses:

$$I(G) = I_0 \exp\{-(\gamma\delta G)^2 D[\Delta - (\delta/3)]\}, \quad (1)$$

where γ is the gyromagnetic ratio of the nucleus (proton), δ is the gradient pulse length, and Δ is the pulse spacing. Depending on the magnitude of the self-diffusion coefficients measured, the constant times δ and Δ were varied from 5 to 10 ms and from 50 to 70 ms, respectively. These times are much longer than the time of molecular exchange between the free and micellar components of the solution. The coefficients D were determined by averaging the values obtained from the ^{1}H NMR signals for different fragments of the surfactants and for the phenyl protons of solubilizate **7**. The errors of D determination were ~2 and ~5% at high and low concentrations of the surfactant, respectively. The temperature of samples was maintained with a thermostatic system of the spectrometer.

^{1}H and ^{31}P NMR spectra were recorded on a Bruker AVANCE 400 instrument (162 MHz); ^{31}P chemical shifts were referenced to H_3PO_4 as the external standard.

The kinetics of the reactions was studied by measuring the increasing optical density of the absorption band of the 4-nitrophenolate anion at 400 nm on a Specord UV-Vis spectrophotometer in temperature-controlled cells. The apparent reaction rate constants k_{app} (s^{-1}) were determined from a first-order equation. The initial concentration of compound **7** was $5 \cdot 10^{-5}$ mol L^{-1}.

Results and Discussion

The plots of the coefficients D of surfactant **3** and the solubilizate (phosphate **7**) in D_2O *vs.* the total concentration of the surfactant C_t are shown in Fig. 1. Below the critical micelle concentration (CMC), the D values for the surfactant and the solubilizate are constant ($4.2 \cdot 10^{-10}$ and $6.2 \cdot 10^{-10}$ m^2 s^{-1}, respectively) (see Fig. 1, inset). Therefore, the molecules of surfactant **3** and phosphate **7** at sub-CMC concentrations diffuse in solution like free monomeric species. In addition, the vicinity of the inflectional areas (see Fig. 1, inset) suggests that the self-diffusion of solubilizate molecules is related to the self-diffusion of surfactant molecules forming micelles.

According to the published data,[13] CMC can be determined most precisely from a plot of D_{obs} *vs.* $1/C_t$ (Fig. 2). The breakpoints on plots *1* and *2* correspond to the surfactant concentrations of $9 \cdot 10^{-4}$ and $1.6 \cdot 10^{-3}$ mol L^{-1}, respectively. The higher CMC determined from plot *2* can be attributed to the fact that the decreasing self-diffusion coefficient of the surfactant reflects not only micellization but also the formation of premicellar aggregates that cannot solubilize organic substrates because of their low aggregation numbers. A similar discrepancy in CMC values is characteristic for probe-involving experiments.[14] Earli-

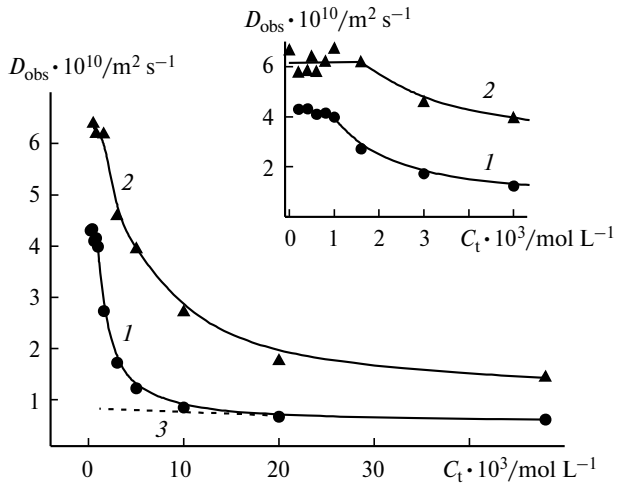


Fig. 1. Plots of the observed self-diffusion coefficient of cationic surfactant **3** (*1*) and phosphate **7** (*2*) *vs.* the surfactant concentration at 30 °C ($C_7 = 10^{-3}$ mol L^{-1}); the tangent to curve *1* at high surfactant concentrations (*3*). The inset: curves *1* and *2* at low surfactant concentrations.

er,[15] we have found that the CMC of cationic surfactant **3** in D_2O containing no substrate is $8.5 \cdot 10^{-4}$ mol L^{-1}; *i.e.*, the presence of phosphate virtually does not change the micelle-forming properties of the surfactant.

When analyzing the experimental results, we used the pseudophase model of micellization, or the two-state model.[13,16,17] Within this approach, the observed D value of the surfactant and the solubilizate (provided that the free and micellar components of a solution undergo rapid exchange) can be represented as the contributions of molecules in the free (D_f) and micellar states (D_m):

$$D_{obs} = p_f D_f + p_m D_m, \quad (2)$$

where p_f and p_m are the relative contents of the free and micellar components in solution, respectively,

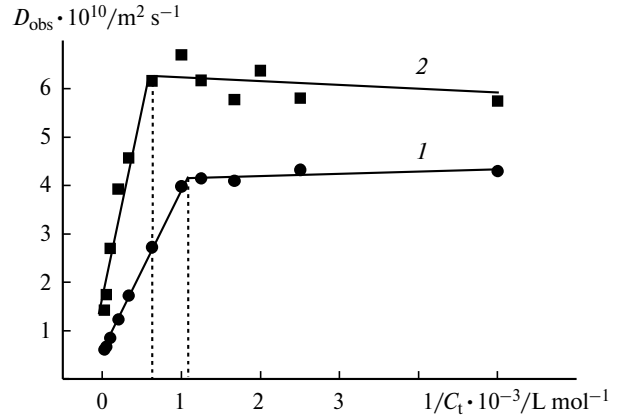


Fig. 2. Plots of the observed self-diffusion coefficient of cationic surfactant **3** (*1*) and phosphate **7** (*2*) *vs.* the reciprocal concentration of the surfactant at 30 °C.

$$p_f = C_f/C_t,\ p_m = C_m/C_t,\ p_f = 1 - p_m. \quad (3)$$

Using Eqs (2) and (3), one can derive an equation for the concentrations of the free (C_f) and micellar components (C_m) of the surfactant and the solubilizate:

$$C_f = C_t - C_m = C_t(D_{obs} - D_m)/(D_f - D_m). \quad (4)$$

It is thought that the self-diffusion coefficient of surfactant molecules is virtually insensitive to micellization and that its value at concentrations above CMC differs only slightly from that at CMC ($D_{f,CMC}$); for super-CMC values, an insignificant correction is applied to allow for the hindrances presented by micelles:

$$D_f = D_{f,CMC}(1 + \phi/2)^{-1},$$

where ϕ is the volume fraction of the micellar surfactant; $\phi = M(C_t - CMC)/\rho$, where M and ρ are the molar mass and density of the surfactant, respectively. Its molar mass (0.393 kg mol^{-1}) was determined with allowance for Br^- counterions bound, the binding degree taken to be 0.7.[18] The molar mass of the solubilizate is 0.275 kg mol^{-1}.

The coefficients D for micelles (D_m) were determined from a tangent *3* (see Fig. 1) at high concentrations of surfactant **3**. This approach is based on data[15] obtained with hexamethyldisiloxane as a hydrophobic probe. We found that the concentration dependence of D for the solubilizate (D_m) coincides with that for the surfactant **3** (D_{obs}) at $C_3 > 10^{-2}$ mol L^{-1}. At $C_3 = 2 \cdot 10^{-3}$—$1 \cdot 10^{-2}$ mol L^{-1}, the concentration dependence of D_m coincides with the tangent to the curve D_{obs} of the surfactant for $C_3 > \sim 10^{-2}$ mol L^{-1}.[15] With known D_{obs}, D_f, and D_m values, one can calculate by formula (4) the concentrations of the surfactant and the solubilizate in the free and micellar states.

The results obtained can be used to estimate the number of surfactant and solubilizate (phosphate) molecules in micelles. The volume of a micelle (V_m) is a sum of the volumes occupied by surfactant (V_{Surf}) and solubilizate molecules (V_{Ph})

$$V_m = V_{Surf} n_{Surf} + V_{Ph} n_{Ph}, \quad (5)$$

where $V_{Surf} = M_{Surf}/(\rho_{Surf} N_A)$ is the volume occupied by a surfactant molecule, $V_{Ph} = M_{Ph}/(\rho_{Ph} N_A)$ is the volume occupied by a solubilizate molecule in the micelle, M_{Surf} and M_{Ph} are the molecular masses of the surfactant and the solubilizate, ρ_{Surf} and ρ_{Ph} are the densities of the surfactant and the solubilizate, n_{Surf} and n_{Ph} are the numbers of surfactant and solubilizate molecules in the micelle, and N_A is Avogadro´s number. With consideration to the aggregation number of the micelle $N = n_{Surf} + n_{Ph}$, Eq. (5) can be rewritten as follows:

$$\frac{V_m}{N} = \frac{M_{Surf}}{\rho_{Surf} N_A} \frac{n_{Surf}}{n_{Surf} + n_{Ph}} + \frac{M_{Ph}}{\rho_{Ph} N_A} \frac{n_{Ph}}{n_{Surf} + n_{Ph}}. \quad (6)$$

If formula (6) is supplemented with a solubilization factor defined as $\beta = n_{Ph}/n_{Surf} = C_{m,Ph}/C_{m,Surf}$, where

$C_{m,Ph}$ and $C_{m,Surf}$ are the concentrations of solubilizate and surfactant molecules in micelles, then the expression for the micelle aggregation number takes the form

$$N = V_m N_A(1 + \beta)/[M_{Surf}/\rho_{Surf} + (M_{Ph}/\rho_{Ph})\beta]. \quad (7)$$

Under the assumption that micelles are spherical species, the micelle volume can be calculated by the formula $V_m = (4/3)\pi R^3$, where R is the radius of "dry" (*i.e.*, deprived of the hydration shell) micelles. With allowance for only one layer of hydrated water present in the hydration shell of micelles, the radius of dry micelles can be defined as a difference between the hydrodynamic radius of micelles R_h calculated by the Stokes—Einstein equation

$$R_h = kT/(6\pi\eta D_m),$$

and the diameter of water molecules d; so $R = R_h - d$. The viscosity coefficient of a micellar solution can be calculated by the Einstein—Simha formula[19]

$$\eta = \eta_0(1 + 2.5\phi),$$

where $\eta_0 = 0.969$ cP is the viscosity of the pure solvent (D_2O at 30 °C).[20] The densities of the surfactant and the solubilizate were taken to be 10^3 kg m^{-3}. The number of solubilizate molecules in micelles was calculated by the formula

$$n_{Ph} = N/(1 + 1/\beta).$$

The experimental and calculated parameters are summarized in Table 1. It can be seen that an increasing concentration of surfactant **3** in solution increases the relative fraction of surfactant molecules in micelles, the total concentration of micelle-bound phosphate molecules, and the radius and aggregation number of micelles but decreases the number of solubilizate molecules in micelles.

Using the data obtained, one can calculate the coefficient of partition of the solubilizate between the micellar and aqueous phases from the ratio of the corresponding molar concentrations: $C_{Ph}/(1 - C_{m,Ph})$, where C_{Ph} is the total concentration of phosphate **7** in solution.[18] It can be seen in Fig. 3 that the fraction of the micelle-bound phosphate on the initial segment of the plot is a linearly increasing function of the surfactant concentration with a tendency toward saturation. The partition coefficient calculated from the slope of the linear segment is 211, which agrees well with previous data.[18]

Table 1. Parameters of the system cationic surfactant **3**—phosphate **7**—D_2O at 30 °C*

$C_t \cdot 10^3$	$C_{m,Surf} \cdot 10^3$	$C_{m,Ph} \cdot 10^4$	$D_m \cdot 10^{11}$	R	β	N	n_{Ph}
mol L^{-1}			/m^2 s^{-1}	/Å			
3	2.2	2.9	7.6	28.3	0.134	151	18
5	4.3	4.5	7.0	30.3	0.095	183	16
10	9.5	6.3	6.6	32.5	0.066	224	14
20	19.5	8.0	6.2	34.3	0.040	261	10
48	47.0	8.5	5.2	37.5	0.018	338	6

* $C_7 = 10^{-3}$ mol L^{-1}.

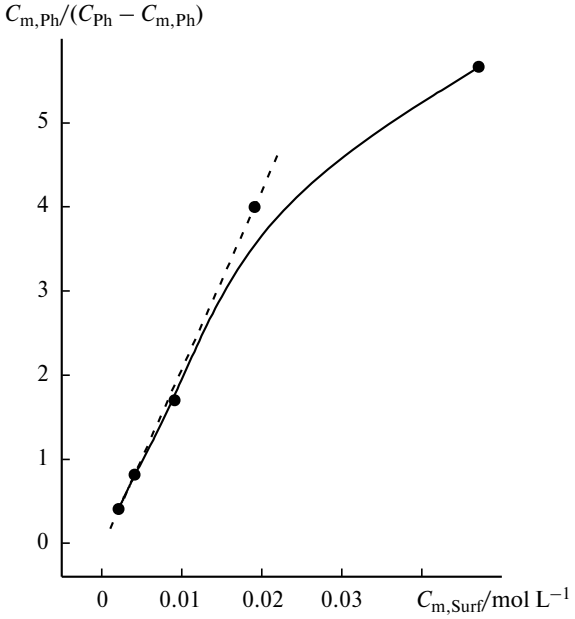


Fig. 3. Plot of the ratio of the molar concentrations of phosphate **7** in the micellar and aqueous phases *vs.* the concentration of cationic surfactant **3** in the micelles.

Solubilization by surfactant micelles changes not only the quantitative characteristics of the micellar system but also the reactivity of the solubilizate, which is evident from the basic hydrolysis of phosphate **7** (see Scheme 1).

The hydrolysis of phosphate **7** gives products **8** and **9**, regardless of the presence of the surfactant in the system (^{1}H and ^{31}P NMR data). The chemical shifts of their signals are given in Table 2.

The hydrolysis kinetics of phosphate **7** was studied by ^{1}H NMR spectroscopy; the data obtained are shown in Fig. 4. Both in the absence (see Fig. 4, *a*) and in the presence of surfactant **3** (see Fig. 4, *b*), we observed the decreasing intensity of the signal for phosphate **7** (the H_α and H_β protons) and the increasing intensities of the signals for products **8** (the OCH_2 protons) and **9** (the H_α and H_β protons) during the hydrolysis reaction (see Scheme 1). The apparent rate constants of the hydrolysis are $2.5 \cdot 10^{-4}$ and $9.1 \cdot 10^{-4}$ s^{-1} in the absence and in the presence of a 0.02 *M* solution of the surfactant, respectively; *i.e.*, a micellar solution of cationic surfactant **3** catalyzes the reaction by a factor of 3.6.

The catalytic effect of micelles **3** on the hydrolysis of phosphate **7** was confirmed by spectrophotometric measurements. The plot of the apparent rate constant of the hydrolysis of phosphate **7** *vs.* the surfactant concentration is shown in Fig. 5; the curve shows a distinct peak. Such a

Table 2. Chemical shifts δ of the ^1H and ^{31}P NMR signals for compounds **7**—**9** in solutions of cationic surfactant **3** (0.02 mol L^{-1}) at 35 °C

Compound	δ_P	δ_H[a]			
		H_α	H_β	OCH_2	CH_3
7	−1.59	7.41; 7.42[b]	8.31; 8.31[b]	4.29; 4.30[b]	1.33[b]
8	2.29	—	—	3.88; 3.89[b]	1.22[b]
9	—	6.40; 6.49[b]	7.95; 8.04[b]	—	—

[a] For the designations of the protons, see Scheme 1.
[b] The chemical shifts in the absence of the surfactant.

profile is typical of micelle-catalyzed processes,[18] suggesting the formation of a micelle—substrate complex *via* binding of phosphate molecules by surfactant aggregates. The kinetic data (see Fig. 5) were analyzed in terms of the pseudophase model of micellar catalysis:[18]

$$k_{2,app} = \frac{k_{2,0} + (k_{2,m}/V)K_{Ph}K_{Nu}(C_t - CMC)}{(1 + K_{Ph}C_t)[1 + K_{Nu}(C_t - CMC)]}, \quad (8)$$

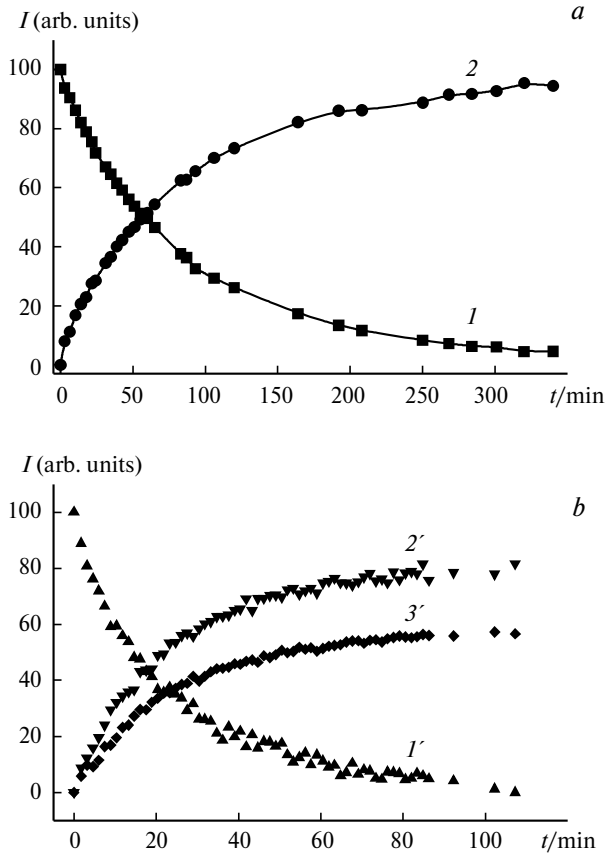


Fig. 4. Integral intensity profiles of the ^1H NMR signals for substrate **7** (*1*, *1′*) and reaction products **8** (*2*, *2′*) and **9** (*3*) during the hydrolysis of phosphate **7** in the absence (*a*) and in the presence of cationic surfactant **3** (*b*) at 35 °C ($C_7 = 10^{-3}$ mol L^{-1}, $C_3 = 0.02$ mol L^{-1}, $C_{NaOH} = 0.02$ mol L^{-1}).

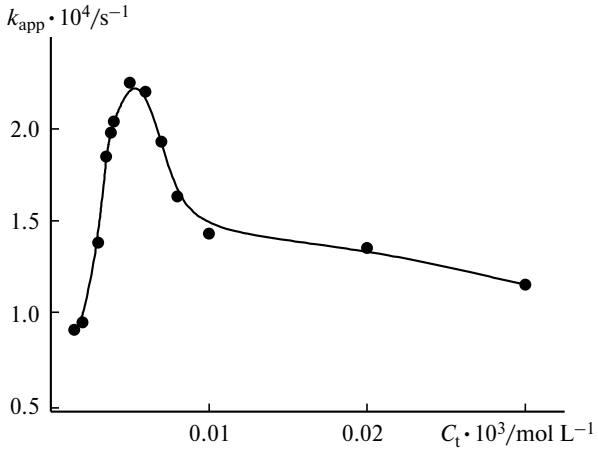


Fig. 5. Plot of the apparent rate constant of the hydrolysis of phosphate **7** *vs.* the concentration of cationic surfactant **3** at 25 °C ($C_{NaOH} = 10^{-3}$ mol L^{-1}).

where $k_{2,app}$ (L mol^{-1} s^{-1}) is the apparent second-order rate constant obtained by dividing k_{app} by the concentration of the nucleophile, $k_{2,0}$ and $k_{2,m}$ (L mol^{-1} s^{-1}) are the second-order rate constants in the solvent bulk and the micellar pseudophase, respectively, V (L mol^{-1}) is the molar volume of the surfactant, and K_{Ph} and K_{Nu} (L mol^{-1}) are the micelle-binding constants of the phosphate and the nucleophile, respectively.

The parameters of the micelle-catalyzed hydrolysis of phosphate **7** in the presence of surfactant **3** at 25 °C for $C_{NaOH} = 10^{-3}$ mol L^{-1} and $C_7 = 5 \cdot 10^{-5}$ mol L^{-1} were calculated by Eq. (8) and are given below

$k_{2,m}$ /L mol^{-1} s^{-1}	K_{Ph}	K_{Nu}	$(k_{app} \cdot k_0^{-1})_{max}$[a]	F_m	F_c
	L mol^{-1}				
0.0012	3800	70	23	0.125	180

[a] k_0 is the apparent rate constant of the hydrolysis of phosphate **7** in the absence of any surfactant.

Note the high micelle-binding constant of phosphate **7** (K_{Ph}) and a 23-fold increase in its hydrolysis rate in the presence of surfactant **3**. In the context of the pseudophase model, the maximum acceleration of the reaction is described by the equation

$$\sim(k_{app}/k_0)_{max} = \frac{k_{2,m}}{k_{2,0}} \frac{K_{Ph}K_{Nu}}{V(K_{Ph}^{0.5} + K_{Nu}^{0.5})^2}, \quad (9)$$

where the first cofactor on the right-hand side reflects the change in the microenvironment of the reagents upon their passage from the solvent into the micellar phase (F_m) and the second cofactor reflects the concentration of the reagents in the micellar phase (F_c).

Clearly, the concentration factor ($F_c = 180$) makes a major contribution to the reaction acceleration, while the microenvironment factor ($F_m < 1$) lowers the reaction rate. Some discrepancy in the quantitative parameters

obtained by NMR spectroscopy and spectrophotometry is quite explainable. The specificity of either technique requires a special algorithm and individual experimental conditions, including the concentrations of the reagents and the reaction times. The slightly higher rate constants determined from NMR data are due to the higher pH value of the solution. The binding constant calculated for phosphate **7** from spectrophotometric data (see above) is higher than that found from NMR data; this may be attributed to the presence of NaOH, which causes salting the organic substrate out of the aqueous phase into the micellar one. At the same time, the revealed trends agree well with, and complement, each other. For instance, the calculated high binding and partition constants of the phosphate provide clear explanation to the catalytic effect of micelles as a result of concentration of the reagents and to the decreasing reaction rate constant with an increase in the surfactant concentration (see Fig. 5) as a result of dilution of the reagents in the micellar phase (see Table 1, the decreasing number of phosphate molecules per micelle). Thus, the micellization of monocationic 1,4-diazabicyclo-[2.2.2]octane derivative **3** substantially affects the apparent rate constants of the basic hydrolysis of micelle-bound phosphate **7**.

Another area of our research is design of polyfunctional nanosystems[21] combining a number of practically useful properties such as solubilizing and catalytic (or inhibitive) activity and anticorrosive and antimicrobial effects. Such systems can be used, *e.g.*, under the operating conditions of oil-field equipment.[22] In this case, the high values of some of a system´s parameters should not serve as a system performance criterion; instead, it is advisable to design systems with a set of properties comparable with those of known analogs. In the context of this approach, we tested alkylated DABCO derivatives for biological activity and tried to find a correlation between their antimicrobial activity and aggregation characteristics.

Surface-active quaternary ammonium salts are known to exhibit antimicrobial activity due to the presence of the positively charged site and an alkyl radical, which ensure electrostatic and hydrophobic interactions with cell membranes.[23] The antimicrobial activity of a surfactant largely depends on the length of its alkyl radical; in homologous series, this activity becomes stronger as the number of carbon atoms (n) increases to 12—16.[24] However, the correlation between antimicrobial and aggregation properties still remains a controversial problem. Here we estimated the bacteriostatic, fungistatic, bactericidal, and fungicidal effects of quaternized DABCO derivatives on the indicator test strains of microorganisms *Staphylococcus aureus-209 P* (*St. aureus*), *Escherichia coli F50* (*E. coli*), *Bacillus cereus 8035* (*B. cereus*), *Pseudomonas aeruginosa 9027* (*Ps. aeruginosa*), *Trichophyton gipseum* (*Tr. gipseum*), *Aspergillus niger* (*Asp. niger*), and *Candida albicans* (*C. alb.*) and studied a relationship between the antimicrobial and micelle-forming properties for the above homologous series. Antibacterial (ciprofloxacin (**10**)) and antifungal drugs (amphotericin B (**11**)), as well as hexadecyl(trimethyl)-ammonium bromide (**12**), which is structurally similar to the cationic surfactants under study, served as reference compounds. The results obtained are summarized in Tables 3 and 4.

We found that the acute toxicity of compounds **1**, **2**, and **3** injected intraperitoneally into laboratory mice is LD_{50} = 61.1, 50.9, and 61.1 mg kg^{-1}, respectively. According to the toxicity classification,[25] these compounds can be rated as moderately toxic (toxicity class III); they are less toxic by a factor of ~2.5 than surfactant **12** (LD_{50} = 24.0).

It turned out that surfactants **1**—**6** exhibit high antimicrobial activity often against both bacterial and fungal strains and are comparable in efficiency with the reference drugs. When the effects of the homologous compounds studied are differential, their antimicrobial properties largely depend on the length of the hydrocarbon radical and are best for n = 18. This trend holds for almost all the test strains and is especially pronounced for static activity. Some of the compounds studied are superior to surfactant **12** in antimicrobial activity. The influence of the head group can clearly be seen with *St. aureus*: replacement of

Table 3. Bacteriostatic and fungistatic effects of mono- and dicationic 1,4-diazabicyclo[2.2.2]octane derivatives

Surfactant	Bacteriostatic and fungistatic effects/μg mL^{-1}						
	St. aureus	*E. coli*	*B. cereus*	*Ps. aeruginosa*	*Tr. gipseum*	*Asp. niger*	*C. alb.*
1	12.5	250	31.3	>500	>500	>500	125
2	3.1	62.5	7.8	>500	500	>500	62.5
3	0.3	6.3	1.9	500	62.5	500	3.1
4	0.3	6.3	1.9	500	15.6	125	0.78
5	0.5	7.8	1.9	500	250	>500	31.3
6	0.5	6.3	1.9	500	62.5	500	3.1
10	0.25	0.5	0.25	0.5	—	–	–
11	—	—	—	—	–	20	–
12	0.5	6.3	3.1	250	31.3	62.5	3.1

Table 4. Bactericidal and fungicidal effects of mono- and dicationic 1,4-diazabicyclo[2.2.2]octane derivatives

Surfactant	Bactericidal and fungicidal effects/μg mL^{-1}						
	St. aureus	*E. coli*	*B. cereus*	*Ps. aeruginosa*	*Tr. gipseum*	*Asp. niger*	*C. alb.*
1	>500	>500	>500	>500	>500	>500	>500
2	500	>500	>500	>500	>500	>500	500
3	5	>500	500	>500	500	>500	50
4	5	>500	500	>500	31.3	500	5
5	50	>500	500	>500	>500	>500	125
6	50	>500	500	>500	500	>500	50
10	0.25	0.5	0.25	0.5	—	–	—
11	—	—	—	—	31.3	20	0.39
12	50	>500	500	>500	500	>500	50

the head group in structure **12** by a bicyclic fragment (cationic surfactant **3**) enhances the bactericidal effect by an order of magnitude (see Table 4). It should also be noted that the antifungal effect of compound **4** on *Tr. gipseum* and *C. alb.* is much higher than those of compounds **12** and **3**. The fungistatic effects of the octadecyl homolog on these strains are higher by factors of 2—4 and 4, respectively, (see Table 3) and its fungicidal effects, by factors of 16 and 10 (see Table 4).

The presence of a second charged site is unfavorable for antifungal activity: compound **5** is less efficient than compound **3** against *Tr. gipseum* and *C. alb.* by factors of 4 and 10, respectively. However, the antimicrobial effect on *B. cereus*, *E. coli*, and *St. aureus* remains virtually unchanged.

Therefore, introduction of a bicyclic fragment into a cationic surfactant is of interest for the design of antimicrobial (notably, antifungal) drugs with low toxicity for mammals; the best effect can be achieved by increasing the alkyl chain length in surfactants of this type to optimize the ratio of their lipophilic and hydrophilic properties.

There are different ways of suppressing the bacterial and fungal activity by synthetic surfactants; these ways are contributed by several components. Obviously, the presence of a positively charged head group ensures, as a first step, electrostatic interactions with both cell membranes and a peptidoglycan layer containing negatively charged carboxy groups. The content of the peptidoglycan component in the cell wall of Gram-positive bacteria is higher by nearly an order of magnitude (90%) than that in Gram-negative bacteria (~10%), which can be responsible for the different levels of activity of the cationic surfactants against bacteria of this type (see Table 3). The impact of a surfactant on the membrane structure integrity of any cell depends on its ability to insert itself into lipid bilayers and thus the lipophilic nature of the surfactant is crucial here. It is not improbable that the specific features of the antimicrobial activity of this homologous series are substantially determined by the bicyclic fragment. When a surfactant is inserted into a lipid cell membrane, the rigid bicyclic structure can irreversibly change the packing of bioamphiphiles. In addition, the activity of a bicyclic surfactant depends on the charge density at its head groups, which can appreciably differ from typical values of cationic surfactants.

The fact that the cationic surfactants exhibit antimicrobial properties in concentrations considerably below CMC suggests the participation of their individual molecules. However, the presence of organic substrates, as well as an increase in the ionic strength of solution, can substantially decrease CMC.[26] Therefore, it is not improbable that the antimicrobial activity is contributed by aggregated surfactant molecules. In addition, interactions of surfactants with bioamphiphiles always implies the formation of local mixed structures and should correlate with the aggregation activity of individual components. In the context of the present work, we studied a relationship between antimicrobial and micelle-forming properties of homologous surfactants and derived the corresponding equations. For this purpose, a regression analysis of our experimental data was performed. Earlier,[12] we have determined the CMC of surfactants **1**, **2**, **3**, and **4** in water at 25 °C by tensiometry (0.011, 0.004, 0.001, and 0.00012 mol L^{-1}, respectively). We found parabolic correlations between bacteriostatic activity (C_{bac}/mol L^{-1}) and logCMC for compounds **1**—**4**:

$$\textit{St. aureus}: \log(C_{bac}^{-1}) = -2.88 + 5.10(-\log CMC) - 0.711(-\log CMC)^2 \quad (R = 0.989),$$

$$\textit{E. coli}: \log(C_{bac}^{-1}) = -4.07 + 5.02(-\log CMC) - 0.700(-\log CMC)^2 \quad (R = 0.989),$$

$$\textit{B. cereus}: \log(C_{bac}^{-1}) = -1.81 + 4.14(-\log CMC) - 0.589(-\log CMC)^2 \quad (R = 0.999),$$

where R is the correlation coefficient.

A linear correlation model gave lower R values: 0.900 (*St. aureus*), 0.900 (*E. coli*), and 0.888 (*B. cereus*).

Correlations between fungistatic activity (C_{fun}/mol L^{-1}) and logCMC are described by parabolic and linear models with very close correlation coefficients:

$$C.\ alb.: \log(C_{fun}^{-1}) = $$

$$= -1.44 + 3.02(-\log CMC) - 0.300(-\log CMC)^2 \ (R = 0.980),$$

$$\log(C_{fun}^{-1}) = 1.03 + 1.24(-\log CMC) \ (R = 0.971).$$

Correlations between bacteriostatic and fungistatic activities and the number of carbon atoms in the surfactant radical are described best by the parabolic model:

$$St.\ aureus: \log(C_{bac}^{-1}) = -7.57 + 1.45n - 0.0382n^2 \ (R = 0.976),$$

$$E.\ coli: \log(C_{bac}^{-1}) = -8.67 + 1.43n - 0.0375n^2 \ (R = 0.976),$$

$$B.\ cereus: \log(C_{bac}^{-1}) = -6.92 + 1.37n - 0.038n^2 \ (R = 0.992),$$

$$C.\ alb.: \log(C_{fun}^{-1}) = 2.40 - 0.139n - 0.0183n^2 \ (R = 0.979).$$

Thus, alkylated 1,4-diazabicyclo[2.2.2]octanes, which are cationic surfactants with a bicyclic head group, form micellar aggregates in water with a high solubilizing tendency toward diethyl 4-nitrophenyl phosphate (an analog of organophosphorus ecotoxicants), produce a considerable catalytic effect on its basic hydrolysis, and are superior to analogs with acyclic head groups in some of the characteristics of antimicrobial activity. We found a correlation between the aggregation and antimicrobial properties. A number of useful properties combined in alkylated 1,4-diazabicyclo[2.2.2]octanes open up new scope for the design of multipurpose nanostructured systems.

References

1. B. Nikoobakht, M. A. El-Sayed, *Chem. Mater.*, 2003, **15**, 1957.
2. A. V. Shvets, N. V. Kas´yan, E. M. Bezuglaya, G. M. Tel´biz, V. G. Il´in, *Teor. Eksp. Khim.*, 2001, **37**, 372 [*Theor. Exp. Chem.* (*Engl. Transl.*), 2001, **37**].
3. E. G. Rakov, *Usp. Khim.*, 2001, **70**, 934 [*Russ. Chem. Rev.* (*Engl. Transl.*), 2001, **70**, 827].
4. S. R. Derkach, G. I. Berestova, T. A. Motyleva, *Vestn. Mosk. Gos. Tekh. Univ.* [*Bulletin of the Moscow State Technical University*], 2010, **13**, 784 (in Russian).
5. R. S. Iskanderov, S. N. Aminov, Kh. T. Avezov, *Khim. Prir. Soedin.*, 1998, 648 [*Chem. Nat. Compd.* (*Engl. Transl.*), 1998].
6. L. Ya. Zakharova, A. B. Mirgorodskaya, E. P. Zhiltsova, L. A. Kudryavtseva, A. I. Konovalov, *Izv. Akad. Nauk, Ser. Khim.*, 2004, 1331 [*Russ. Chem. Bull., Int. Ed.*, 2004, **53**, 1385].
7. H. J. Cristau, J. F. Ginieys, E. Torreilles, *Bull. Soc. Chim. Fr.*, 1991, 712.
8. A. F. Popov, *Pure Appl. Chem.*, 2008, **80**, 1381.
9. A. V. Zakharov, M. A. Voronin, F. G. Valeeva, E. F. Gubanov, in *Struktura i dinamika molekulyarnykh sistem* [*Structures and Dynamics of Molecular Systems*], 2003, **10**, Part 2, p. 89.
10. C. A. Bunton, A. A. Kamego, M. J. Minch, J. L. Wright, *J. Org. Chem.*, 1975, **40**, 1321.
11. F. M. Menger, R. A. Persichetti, *J. Org. Chem.*, 1987, **52**, 3451.
12. T. N. Pashirova, E. P. Zhiltsova, R. R. Kashapov, S. S. Lukashenko, A. I. Litvinov, M. K. Kadirov, L. Ya. Zakharova, A. I. Konovalov, *Izv. Akad. Nauk, Ser. Khim.*, 2010, 1699 [*Russ. Chem. Bull., Int. Ed.*, 2010, **59**, 1745].
13. M. Jansson, P. Stilbs, *J. Phys. Chem.*, 1985, **89**, 4868.
14. L. Ya. Zakharova, Yu. R. Kudryashova, N. M. Selivanova, M. A. Voronin, A. I. Ibragimova, S. E. Solovieva, A. T. Gubaidullin, A. I. Litvinov, I. R. Nizameev, M. K. Kadirov, Yu. G. Galyametdinov, I. S. Antipin, A. I. Konovalov, *J. Membrane Sci.*, 2010, **364**, 90.
15. N. K. Gaisin, O. I. Gnezdilov, T. N. Pashirova, E. P. Zhiltsova, S. S. Lukashenko, L. Ya. Zakharova, V. V. Osipova, V. I. Dzhabarov, Yu. G. Galyametdinov, *Kolloidn. Zh.*, 2010, **72**, 755 [*Colloid J.* (*Engl. Transl.*), 2010, **72**].
16. M. Jansson, P. Stilbs, *J. Phys. Chem.*, 1987, **91**, 113.
17. A. I. Rusanov, *Mitselloobrazovanie v rastvorakh poverkhnostno-aktivnykh veshchestv* [*Micellization in Solutions of Surfactants*], Khimiya, St. Petersburg, 1992, 280 pp. (in Russian).
18. I. V. Berezin, C. Martinec, A. K. Yatsimirskii, *Usp. Khim.*, 1973, **42**, 1729 [*Russ. Chem. Rev.* (*Engl. Transl.*), 1973, **42**, 787].
19. C. Chachaty, T. Ahlnas, B. Lindstrom, H. Nery, A. M. Tistchenko, *J. Colloid Interface Sci.*, 1988, **122**, 406.
20. *Spravochnik khimika* [*A Chemist´s Handbook*], Goskhimizdat, Leningrad—Moscow, 1963, Vol. **1**, p. 987 (in Russian).
21. L. Zakharova, A. Mirgorodskaya, E. Zhiltsova, L. Kudryavtseva, A. Konovalov, in *Molecular Encapsulation: Organic Reactions in Constrained Systems*, Eds U. H. Brinker, J. L. Mieusset, John Wiley and Sons, Chichester, 2010, p. 397.
22. D. B. Kudryavtsev, A. R. Panteleeva, A. V. Yurina, S. S. Lukashenko, Yu. P. Khodyrev, R. M. Galiakberov, D. N. Khaziakhmetov, L. A. Kudryavtseva, *Neftekhimiya*, 2009, **49**, 211 [*Petrol. Chem.* (*Engl. Transl.*), 2009, **49**, 193].
23. V. M. Bakhir, B. I. Leonov, S. A. Panicheva, V. I. Prilutskii, N. Yu. Shomovskaya, I. I. Strel´nikov, Yu. G. Suchkov, *Dezinfektsionnoe delo* [*Disinfection Science*], 2004, 44 (in Russian).
24. E. P. Tishkova, S. B. Fedorov, L. A. Kudryavtseva, Zh. V. Molodykh, N. L. Kucherova, Sh. M. Yakubov, S. M. Gorbunov, A. M. Zotova, L. V. Teplyakova, A. A. Abramzon, *Khim.-Farm. Zh.*, 1989, 592 [*Pharm. Chem. J.* (*Engl. Transl.*), 1989, 418].
25. N. F. Izmerov, I. V. Sanotskii, K. K. Sidorov, *Parametry toksikometrii promyshlennykh yadov pri odnokratnom vvedenii: spravochnik* [*Toxicometric Parameters of Singly Administered Industrial Poisons: A Handbook*], Meditsina, Moscow, 1977, p. 197 (in Russian).
26. K. Holmberg, B. Jonsson, B. Kronberg, B. Lindman, *Surfactants and Polymers in Aqueous Solution*, J. Wiley and Sons, Ltd, Chichester, 2002, 562 pp.

Received January 18, 2011;
in revised form August 11, 2011