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Self-assembling systems based on quaternized derivatives of 1,4-diazabicyclo[2.2.2]octane in nutrient broth as antimicrobial agents and carriers for hydrophobic drugs

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ABSTRACT

Aggregation properties of mono (mono-CS) and dicationic (di-CS) surfactants, namely quaternised derivatives of 1,4-diazabicyclo[2.2.2]octane (DABCO), have been evaluated in water and in nutrient broths of different pH, i.e. in Hottinger broth (pH = 7.2) and Sabouraud dextrose broth (pH = 5.6). Aggregation capacity of surfactants was shown to be responsible for the solubilization properties of a complex composed of a hydrophobic probe (Sudan I) and a selected drug (quercetin), contributing to the antimicrobial activity of this surfactant system. The effect of N-methyl-D-glucamine (NmDg) additive on the antimicrobial activity of mono-CS, and its aggregation and solubilization parameters, has also been evaluated. A substantial decrease in critical micelle concentration (CMC) of cationic surfactants in nutrient broths (up to 60 times) has been reported. Twofold dilution of monocationic surfactant by NmDg slightly changed the CMC of surfactant; however, it provided a remarkable increase in solubilization capacity (~by 4 times) and decrease in its toxicity. The data anticipate the potential use of DABCO quaternized derivatives as innovative non-toxic delivery systems for hydrophobic drugs.

1. Introduction

One of the key properties of surfactants responsible for their wide application in modern technologies [1–4] is their ability to form aggregates in solution above the critical micelle concentration (CMC) [5]. This fundamental property of surfactants resulting from their amphiphilic nature has received much attention in recent papers [6,7]. In aqueous media, the so-called direct micelles are formed due to a hydrophobic effect, with nonpolar fragments of surfactant molecules isolated within micellar interior and polar head groups facing towards bulk water. Different factors are known to control the aggregation in water, namely, the structure of surfactants (packing parameter), ionic strength, the presence of additives and co-surfactants. Cationic surfactants (CSs) have been used as antibacterial [8–10] and antifungal [11,12] agents, as well as denaturants of protein molecules [13,14] in biomedical and biochemical studies [15–17], and in the design of new functional materials [18,19], drug delivery systems [20–23], and as corrosion inhibitors [24].

The recent literature, focusing on the design of antimicrobial agents [25,26], emphasizes two main trends in this research field: (i) the investigation of the mechanism of biocidal activity of amphiphilic salts, including their influence on different biological events involved, i.e. adsorption of amphiphilic cations on

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the bacterial cells, diffusion through the cell wall, and disruption of cytoplasmic membrane [27]; and (ii) the investigation of new effective agents, which is motivated by the increasing resistance of microorganisms [28,29]. To answer the criteria of green chemistry, different types of cationic [8,30–35] and nonionic [36] surfactants of lower toxicity have been designed, among which ionic liquids [31,32] and surfactants bearing natural fragments [33–35] are reported. These studies are mainly devoted to the elucidation of the structure-activity relationship [8,30–37]. In particular, the relationship between CMC and the minimum inhibitory concentration (MIC) has been examined. As documented, the structure of head groups plays a minor role, while hydrophobicity of surfactants markedly influences their antimicrobial activity. In particular, MIC generally decreased with an increase in the alkyl chain length [8,30,31,35]. In the case of geminis, both the hydrophobicity and spacer length can influence the activity [8,33,34], with dimeric analogs typically showing advanced antimicrobial properties. The size of aggregates and their morphology are also key factors controlling this activity [33].

The present work is focused on the study of the relationship between the structural behaviour and the antimicrobial activity of cationic surfactants. The establishment of the relationship between aggregation and antimicrobial properties of CSs is of scientific interest in order to reveal structural features of detergents, which determine their antimicrobial activity. There are however some difficulties to obtain such correlation, since self-assembly studies are typically carried out in aqueous or buffer solutions, while antimicrobial tests assume the use of nutrient broths [38,39] composed of polymers and electrolytes capable of markedly affecting the surfactant self-assembly. Therefore, combined aggregation/antimicrobial study is expected to provide valuable information. Besides, as mentioned above, micellization and properties of formed aggregates are known to depend markedly on the characteristics of the medium e.g. ionic strength, pH, and temperature [40,41], and also by the presence of various inorganic [42] and organic [43,44] substances, that are ample in nutrient broths. Therefore, although antimicrobial activity is usually exhibited at low surfactant concentrations, the problem should be considered whether antimicrobial effect is mediated by associated cationic surfactants, or if it is a result of the molecular forms of amphiphilic agents.

Among known CSs, quaternized derivatives of 1,4-diazabicyclo[2.2.2]octane (DABCO) were selected for the present work, because of their wide applications [45–47] including their use as antibacterial agents [48,49]. We have previously shown that monoquaternized derivatives of 1,4-diazabicyclo[2.2.2]octane (DABCO-R, where R = n-$C_{12}H_{25}$, n-$C_{14}H_{29}$, n-$C_{16}H_{33}$, n-$C_{18}H_{37}$,) demonstrated the high degree of both antibacterial and fungicidal activity [50]. The aggregation behaviour of a homological series of monoquaternized derivatives of 1,4-diazabicyclo[2.2.2]octane with various degrees of hydrophobicity was studied in aqueous solution, with the CMC and the sizes of aggregates determined. It was shown that these CSs are characterized by unusual morphology of aggregates in solution [51,52]. A correlation of CMC with biological activity of monoquaternized derivatives of 1,4-diazabicyclo[2.2.2]octane has been demonstrated. To support the growth of microorganisms special liquids are used. The most common growth media for microorganisms are *nutrient broths* and agar plates, e.g. Hottinger broth for bacteria and Sabouraud dextrose broth for fungal strains. Nutrient media are composed of components that most bacteria need for growth and are non-selective, so they are used for the general cultivation and maintenance of bacteria kept in laboratory culture collections. Therefore, to estimate the medium effect, the aggregation properties of biologically active surfactants were assessed in two nutrient broths, namely, Hottinger broth (pH = 7.2) and Sabouraud dextrose broth (pH = 5.6). The effect of organic additives on the properties of surfactant in various media was studied with the aid of N-methyl-D-glucamine (NmDg). Hydroxyl-containing amine of this structure is of interest as an active nontoxic component used with the main active ingredient in pharmaceutical dosage forms. It is known that NmDg is able to stabilize and dissolve drugs [53,54] and form supramolecular adducts [55].

2. Materials and methods

2.1. Materials

1-Phenylazo-2-naphthol (Sudan I, ACROS Organics, NJ, USA), N-Methyl-D-glucamine (99%, ACROS Organics, NJ, USA), 1,6-Diphenyl-1,3,5-hexatriene (DPH, 98%, Sigma–Aldrich, NJ, USA), Quercetin (≥95% (HPLC), solid, Sigma–Aldrich, Saint Louis, USA) were used. Monoquaternized derivatives of 1,4-diazabicyclo[2.2.2]octane (mono-CS) was prepared by the reaction of 1,4-diazabicyclo[2.2.2]octane (DABCO) (3 g, 0.0267 mol) with 1-bromohexadecane (6.65 g, 0.0257 mol) in acetone (20 cm^3). The temperature of the reaction mixture was maintained at 18–20 °C. The resulting reaction mixture was stirred for 1 h at 20 °C and for 30 min at 45–50 °C. Solvent excess was distilled *in vacuum*; and diquaternized derivatives of 1,4-diazabicyclo[2.2.2]octane (di-CS) by quarterisation of mono-CS (3 g, 7.2 mmol) with 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 vacuum* according to the procedure described earlier [50,52].

2.2. Methods

2.2.1. Surface tension

The analysis of the surface tension was performed using the du Nouy ring detachment method (Kruss GmbH K6 Tensiometer, Hamburg, Germany). The experimental details are described elsewhere [56]. Briefly, the planar and spherical ring was placed parallel to the air/aqueous interface. Between the surface tension analyses, the ring was cleaned by rinsing it with double-distilled water, followed by soaking it in nitric acid for 5–7 min, rinsing again with double-distilled water, and finally flame-drying. All glassware was soaked in nitric acid to avoid any contaminants, thoroughly rinsed with double-distilled water, and then steamed before use. Temperature was kept at 25 ± 0.2 °C during all experiments.

2.2.2. Electrical conductivity

Electrical conductivity was measured using a WTW InoLab Cond 720 precision conductivity meter (WTW Gmb, Weilheim, Germany). Reproducibility was checked for selected samples, and no significant differences were observed. All samples were studied at 25 ± 0.1 °C. Purified water (18.2 MΩ cm resistivity at 25 °C) from Direct-Q 5 UV equipment (Millipore S.A.S. 67120 Molsheim-France) was used for all sample preparations.

2.2.3. Dynamic light scattering

Dynamic light scattering (DLS) measurements were performed using the Malvern Instrument Zetasizer Nano (Worcestershire, UK). The measured autocorrelation functions were analysed by Malvern DTS software, applying the second-order cumulant expansion methods. The effective hydrodynamic radius (R_H) was calculated according to the Einstein-Stokes equation: $D = k_BT/6\pi\eta R_H$, in which D is the diffusion coefficient, k_B is the Boltzmann constant, T is the absolute temperature, and η is the viscosity. The diffusion coefficient was measured at least three times for each sample. The average error in these experiments was approximately 4%. The

solutions were filtered with Millipore filters to remove dust particles from the scattering volume.

2.2.4. Dye and drug solubilization studies

The solubilization studies of the dye were performed by adding an excess of crystalline Sudan I to the solutions. These solutions were allowed to equilibrate for about 48 h at room temperature, followed by filtration, and the absorbance measured at 485 nm using the spectrophotometer Specord 250 Plus (Analytik Jena AG, Germany). Quartz cuvettes containing the sample were used, with a cell length of 0.1 cm.

For the solubilization studies of quercetin, saturated drug solutions were prepared in glass vessels by mixing of the excess of powdered quercetin with solutions with different substance concentrations. The samples were allowed to equilibrate at room temperature for 48 h before filtering (Millipore, 0.45 mm) to remove any non-solubilised species. The spectra were recorded in the range of wavelengths 200–1100 nm using a spectrophotometer Specord 250 Plus (Analytik Jena AG, Germany). Absorbance was measured at the optimum wavelength 380 nm. Quartz cuvettes containing the sample were used with a cell length of 0.1 cm.

2.2.5. Fluorescence

Steady-state fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene (DPH) was measured on a Cary Eclipse (spectrometer (Varian, Inc., California, USA)) equipped with filter polarizers. DPH were excited at 361 nm, and the fluorescence intensity was measured at 450 nm. The excitation and emission slit widths were 2.5 and 5 nm, respectively. The embedded software automatically determined the correction factor and anisotropy value. A quartz cell of 1 cm path length was used for all fluorescence measurements. Temperature of 25 °C was maintained. Surfactant solutions were prepared by the stepwise dilution of a stock sample, with a wide concentration range from molecular solution to 50 mM covered. A fixed concentration of 0.175 mM of fluorescence probe DPH was used.

2.2.6. Biological evaluation

2.2.6.1. Antibacterial and antifungal activity. The *in vitro* antibacterial and antifungal activity of the cationic surfactants was evaluated against pathogenic representative Gram-positive bacteria (*S. aureus 209p*) and yeast (*C. albicans 885-653*). The MIC values were estimated by conventional dilution methods for bacteria and fungi [42,43]. The antibacterial and antifungal assays were performed in Hottinger broth and Sabouraud dextrose broth (bacteria and yeast 3×105 cfu/ml).

The *in vitro* antibacterial and antifungal activity of CS were investigated against pathogenic representative Gram-positive bacteria (*S. aureus 209p*) and yeast (*C. albicans 885-653*). Minimal inhibitory concentrations (MICs) were estimated by conventional dilution methods for bacteria and fungi [57,58]. The antibacterial and antifungal assays were performed in Hottinger broth (HiMedia Laboratories Pvt. Ltd Mumbai, India) and Sabouraud dextrose broth (HiMedia Laboratories Pvt. Ltd Mumbai, India) (bacteria 3×105 cfu/ml and yeast 2×104 cfu/ml). The components of Hottinger broth (Lactalbumin hydrolysate (10 g), Peptone (10 g), NaCl (5 g)) were dissolved in one liter of distilled water, autoclaved for 20 min at 121 °C. The pH was adjusted to 7.2 prior to autoclaving. The components of Sabouraud dextrose (Peptone (10 g), glucose (40 g)) were dissolved in one liter of distilled water, autoclaved for 15 min at 121 °C. The pH was adjusted to 5.6 prior to autoclaving.

2.2.6.2. Toxicity assay. The toxicity of CS and its compositions with NmDg were tested for their haemolytic activities against human red blood cells (hRBC). Fresh hRBC with heparin were rinsed 3 times with PBS (35 mM phosphate buffer/0.15 M NaCl, pH 7.3) by centrifugation for 10 min at 800 g and re-suspended in PBS. Test compounds dissolved in PBS (concentrations 0.98–500 μg/ml) were then added to 0.5 mL of a solution of the stock hRBC in PBS to reach a final volume of 5 mL (final erythrocyte concentration, 10% v/v). The resulting suspension was incubated under stirring for 1 h at 37 °C. The samples were then centrifuged at 2000 g for 10 min. Release of haemoglobin was monitored by measuring the absorbance of the supernatant at 540 nm. Controls for zero haemolysis (blank) and 100% haemolysis consisted of hRBC suspended in PBS and distilled water, respectively.

3. Results and discussion

In Fig. 1A, the correlation between the concentration of mono-CS and di-CS and the surface tension is given in various media containing NmDg. All the recorded isotherms are typical for conventional surfactants. It is evident that micelle formation of CSs was enhanced in the nutrient broths under study. The obtained CMC values are given in Table 1.

CMC for mono-CS was shown to be 0.07 mM in Hottinger broth and 0.06 mM in Sabouraud dextrose broth, values that are by ~15-fold lower than those obtained in water. The similar tendency was observed for di-Cs; di-CS aggregates in Hottinger broth at the concentration of 0.05 mM, which is 60-fold lower than in water. The decrease in CMC observed for CSs in nutrient broths is in accordance to known behaviour of CSs in polymer solutions. The protein representing nucleoprotein (in Hottinger broth) or polypeptide (in Sabouraud dextrose broth) acts as a polymer in these solutions. The change of CMC of CSs may be provided by inorganic salt (NaCl), which exists in the broth.

The measurement of specific electroconductivity (Fig. 1B) and surface tension of mono-CS solutions with NmDg (Fig. 1A, lines 2 and 4) demonstrates that the presence of NmDg did not influence CMC values, both in water and in Hottinger broth (Table 1).

In order to prove the formation of CS assemblies in solution, the size behavior was monitored by dynamic light scattering (DLS) for mono-CS and di-CS in Hottinger broth and Sabouraud dextrose broth (data not shown). Large aggregates ~100÷300 nm are formed in CS solution near CMC. With the increase in the concentration of CS, particle sizes decrease about to 4–5 nm (Table 2). This behaviour may be related to the presence of proteins in nutrient broths. Scientific literature is limited with respect to methods describing the formation of surfactant–protein assemblies because of the large variety of protein types. Nevertheless, it has been demonstrated that protein denaturation usually occurs in the presence of surfactants, and this phenomenon has been pointed out as the main factor contributing to the formation of larger aggregates, which are detected in the range of low concentrations of CSs in nutrient broths. The decrease of the size of aggregates in the high-concentration range of surfactant has been attributed to the compacting of protein molecules modified by CS micelles. This behaviour results from the change of the morphology of aggregates, i.e. by the spontaneous formation of vesicular structures within the CMC range, followed by the transition to more compact micellar form with the increase in the content of surfactant [59].

The vesicle-to-micelle transitions have been described previously [60,61]; however, to describe the type of structures formed in CMC range requires additional experimental verification. One of the methods successfully used to determine the morphology of aggregates makes use of DPH fluorescent probes [62–64]. It is known that fluorescence anisotropy of micelles composed of ionic surfactants is low (below 0.1); whereas fluorescence anisotropy of vesicles is usually higher than 0.14 [65]. As shown in Fig. 2, fluorescence anisotropy values were close to 0.15, within the concentration range of mono-CS, where bulky aggregates were recorded. This is

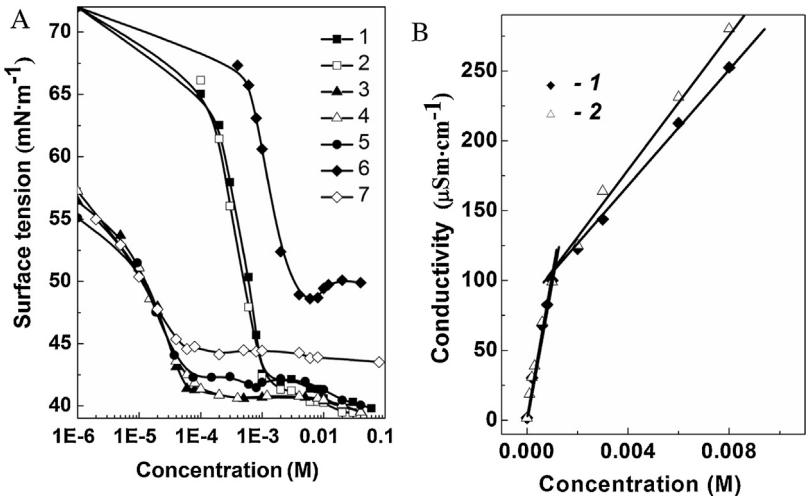

Fig. 1. Surface tension isotherms of aqueous solutions of mono-CS (1-5) and di-CS (6, 7) in medium: water (1, 2, 6), in nutrient broth (3, 4, 7), in Sabouraud dextrose broth (5) in absence (1, 3, 5-7) and in the presence (2, 4) of NmDg in the (1:1) molar ratio; 25 °C (A); Specific electroconductivity of aqueous solutions of mono-CS vs. its concentration in absence (1) and presence NmDg (2) at the ratio 1:1, 25 °C (B).

Table 1
CMC values, parameter of slope (B) and solubilisation power (S) of quaternized derivatives DABCO in different media, in the absence and presence NmDg at a 1:1 ratio; 25 °C. The coefficients of variation of CMC values determined by tensiometry, conductivity and spectrophotometry were calculated from three independent determinations of CMC and were found to be ≤5%, ≤7% and ≤3%, respectively.

Compound	Medium	Additive	CMC × 10^3 (mol/L)			S × 10^3 (mol cm L^{-1})
			Tensiometry	Conductometry	Spectrophotometry	
mono-CS	water	–	1[a]	1; 11[a]	0.5	107
mono-CS	in Hottinger broth	–	0.07	–	2.0	82
mono-CS	in Sabouraud dextrose	–	0.06	–	–	–
di-CS	water	–	3[a]	3.1; 10[a]	–	–
di-CS	in Hottinger broth	–	0.05	–	–	–
mono-CS	water	NmDg	1	1	0.4	377
mono-CS	in Hottinger broth	NmDg	0.07	–	1.1	119

[a] Data from[44]

Table 2
The distribution of sizes (hydrodynamic diameter, nm) of CS in Hottinger broth and Saburo dextrose broth and polydispersity index; using the number and intensity parameters, 25 °C. Mean ± standard deviation from three independent samples.

Surfactants	Medium	C_{CS}, mM	Hydrodynamic diameter ± SD, nm		Polydispersity index ± SD
			Intensity	Number	
mono-CS	Hottinger broth	0.515102040	220 ± 35	142 ± 24	0.2 ± 0.02
			459 ± 73	342 ± 55	0.17 ± 0.02
			7.5 ± 0.8	3.6 ± 0.8	0.26 ± 0.002
			6.5 ± 0.9	3.6 ± 0.7	0.16 ± 0.02
			10.1 ± 1.5	5.6 ± 1.4	0.22 ± 0.015
			7.5 ± 1	4.2 ± 1	0.22 ± 0.015
mono-CS	Sabouraud dextrose broth	0.20.30.40.540	122 ± 24	91 ± 22	0.065 ± 0.009
			142 ± 27	91 ± 25	0.065 ± 0.018
			255 ± 60	220 ± 51	0.08 ± 0.02
			220 ± 43	164 ± 33	0.069 ± 0.007
			7.5 ± 0.9	3.6 ± 0.7	0.238 ± 0.005
di-CS	Hottinger broth	0.060.10.510204060	164 ± 28	106 ± 23	0.133 ± 0.013
			190 ± 25	106 ± 18	0.185 ± 0.01
			220 ± 32	122 ± 23	0.251 ± 0.078
			8 ± 1	4 ± 0.9	0.281 ± 0.05
			6.5 ± 1	4 ± 1	0.22 ± 0.019
			6.5 ± 1	3.6 ± 0.9	0.137 ± 0.019
			6.5 ± 1	3.6 ± 0.9	0.119 ± 0.012

in agreement with the formation of vesicles in solution. Further increase of the concentration of mono-CS resulted in a decrease of the fluorescence anisotropy parameter to ca. 0.1. The latter testifies the lower microviscosity typical for micellar structures.

The study of solubilization of the hydrophobic dye Sudan I (Fig. 3) in solutions of mono-CS showed that with the increase of the concentration of mono-CS, the absorption band intensity of Sudan I probe (~485 nm) increased. The increase of the absorbance of solution is due to the formation of mono-CS aggregates with hydrophobic domain capable of solubilizing the water-insoluble dye Sudan I. The solubilization effect of the surfactants is visualized in Fig. 3 and can be quantitatively characterized by the value

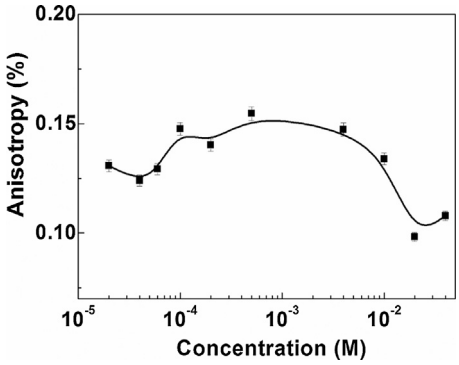

Fig. 2. Variation of fluorescence anisotropy of fluorescence probe DPH as function of mono-CS concentration in Hottinger broth, 25 °C. Error bars indicate standard deviations of the mean values ($n = 3$).

of solubilization power (S), i.e. the number of dye molecules per aggregate: $S = B/\varepsilon$, where B is the slope of linear fragment corresponding to sharp increase in the absorbance in Fig. 3, and ε is the extinction coefficient.

Table 1 summarizes the CMC values of mono-CS, which were determined from the solubilization capacity data of mono-CS aggregates. It is clear that CMC of mono-CS in Hottinger broth is higher than that in water, regardless the presence of NmDg. Thus, absorption band intensity of Sudan I is exhibited at the concentration above CMC, i.e. in the concentration range of mono-CS, where only small aggregates were detected (4–6 nm), and the tendency to decrease the fluorescence anisotropy has been observed (Fig. 2). In the concentration range below CMC (in which only large aggregates exist) (140–340 nm) the fluorescence anisotropy values were close to 0.15 (Fig. 2), and solubilization of hydrophobic dye did not occur. This suggests the change of morphology of mono-CS aggregates with the increase of its concentration, or the assumption of the existence of CS-polymer mixed aggregates according to general model of «pearl necklace» [5]. The latter show a lower solubilization capacity toward hydrophobic probes compared to individual CS aggregates.

The estimation of solubilization capacity of mono-CS aggregates showed that solubilization power of mono-CS aggregates in water and nutrient broth differ by ~1.3 times. It is evident that the medium plays a crucial role in the dissolution of Sudan I dye. The effect of the polarity of medium on the dissolution of dye is well documented [66].

The addition of glucamine leads to insignificant decrease in CMC for mono-CS (Table 1). It should be noted that solubilization power of mono-CS aggregates with respect to Sudan I in the presence of NmDg increases both in water (by 4 times) and in Hottinger broth (1.5 times).

The ability of the systems under study to solubilize drugs was tested using the flavonoid quercetin. This drug is an anionic polyphenol possessing antioxidant and chelating properties [67]. In addition, quercetin is an effective anti-inflammatory, anti-tumor, and heart disease drug [68,69]. Quercetin is low-soluble in water and it is sensitive to the changes of factors, such as temperature and pH, as well as in the presence of enzymes, organic solvents, micellar solutions [70,71]. Various systems for quercetin flavonoid delivery, which can improve bioavailability of drug and prevent its degradation, have been developed [72,73]. In order to improve the solubility of quercetin, the following systems have suggested in the present work, namely, mono-CS, NmDg, and mono-CS-NmDg mixed composition at a 1:1 ratio. The absorbance of quercetin solutions in the studied systems was measured by spectrophotometry. In Fig. 4(a), the absorption spectra of quercetin in micellar solution of mono-CS are given. With the increase of the concentration of mono-CS, intensities of absorption bands at 260 and 380 nm increased. This proves the dissolution of quercetin in micellar solution of mono-CS.

The absorption spectra of quercetin in NmDg solution with the maxima at 260 and 475 nm are given in Fig. 4(b). With the increase of the concentration of NmDg, pH of the studied solutions increased in the range of 8 to 10. It is known that alkali solutions enable the dissolution of quercetin due to ionization of hydroxyl groups in phenol molecule [71,74], while the probability occurs that the oxidized quercetin can be formed as well.

In NmDg solutions with and without mono-CS, the maxima of absorbance of quercetin have been observed at 290 and 330 nm, as well as 280 and 340 nm (Fig. 4(b and c)) with an increase in the NmDg concentration. The increase of the intensity of these absorption bands can be related to the formation of supramolecular complexes quercetin–glucamine. Examples of the formation of supramolecular structures with NmDg have been already reported [55]. This can be due to the products of chemical reaction.

The dependences of absorbance of quercetin at 380 nm are given in Fig. 5 for micellar solutions of individual mono-CS and mono-CS-NmDg mixed composition. It is evident that the similar tendency is observed for quercetin. In the range above CMC of mono-CS, when mono-CS-NmDg mixed composition is used, a 3-fold increase in solubility of quercetin was observed relative to individual solution of mono-CS.

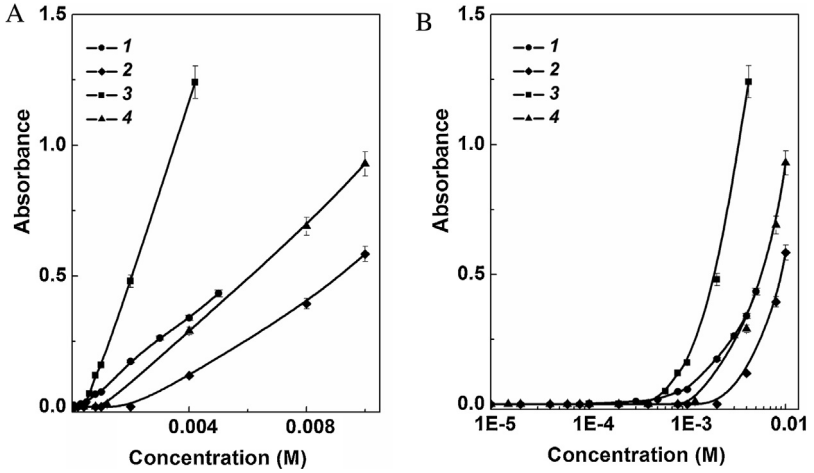

Fig. 3. Optical density of Sudan I in solutions of mono-CS in water (1, 3), in Hottinger broth (2, 4) vs. concentration of mono-CS in the absence (1, 2) and presence of NmDg (3, 4), 25 °C, $L = 0.1$ cm. Error bars indicate standard deviations of the mean values ($n = 3$).

Table 3
Effect of the ratio of mono-CS and NmDg on the antimicrobial activity (mg/L) in *Staphylococcus aureus* and in *Candida albicans*, and Haemolysis human red blood (%). The values are the mean of three independent experiments and the standard deviation is also given) (Mean ± SD, $n = 3$).

Ratio of components (%)		Antimicrobial activity (mg/L)		Haemolysis human red blood (%)	
		Microorganisms		The concentration of the antimicrobial components (mg/L)	
mono-CS	NmDg	*S. aureus 209 P*	*C. albicans 885-653*	0.3	3.1
100	–	0.3 ± 0.029	3.1 ± 0.3	0	2.8 ± 0.027
–	100	>500	>500	0	0
80	20	0.3 ± 0.03	3.1 ± 0.3	0	0.98 ± 0.096
50	50	0.3 ± 0.029	3.1 ± 0.29	0	0.87 ± 0.086
20	80	0.75 ± 0.074	6.3 ± 0.63	0	0.65 ± 0.064

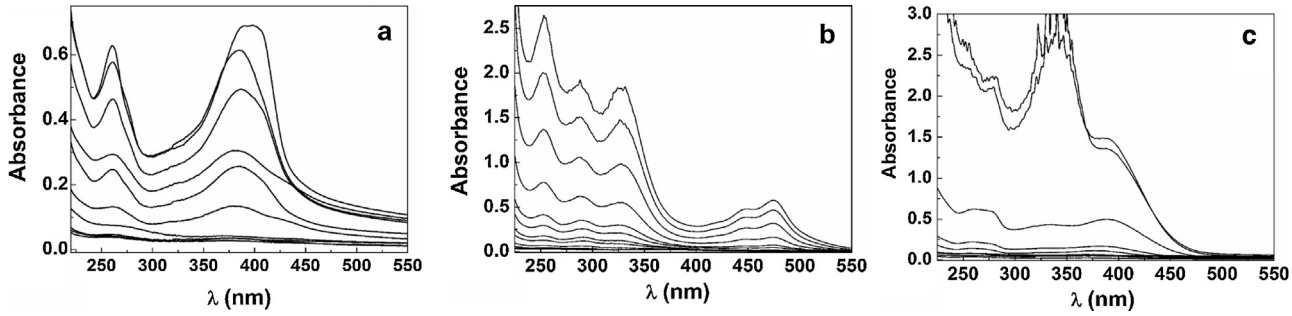

Fig. 4. Absorption spectra of quercetin in aqueous solution of mono-CS (a), NmDg (b) and mono-CS with NmDg at a 1:1 ratio (c) in the concentration range of 0.1/5 mM, 25 °C, $L = 0.1$ cm.

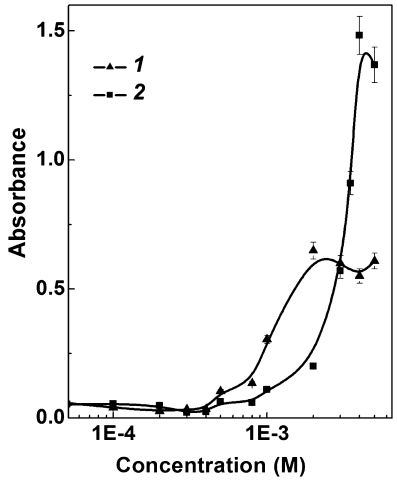

Fig. 5. Dependences of quercetin absorption band intensity (λ = 380 nm) in aqueous solutions of mono-CS(1) and mono-CS-NmDg composition (2) at a 1:1 ratio vs. surfactant concentration, 25 °C, $L = 0.1$ cm. Error bars indicate standard deviations of the mean values ($n = 3$).

The DLS data prove that the addition of NmDg in the solution of mono-CS in water or in Hottinger broth led to the increase of aggregate sizes, which are formed in the concentration range near or below CMC up to 300 nm in water and 600 nm in broth (data not shown). When NmDg is added, the CS-NmDg mixed structures are formed both in water and in Hottinger broth. With the increase of the CS concentration, a tendency to decrease of aggregate sizes was observed.

The data about biological activity and toxicity of the compositions based on mono-CS with and without NmDg at their various ratios are given in Table 3. Although NmDg itself shows no biological activity, its addition to CS enabled the decrease of the concentration of the active ingredient (mono-CS) in antimicrobial composition by 2-fold thereby reducing the toxicity of cationic surfactant in human red blood cells. The observed maximum degree of antimicrobial activity of mono-CS is observed at the concentrations which are by two orders lower than CMC of CS in nutrient broths. As discussed above, in this concentration range bulk aggregates with no hydrophobic cavity, rather than the monomeric amphiphiles, would act as active ingredients.

The possibility of the formation of these bulk nano-assemblies near or onto the surface of bacterial cell should not be excluded. At the primary step, the local adsorption of CS on negatively charged cell surface due to electrostatic interaction is highly feasible. The subsequent formation of bulk aggregates around this "adsorbed center" may have an adverse effect on its metabolism finally resulting in the death of microorganism.

4. Conclusions

We have demonstrated that mono and diquaternized DABCO derivatives form aggregates in nutrient broths at a concentration one order of magnitude below than that in aqueous medium. Nanosized structures of monoquaternized DABCO derivative are able to solubilize the hydrophobic dye Sudan I at a concentration above its critical micelle concentration. The introduction of equimolar amount of N-methyl-D-glucamine to solution with monoquaternized DABCO derivative led to slightly change of the critical micelle concentration of the detergent; however, it contributes to the increase of the solubilization capacity (by ~4-fold) and aggregate sizes, and also allows the decrease of the detergent concentration in antimicrobial composition by 2-fold thus decreasing its toxicity. Based on the study of aggregation properties of cationic surfactants, important results of practical significance were obtained: (i) nanocontainers for water insoluble guests, i.e. hydrophobic probe Sudan I and the drug quercetin were developed; (ii) the role of the surfactant aggregation in the antimicrobial activity was demonstrated; (iii) the way to decrease the toxicity of biologically active formulations with the preservation of their useful properties is anticipated. The obtained results open the perspective of using quaternized DABCO derivatives as delivery systems for hydrophobic drugs.

Conflict of Interests

The authors report no conflict of interests.

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