



# 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.

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## 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<sub>12</sub>H<sub>25</sub>, n-C<sub>14</sub>H<sub>29</sub>, n-C<sub>16</sub>H<sub>33</sub>, n-C<sub>18</sub>H<sub>37</sub>.) 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 ( $\geq 95\%$  (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<sup>3</sup>). 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 quaternisation 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 GmbH, 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:  $D = k_B T / 6\pi\eta R_H$ , in which  $D$  is the diffusion coefficient,  $k_B$  is the Boltzmann constant,  $T$  is the absolute temperature, and  $\eta$  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

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