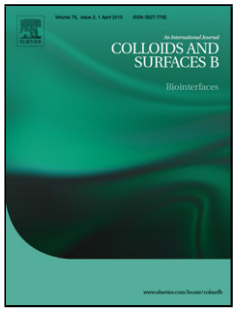
Accepted Manuscript



Title: Structure-Property Relationship of Quinuclidinium Surfactants - Towards Multifunctional Biologically Active Molecules

Author: Mirjana Skočibušić Renata Odžak Zoran Štefanić Ivana Križić Lucija Krišto Ozren Jović Tomica Hrenar Ines Primožič Darija Jurašin

PII: S0927-7765(15)30306-4
DOI: http://dx.doi.org/doi:10.1016/j.colsurfb.2015.11.023
Reference: COLSUB 7478

To appear in: *Colloids and Surfaces B: Biointerfaces*

Received date: 27-8-2015
Revised date: 2-11-2015
Accepted date: 12-11-2015

Please cite this article as: Mirjana Skočibušić, Renata Odžak, Zoran Štefanić, Ivana Križić, Lucija Krišto, Ozren Jović, Tomica Hrenar, Ines Primožič, Darija Jurašin, Structure-Property Relationship of Quinuclidinium Surfactants - Towards Multifunctional Biologically Active Molecules, Colloids and Surfaces B: Biointerfaces http://dx.doi.org/10.1016/j.colsurfb.2015.11.023

Structure-Property Relationship of Quinuclidinium Surfactants – Towards Multifunctional Biologically Active Molecules

Mirjana Skočibušić[1], Renata Odžak[2], Zoran Štefanić[3], Ivana Križić[4], Lucija Krišto[4], Ozren Jović[4], Tomica Hrenar[4], Ines Primožič[4*] ines.primozic@chem.pmf.hr, Darija Jurašin[3*] djurasin@irb.hr

[1]Department of Biology, Faculty of Science, University of Split, Teslina 12, 21 000 Split, Croatia

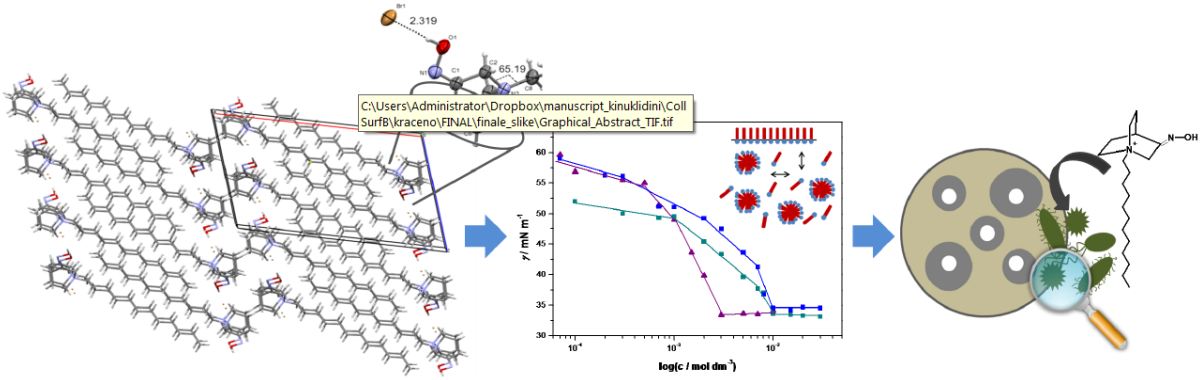
[2]Department of Chemistry, Faculty of Science, University of Split, Teslina 12, 21 000 Split, Croatia

[3]Division of Physical Chemistry, Ruđer Bošković Institute, Bijenička cesta 54, 10 000 Zagreb, Croatia

[4]Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, 10 000 Zagreb, Croatia

[*]Corresponding authors at: Laboratory for synthesis and self-assembly processes of organic molecules, Division of Physical Chemistry, Ruđer Bošković Institute, Bijenička cesta 54, 10 000 Zagreb, Croatia (Darija Jurašin); Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, 10 000 Zagreb, Croatia (**Ines Primožič**)

Graphical Abstract



Highlights

- Novel oxime functionalized quinuclidinium surfactants were synthesized (C_nQNOH)
- Crystal structure, surface properties and antimicrobial efficacy were determined
- C_nQNOH demonstrated high adsorption efficiency and relatively low cmc
- C_nQNOH have potent broad spectrum antimicrobial activity
- Bicyclic headgroup with oxime moiety has dominant effect on characteristics

Abstract

Motivated by diverse biological and pharmacological activity of quinuclidine and oxime compounds we have synthesized and characterized novel class of surfactants,3-hydroxyimino quinuclidinium bromides with different alkyl chains lengths (C_nQNOH; n = 12, 14 and 16). The incorporation of nonconventional hydroxyimino quinuclidinium headgroup and variation in alkyl chain length affects hydrophilic-hydrophobic balance of surfactant molecule and thereby physicochemical properties important for its application. Therefore, newly synthesized surfactants were characterized by the combination of different experimental techniques: X-ray analysis, potentiometry, electrical conductivity, surface tension and dynamic light scattering measurements, as well as antimicrobial susceptibility tests. Comprehensive investigation of C_nQNOH surfactants enabled insight into structure-property relationship, *i.e.* way in which the arrangement of surfactant molecules in the crystal phase correlates with their solution behavior and biologically activity. The synthesized C_nQNOH surfactants exhibited high adsorption efficiency and relatively low critical micelle concentrations. In addition, all investigated compounds showed very potent and promising activity against Gram-positive and clinically relevant Gram-negative bacterial strains compared to conventional antimicrobial agents: tetracycline and gentamicin. The overall results indicate that bicyclic headgroup with oxime moiety, which affects both hydrophilicity and hydrophobicity of C_nQNOH molecule in addition to enabling hydrogen bonding, has dominant effect on crystal packing and physicochemical properties. The unique structural features of cationic surfactants with hydroxyimino quinuclidine headgroup along with diverse biological activity have made them promising structures in novel drug discovery. Obtained fundamental understanding how combination of different functionalities in a single surfactant molecule affects its physicochemical properties represents a good starting point for further biological research.

Keywords: cationic surfactants; quinuclidinium oximes; biologically active compounds; structure-property relationship; crystal structure; antimicrobial efficacy.

1. Indroduction

Surfactants are organic compounds containing in one molecule both lyophobic (hydrophobic) and lyophilic (hydrophilic) parts. In solutions surfactants self-assemble in the variety of three-dimensional nano and micro structures like micelles, vesicles and liquid crystalline phases [1]. Different surfactant phases are of interest in pharmaceutical applications either as drug carriers or as targeted drug delivery systems [2–4].

Contemporary investigations in surfactant science are driven by the requirements to design surfactants that possess enhanced physicochemical properties and can be utilized in complex systems as well as for specific applications in modern technologies [5–11]. On the other hand, there has been increased interest in the development of drugs with polypotent chemical structures which result in interaction with various molecular targets or receptors and multifunctionality [12]. Due to their functionalities drug molecules themselves often behave as surfactants. Nano structures formed by self-assembling of biologically active amphiphilic molecules can be at the same time efficient therapeutics as well as nano carriers.

A class of compounds that has been attracting increased attention in modern drug discovery is the quinuclidine based derivatives. The quinuclidine is a saturated bicyclic alkaloid found in several plant-based natural products with a broad spectrum of biological activities: (i) a large number of quinuclidine-based compounds are found to be an attractive isostere of lipid-lowering agents which inhibit squalene synthase activity, leading to reduced cholesterol biosynthesis in animals [13,14], (ii) they are also identified as promising classes of anticancer agents against several cancer cell lines [15], (iii) it has been demonstrated that several quinuclidine scaffolds, including arylquinuclidine have potent activity against parasitic protozoa in concentrations varying from the nanomolar to subnanomolar range [16–18].

Oximes and their complexes are another class of compounds that have recently drawn compelling interest owing to their biological and pharmacological activities. Oximes have been recognized as valuable structures for many important pharmaceutical and synthetic chemistry applications, and often act as chemical building blocks for the synthesis of some relevant antimicrobial and antihypertensive agents as well as insectides [19,20]. Besides that, oximes and oxime functionalized surfactants have been firstly reported as compounds with a great potential in the treatment of organophosphorus compounds poisoning, including insecticides and nerve agents, by acetylcholinesterase (AChE) reactivation [21,22]. Several 3-substituted quinuclidinium derivatives, including quinuclidinium oximes, showed antidotal

efficacy as a result of their interaction with AChE and/or other receptors in the cholinergic system, *e.g.* suppression of presynaptic synthesis of acetylcholine [23,24].

Despite considerable interest for quinuclidine and oxime functionalities, studies of surfactants containing these moieties are scarce. In order to obtain oxime functionalized surfactants and estimate their physicochemical properties Singh *et al.* have synthesized and characterized 3-hydroxyiminomethyl-1-alkylpyridinium bromides [25]. Hydrophobized derivatives of 1,4-diazabicyclo[2.2.2]octane, which are cationic surfactants with quinuclidinium headgroup, have been investigated by Zakharaova *et al.* [26]. Dymond and Attard investigated vastly different cationic amphiphiles as modulators of membrane curvature elastic stress [27]. They have shown that alkylquinuclidinium compounds exhibit good cytotoxic efficacy towards HL-60 cells. Studies by differential scanning calorimetry and Raman spectroscopy showed that alkylquinuclidinium bromides exhibit two or three thermal phase transitions depending on alkyl chain length [28]. Recently, Bhadani *et al.* investigated self-aggregation and liquid crystalline behavior of ester-functionalized quinuclidinium surfactants [29]. They showed that physicochemical properties of these surfactants are greatly affected by unique structure of quinuclidinium headgroup.

Motivated by diverse biological and pharmacological activity of quinuclidine and oxime compounds we have prepared and characterized novel series of homologous 3-hydroxyimino quinuclidinium bromides with different alkyl chains lengths (C_nQNOH, n = 12, 14 and 16, Scheme 1). It was expected that incorporation of nonconventional hydroxyimino quinuclidinium group into the molecular structure of surfactants will take advantage of biological activity of quinuclidinium oximes and amphiphilic nature of surfactant. Since surfactant properties can be tailored by changing hydrophilic-hydrophobic balance of molecule by changing alkyl chain length, the influence of increasing number of C atoms in alkyl chain on physicochemical and biological properties was investigated.

The manner in which surfactant molecules are arranged in the crystal phase correlates with their solution behavior as well as their adsorption and aggregation properties reflects on their biologically activity. Therefore, to establish favorable structure-property relationship is not easy task because it is often difficult to change the surfactant structure in one specific way without changing some of their physicochemical properties in undesirable direction. The structure-property investigations are essential in order to understand the behavior of the complex surfactant systems and to be able to effectively synthesize new surfactants for targeted application. In order to determine structure-property relationship of newly synthesized C_nQNOH surfactants comprehensive characterization from their crystal structure

to adsorption and aggregation behavior in solution was performed. In addition, to confirm antimicrobial profile C_nQNOH surfactants were evaluated against a panel of laboratory reference Gram-positive bacteria and clinically relevant antibiotic resistant Gram-negative strains by both disk diffusion and broth microdilution assays. The fundamental understanding how combination of different functionalities in a single molecule affects their physicochemical and biological properties is a first step to its larger-scale implementation.

2. Experimental

2.1. Synthesis of novel C_nQNOH surfactants. Three quaternary quinuclidinium oximes C_nQNOH (n = 12, 14 and 16) which were not previously described were synthesized and characterized (Scheme 1). All already known compounds were identified by comparison of their physical and spectral data with those reported in the literature. The synthesis started from the commercially available quinuclidine-3-one and the corresponding oxime was prepared in a solvent free reaction (a catalytic amount of the solvent was added) using ball grinding without adding any base. This mechanochemical approach using hydroxylamine hydrochloride proved to be an excellent alternative for the classical oxime synthesis of *N*-heterocyclic carbonyl compounds. Finally, quaternary compounds were synthesized by the Menshutkin reaction of the prepared 3-hydroxyiminoquinuclidine, QNOH, with the appropriate alkyl bromide in a good yields.

If not otherwise stated, all chemicals were obtained from Sigma-Aldrich Co. and used without further purification. Quinuclidin-3-one [30] was prepared as described previously from the commercially available quinuclidin-3-one hydrochloride (Fluka). The mechanochemical synthesis of QNOH HCl was carried out according to the published procedure [31]. Barium hydroxide was used to remove HCl and obtain QNOH. QNOH (1.5 mmol) was dissolved in dry acetone. *N*-dodecyl bromide, *N*-tetradecyl bromide, or *N*-hexadecyl bromide respectively (2.0 mmol) was slowly added to the solution. The mixture was heated for 48 h at 50 °C. After cooling at the room temperature, acetone was evaporated under reduced pressure in a rotary evaporator at 40 °C. Crude reaction product was washed 3 times with hexane. All three compounds were obtained as white crystals after recrystallization from acetonitrile. The compound characterization (IR, ^{1}H and ^{13}C NMR, CHN) is given in Supplementary material.

2.2. Methods

2.2.1. Single crystal analysis. Crystals of C_nQNOH surfactants suitable for single-crystal X-ray analysis were grown from nearly saturated acetonitrile solutions by slow evaporation at room temperature. All measurements were performed on Xcalibur Nova X-ray diffractometer with multilayer optics and Cu Ka radiation (λ = 1.5412 Å) at room temperature. Crystallographic data for the structures C_{12}QNOH, C_{14}QNOH and C_{16}QNOH are deposited in the Cambridge Structural Database under the CCDC codes 1413313, 1413315, and 1413316, respectively.

2.2.2. Krafft temperature. The Krafft temperature (t_{Krafft}) of C_nQNOH surfactants was determined by measuring the change of electrical conductivity (Conductivity Meter, Methron, Switzerland) with temperature by heating and cooling of 1 wt % solution [32].

2.2.3. Electrical conductivity and surface tension measurements. The electrical conductivity (κ) measurements were performed with a Conductivity Meter (Methron, Switzerland) in a temperature-controlled double-walled glass container with a circulation of water and coverlid. The surface tension (γ) measurements were carried out with an Interfacial Tensiometer K100 (Krüss, Germany) using the Du Noüy ring method.

2.2.4. Determination of Acid Dissociation Constants. pK_a of synthesized compounds was determined by potentiometric titrations using Metrohm 827 pH/Ion meter.

2.2.5. Light scattering and zeta potential measurements. The size distribution and zeta potential of C_nQNOH micelles were determined by means of a dynamic light scattering (DLS) technique using a photon correlator spectrophotometer equipped with a 532 nm "green" laser (Zetasizer Nano ZS, Malvern Instruments, UK).

2.2.6. Biological tests. All of the newly synthesized target C_nQNOH surfactants were evaluated for their *in vitro* antibacterial activity. The tested microorganisms were obtained from the culture collection at the American Type Culture Collection (ATCC) (Rockville, MD, USA) and at the Microbiology laboratory, Department of Biology, Faculty of Natural Science, University of Split, Croatia (FNSST). The assayed collection included four Gram-positive bacteria *Bacillus cereus* (ATTC 11778), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923) and *Clostridium perfringens* (FNSST 4999) in addition to four of key Gram-negative ampicillin-resistant bacterial strains; *Escherichia coli* (FNSST 982), *Klebsiella pneumoniae* (FNSST 011), *Pseudomonas aeruginosa* (FNSST 982) and *Chronobacter sakazakii* (FNSST 014).

More detailed description of experimental setups and used methods as well as data interpretation is given in Supplementary material.

3. Results and discussion

3.1. Crystal structure. The way in which surfactant molecules are packed in the crystal phase correlates with their solution behavior *i.e.* there is a strong relationship between the structure of a surfactant molecule, its crystal structure and adsorption and aggregation properties in aqueous solutions. The structure of the newly synthesized C_nQNOH surfactants was determined by single-crystal X-ray diffraction (Fig. 1). All three investigated compounds crystallized in the monoclinic space group $P2_1/c$ and displayed layered arrangements with interdigitated hydrocarbon chains. Crystallographic data, data collection, and structure refinement details are given in Table 1.

The variation in alkyl chain length leads to small changes in molecular packing of C_nQNOH surfactants (Fig. 1a, Table 1). The asymmetric unit of crystal structure of all investigated compounds consists of one amphiphilic cation and one bromide ion counterbalancing the charge (Fig. 1b, only the crystal packing for C_{12}QNOH is shown due to small changes in crystal arrangement with variation in the alkyl chain length). The hydrocarbon chains are in all-*trans*, fully extended conformation aligned in the direction of the long axis and parallel to one another while headgroups are oriented in opposite direction. Due to their amphiphilic character the orientation of the cations alternates from layer to layer separating hydrophobic (alkyl tails) and hydrophilic (the quinuclidine headgroup with quaternary ammonium cation and bromide ion) regions. The terminal oxygen atom O1 from oxime moiety in quinuclidine headgroup forms hydrogen bond with bromide ion in all investigated crystal structures (Fig. 1a, Table S1 in Supplementary material).

The overall arrangement of C_nQNOH surfactants in crystal could be described as tilted interdigitaded bilayers with a thickness equal to the dimension of the unit cell *a* axis. The tilt angle of long alkyl chains with respect to the bilayer normal was approximately 45°. The crystal structure of all investigated compounds is composed of bilayers stacked along *a* axis forming a bulk crystalline phase. The variation in the alkyl chain length in C_nQNOH molecules leads only to increase in the thickness of a bilayer, *i.e.* to the elongation of the unit cell *a* axis and slight change in β angle, while *b* and *c* axis lengths remain almost constant (Table 1).

The stacked bilayered structures with interdigitaded hydrocarbon chains are characteristic for *n*-alkyl amphiphilic compounds, *e.g.* trimethylammonium bromides (C_nTAB); dodecyl (C_{12}TAB) [33], tetradecyl (C_{14}TAB) [34] and hexadecyl (C_{16}TAB) [35]. As already mentioned, other than small change in the interlayer distance there is hardly any

difference in atom positions and crystal packing with increasing chain length of C_nQNOH surfactants. On the contrary, two additional methylene units in the alkyl chains of C_nTAB cause more significant changes in surfactants molecular packing. C_{12}TAB crystallizes in $P2_1$, while C_{14}TAB and C_{16}TAB as well as C_nQNOH surfactants crystallize in centrosymmetric $P2_1/c$ space group [33–35].

Regardless of the number of carbon atom in alkyl tail, the thickness of C_nQNOH bilayers (Fig. 1b, Table 1) is close to the one found for C_nTAB surfactants [33–35]. These results were somewhat unexpected having in mind large and rigid quinuclidinium unit compared to conventional trimethyl quaternary ammonium headgroup. They indicate high extent of alkyl chain interdigitation and dense molecular packing of C_nQNOH surfactants despite steric effects. Due to the lack of proton donors, no classical hydrogen bonds are found in C_nTAB structures. The hydrogen bond between oxime moiety in quinuclidine headgroup and bromide ion includes more favorable interactions and consequently contribute to a dense crystalline pattern of C_nQNOH. It is likely that increased hydrophobicity of the quaternary ammonium cation environment also contributes to the high packing density of C_nQNOH molecules. These findings point to the conclusion that dominant role in determining C_nQNOH crystal structure has bicyclic headgroup with hydroxyimino moiety which act as proton donor in hydrogen bond with counter ion making the ordering in the headgroup region more tightly.

3.2. Acid Dissociation Constant (pK_a). In order to determine acid-base properties of the oxime group in C_nQNOH surfactants potentiometric titrations were performed. The determined p$K_{a,1}$ and p$K_{a,2}$ values for QNOH (Scheme 1) at 25 °C were 8.072 ± 0.034 and 10.805 ± 0.030, respectively (Fig. S1 in Supplementary material). The first p$K_{a,1}$ value correspond to the quinuclidine nitrogen atom, whereas the second p$K_{a,2}$ is for the oxime moiety. This value is similar to the known values for ketoxime compounds [36]. As expected, upon the quaternization, the acidity of oxime group is increased. Due to high Krafft temperature of C_{12}QNOH pK_a value was measured at 65 °C and is equal to 9.95 ± 0.20. The resulting oxyanion is stabilized by the positive charge of the quinuclidinium nitrogen atom. The measured value is somewhat higher than those determined for 3-hydroxyiminomethyl-1-alkylpyridinium bromides at 27 ^{o}C [25]. The screening of all C_nQNOH compounds provided similar values confirming the fact that pK_a is not affected by elongation of the alkyl chain length. Since the pH values measured in monomer and micellar solutions of C_nQNOH surfactants remained almost constant and did not exceeded 6.7, it is safe to argue that 3-hydroxyimino group is not dissociated at the given experimental conditions.

3.3. Krafft temperature. The solubility of ionic surfactants in water is highly temperature dependent. In the case of ionic surfactants the solubility undergoes a sharp, discontinuous increase at characteristic temperature, named the Krafft temperature (t_{Krafft}) due to formation of the micelles [1]. Knowledge of the Krafft temperature is crucial in many applications since below t_{Krafft} the surfactant overall effectiveness is markedly reduced.

The Krafft temperatures of investigated surfactants determined from the electrical conductivity measurements and visually are presented in Table 2. Obtained results are in accordance with general trend observed for homologue series of ionic surfactants with increasing chain length. The Krafft temperature usually increases approximately 10 ^{o}C for every two additional methylene units in the straight alkyl chain of ionic surfactants [1]. In all cases determined t_{Krafft} values were well above the room temperature and substantially higher than those reported for the structurally similar quinuclidinium surfactants without hydroxyimino group [27] and C$_n$TAB [37,38] (Table 2).

The Krafft temperature reflects the solubility of surfactant monomers in the presence of hydrated crystals [37]. It is highly dependent on surfactant molecular structure and primarily determined by the strength of the crystal lattice of the solid surfactant. Therefore only a small modification in surfactant structure can lead to variation in packing and interactions in the solid state that can significantly change the t_{Kraff} value. The linear increase of t_{Krafft} for C$_n$QNOH surfactants (Fig. S2 in Supplementary material) can be ascribed to a decrease in monomer solubility due to the increasing number of methylene groups in alkyl chains. Likewise, introducing quinuclidinium instead of conventional trimethyl quaternary ammonium headgroup in molecular structure makes molecule more hydrophobic and monomer solubility is decreased. On the other hand, due to the presence of large and rigid headgroup Columbic interactions between the charged nitrogen and the bromide counterion are reduced, which should result in lower t_{Krafft} values. In this case, high t_{Krafft} may be attributed to decreased headgroup hydration, as a result of its large geometric frame which prevents water contact with the charged nitrogen atom, thus reducing the monomer solubility [37]. Nevertheless, main factor causing higher t_{Krafft} of investigated homologue series quinuclidinium surfactants compared to C$_n$TAB and structurally similar quinuclidinium surfactants can be attributed to the hydrogen bonding between terminal oxygen atom O1 and bromide ion (Fig. 1a) which provides extra stability to the crystal structure. Although the hydroxyimino group is hydrophilic and can enhance monomer solubility, the hydrogen bonds O−H···Br$^-$ stabilizes the solid state. A strong headgroup-counter ion interaction and a dense packing in a crystal lattice contribute to the crystals stability and consequently, the t_{Krafft} is

significantly elevated. Although high Krafft temperature of the investigated surfactants hinders their application at room and/or body temperature, this can be circumvented by applying the surfactant mixture, *e.g.* ionic-nonionic formulations generally shows lower t_{Krafft} [39].

3.4. Adsorption at the air/solution interface. Due to its bulky and rigid structure the 3-hydroxyimino quinuclidinium headgroup was expected to influence significantly the aggregation and surface properties of investigated surfactants. Fig. 2a presents the surface tension (γ) isotherms for C_{12}QNOH and C_{14}QNOH. The corresponding dependencies of the electrical conductivity (κ) *vs.* surfactant concentration are shown in Fig. 2b and c. Due to very high Krafft temperature the measurements for C_{16}QNOH were carried out at 70 ^{o}C. For that reason the surface tension measurements for C_{16}QNOH was not performed and only electrical conductivity was measured at 70 ^{o}C (Fig. 2c). The working temperature during the conductivity measurements was easier to maintain in temperature-controlled double-walled glass container while coverlid reduced sample evaporation.

All γ *vs.* log c curves (Fig. 2a) exhibit a typical course, the surface tension initially gradually decreased with increasing surfactant concentration up to a plateau region, above which an almost constant value was obtained indicating the formation of micelles. The parameters obtained from surface tension measurements are listed in Table 3. For the sake of comparison data for conventional straight-chain cationic, *i.e.* C_nTAB surfactants are also given [5,40–42]. It enabled us further insight in how changes in headgroup structure, by introducing different functional groups, influences surfactant properties.

The Γ_{max} value is a useful measure of the effectiveness of adsorption at the air/solution interface, since it is the maximum surfactant concentration that can be adsorbed [1]. The effectiveness of adsorption is inversely proportional to a_{min} and mainly depends on the surfactant structure and its orientation at the interface. The Γ_{max} value of C_nQNOH surfactants increases and a_{min} consequently decreases with increasing alkyl chain length. More tightly packed C_{14}QNOH monolayer can be attributed to the increase in repulsion forces between alkyl chains and water molecules and to the increase in chain-chain lateral interactions due to increased hydrophobicity of the molecule.

The difference in the a_{min} values between C_nQNOH and C_nTAB surfactants is not as large as expected considering the difference in the headgroups structure and size (Table 3). In the case of C_{14}QNOH the a_{min} value is even smaller than for C_{14}TAB. It would be expected that bulky quinuclidinium group with oxime moiety occupies larger surface area in adsorbed monolayer compared with trimethylammonium headgruop. In addition, it should be noted that

the Γ_{max} and a_{min} values for these two homologue surfactants series are determined at very different temperatures. The a_{min} value increase with temperature due to the increase of thermal motion of molecules in the surface monolayer, as seen from results obtained for C_{12}QNOH at different temperatures. It would be expected that due to higher experimental temperature the a_{min} values for C_{12}QNOH and C_{14}QNOH are somewhat larger compared to the ones of C_nTAB. It seems that the presence of proton donor hydroxyimino group in molecular structure of investigated surfactants which enables hydrogen bonding with available proton acceptors, such as neighboring =N-OH groups and counter ions, facilitates dense packing of rather large quinuclidinium headgroup in surface monolayer. It is likely that enhanced hydrogen bonding compensates steric and electrostatic repulsions between C_nQNOH headgroups. Likewise, as a consequence of intermolecular hydrogen bonding the unexpectedly small a_{min} values was found for the straight-chain surfactant containing amide and cyclohexane group in its structure [11]. Importantly, Bhadani *et al.* showed that due to its geometry quinuclidinium headgroups in ester-functionalized surfactants (C_nQEsCl) creates molecular cavities between the extended carbon arms of this moiety [29]. The exceptionally small a_{min} values of C_nQEsCl are result of accommodation the adjacent quinuclidinium carbon arms in these cavities leading to dance packing of molecules at the interface. Bearing in mind calculated a_{min} values it is likely that same effect is present in C_nQNOH systems only to a somewhat lesser extent.

The γ_{cmc} values also reflect dense molecular packing of C_nQNOH surfactants in adsorbed monolayer. The γ_{cmc} value indicates maximum reduction of surface tension caused by the dissolution of surfactant molecules and is a measure of the surfactant's effectiveness to lower the surface tension of the solvent. As it can be seen, C_nQNOH surfactants with 12 and 14 carbon atoms in alkyl chain are very efficient in adsorption at the air/solution interface, *i.e.* they significantly reduced the surface tension of water.

3.5. Solution properties below the cmc. The electrical conductivity measurements are sensitive to interionic interactions and, therefore, suitable for obtaining at least qualitative evidence of structural transformation in solution such as ion-pairing, complexation, premicellar association, etc. [6,43]. If C_nQNOH surfactants were completely dissociated, at the concentrations below cmc, the conductivity (κ) would be related to their concentration (c_{C_nQNOH}) as: $\kappa = (\lambda_{Br^-} + \lambda_{C_nQNOH^+})\, c_{C_nQNOH}$, meaning that the slope of the first part of the κ *vs.* c_{C_nQNOH} curve should equal to the sum of the equivalent ionic conductivities of bromide

(λ_{Br^-}) and surfactant ions ($\lambda_{C_nQNOH^+}$). All slopes in the κ *vs.* c_{C_nQNOH} plots below the cmc were smaller than the values for the equivalent conductivity of Br^- ion at infinite dilution calculated from literature data [44] at given temperature. Observed decrease in conductivity indicates that C_nQNOH surfactants are not fully dissociated at concentrations below the cmc.

To confirm these observations measured conductivity data were also studied by means of the molar conductivity *vs.* the square root of concentration plots (Fig. S3 in Supplementary material). It may be noted that marked cmc for C_{16}QNOH in the λ *vs.* $c^{1/2}$ plot (Fig. S3c) is not identical with the onset of molar conductivity decrease in the micellar region. The marked cmc value for C_{16}QNOH was determined from the intersection of the two straight lines drawn at low and high concentration regions in the κ *vs. c* curve (Fig. 2c, Table 2). It is known that the same experimental data can give different values for the cmc depending on how they are plotted. The discrepancy is due in large part to the fact that, a straight line on one plot is curve on the other, so that different points are chosen as the basis for extrapolation [45].

Plotted λ *vs.* $c^{1/2}$ curves at concentration below cmc for all investigated compounds showed curvature toward the concentration axis indicating ion pairing of C_nQNOH at low concentration [7,43]. Binding of Br^- to a quinuclidinium surfactant cation results in a neutralization of electrical charges and thus a loss of conductivity. It is known that ion pairs have high tendency to form when molecule hydrophobicity is increased; typical examples are dimeric quaternary ammonium surfactants [6,43]. In the case of investigated surfactants both quinuclidinium headgroup and lengthening of alkyl chain contribute to increase of hydrophobicity and consequently to the ion pairing. However, the change of hydrophilic-hydrophobic balance was not sufficient to cause premicellar aggregation, as was evidenced by the lack of a maximum in the λ *vs.* $c^{1/2}$ plot [43]. The λ *vs.* $c^{1/2}$ plot very similar to the ones obtained for C_nQNOH surfactants was found for single chain pyridinium based cationic surfactants [7]. This class of surfactants also contains a bulky and hydrophobic headgroup in their molecular structure. On the contrary, conventional single chain cationic surfactant with 12 carbon atoms in alkyl chain, C_{12}TAB, shows no ion paring [43]. It can be concluded that the solution behavior of C_nQNOH at concentrations below cmc is closer to the dimeric than the single-chain cationic surfactants confirming large effect of nonconventional headgroup on their properties.

3.6. Aggregation properties. The C_{12}QNOH and C_{14}QNOH cmc values obtained from the surface tension (cmc_γ) and the electrical conductivity (cmc_κ) measurements were in good agreement (Table 3). Increase of the hydrocarbon chain length shifts cmc of C_nQNOH

surfactants to lower values (Table 3). The log cmc values linearly decreased with the increase in the number of carbon atoms in C_nQNOH surfactants as shown in Fig. S2 in Supplementary material.

The greatest difference in the cmc values in favor of C_nQNOH surfactants, compared to C_nTAB, was found for surfactants containing 12 carbon atoms in alkyl chain. With increase in alkyl chain length the differences in the cmc values between these two series of surfactants decreases. The cmc value for the most hydrophobic compound in the quinuclidinium series, C_{16}QNOH is even higher than the value determined for C_{16}TAB. However, it should be noted that the cmc values for C_nQNOH were determined at higher temperatures.

The inserting quinuclidinium headgroup with oxime moiety instead trimethylammonium cation increased the spatial effect of molecular structure on the micelle formation. The bulky and rigid quinuclidinium headgroup may sterically hinder surfactant molecule to become part of the micelle core. On the other hand, both hydrophobicity and hydrophilicity in headgroup region of micelle is increased. In order to minimalize contact with water the more hydrophobic headgroup enhanced micellization. In addition, when dimension of the headgroup increases the electrostatic repulsions among the headgroups is reduced enabling tighter packing of surfactant molecules. Modification of the headgroups with hydroxyimino group also affects the molecular interactions due to hydrogen bonding enabling denser molecular arrangement at the micelle/solution interface and consequently stronger aggregation ability. Evidently, these effects affect micellization of C_nQNOH surfactants more than the steric effect. It is likely that due to structural constraints imposed by quinuclidinium headgroup the additional methylen units in alkyl chains of C_nQNOH surfactants to a lesser extent contribute to the reduction of cmc in comparison with C_nTAB series.

The cmc values determined for C_nQNOH are close to the ones measured for the structurally similar quinuclidinium surfactants without hydroxyimino group at lower temperatures [26,27]. Conversely, the cmc values of C_nQEsCl surfactants at 25 ^{o}C are more than twice lower that those determined for C_nQNOH series [29]. This indicates that presence of ester functionality in the structure of quinuclidinium surfactant affects micelization at the larger scale than its ability to create molecular cavities locating at interfaces.

The particle size measurements at concentrations 2-fold of the cmc showed that the value of hydrodynamic diameter of the C_nQNOH micelles increased with increasing alkyl chain length from 2.7 ± 0.2 nm (C_{12}QNOH) to 5.2 ± 1.3 nm (C_{16}QNOH). Obtained d_h values were significantly different at $P < 0.05$. From DLS measurements it is not possible to discern

the shape of surfactant aggregates. However, having in mind their measured size and preferred morphology predicted by the packing parameter for single-chain surfactants with large headgroup [46], it is reasonable to assume that all three surfactants form spherical micelles at concentrations close to the cmc .

The degree of counterion association to the micelle/solution interface (β), determined from the electrical conductivity measurements, increased with increasing alkyl chain length (Table 3). Somewhat lower β value of C_{16}QNOH than C_{14}QNOH may be ascribed to the fact that the former refers to measurements at higher temperature as can be seen from results obtained for C_{12}QNOH at different temperatures. The same trend was observed for C_nTAB surfactants; the β value gradually decreases with increase of temperature [38,42]. Higher β value is a result of the increase in charge density at the micelle/solution interface as revealed from the zeta potential (ζ) values. The ζ potential of the C_nQNOH micelles increased with increasing alkyl chain length from 27 ± 4.3 mV (C_{12}QNOH) to 34 ± 6.7 mV (C_{16}QNOH). Obtained ζ values were significantly different at $P < 0.05$.

3.7. Antimicrobial efficacy. The antibacterial activity of cationic surfactants is well known [8–10]. In general, due to the presence of negatively charged phospholipids cationic surfactants interact with cell wall of bacteria by electrostatic interactions. In addition, long alkyl tails of surfactants *via* hydrophobic interactions can penetrate into the membrane interior. As a result permeability of bacteria membrane is altered after which death occurs.

The quaternary ammonium surfactants containing long alkyl chains exert bactericidal and fungicidal properties [8,10]. Furthermore, quinuclidinium and oxime based compounds are known to possess a broad range of biological and pharmacological activities [15–20]. These properties have been exploited as starting point for the design of antibacterial surfactants.

In order to obtain antimicrobial profile of C_nQNOH surfactants and determine influence of the alkyl chain length on their antimicrobial potential, their antimicrobial efficacy was preliminary screened against a diverse panel of laboratory reference antibiotic susceptible Gram-positive and clinically relevant antibiotic resistant Gram-negative species using disc diffusion bioassay and measuring minimum inhibitory concentration (MIC). For comparison, tetracycline and gentamicin, important antibiotics that are currently used in clinical treatment, were also included in the assays as positive controls.

As shown in Table 4, the results of the preliminary *in vitro* disc diffusion bioassay showed that all C_nQNOH surfactants demonstrated significant antibacterial efficacy on a wide range of the antibiotic susceptible Gram-positive and resistant Gram-negative bacteria. In

addition, results obtained with 250 μg / disc and at 500 μg / disc showed that the response is dose dependent. The mean zones of inhibition of C_nQNOH surfactants against all tested bacteria were to ranging from 11.7 ± 0.7 to 41.2 ± 0.4 mm.

These data demonstrate that target compounds are not only active against Gram-positive bacteria, but also demonstrated potent and broad antibacterial spectrum against antibiotic resistant Gram-negative strains. Since membrane of Gram-negative bacteria has complex structure consisting of two bilayers that hinders adsorption they are often less susceptible to treatment with surfactants than Gram-positive bacteria as also evident from obtained results. Among the Gram-negative bacteria tested, two strains namely *Escherichia coli* and *Pseudomonas aeruginosa* showed relative high sensitivity towards all the tested compounds with considerable growth inhibition zones. Moreover, C_nQNOH surfactants were even more effective than both tetracycline and gentamicin. It is particularly important that the all C_nQNOH surfactants displayed potent antimicrobial activity against *Pseudomonas aeruginosa*. This bacteria is a leading nosocomial pathogen, has the distinctive capacity *via* multiple mechanisms to become resistant to virtually all the antibiotics available commercially and is responsible for 10% of all hospital-acquired infections resulting in significant morbidity and mortality [47]. The effect of alkyl chain length of C_nQNOH surfactants on the size of inhibition zones indicated some interesting structure-antibacterial activity relationships. No significant differences in antibacterial efficacy between C_{12}QNOH and C_{14}QNOH were observed, but it was found that zones of inhibition somewhat decreased with the increase in chain length from 14 to 16 carbon atoms in alkyl chains.

In addition to disc diffusion bioassay, the antimicrobial activity was also determined by measuring minimum inhibitory concentrations (MIC) using a standardized broth microdilution assay. Due to relatively large differences in molecular weight of C_nQNOH surfactants, in order to obtain better insight in antimicrobial activity within this homologue series, determined MIC were expressed in molar concentrations (μmol L^{-1}) and presented in Table 5. According to the results given in Table 5, all C_nQNOH surfactants exhibited significant antibacterial activity against a broad spectrum of tested bacterial species. The MIC values were in the range from 1.23 to 280.57 μmol L^{-1}. Their efficiency as antimicrobial agents decreased with the increasing hydrophobicity of the amphiphilic cation, C_{12}QNOH being the most active compounds as well as in *in vitro* disc diffusion bioassay. Antibacterial efficacy against *E. coli* proved to be exception. The MIC values obtained for *E. coli*, decreased from 80.25 μmol L^{-1} to 37.42 μmol mL^{-1} with the increase in the alkyl chain length from 12 to 14 carbon atoms and then increased to 112.23 μmol L^{-1} for C_{16}QNOH. In general,

C_{12}QNOH displayed more potent and broad-spectrum activity against the Gram-positive (1.23 – 80.25 μmol L^{-1}) than the Gram-negative bacteria (10.02 - 80.25 μmol L^{-1}). C_{12}QNOH also possess a promising antimicrobial potential for other important pathogens such as *Enterococcus faecalis* (1.23 μmol L^{-1}), *Staphylococcus aureus* (2.49 μmol L^{-1}), *Clostridium perfringens* (20.06 μmol L^{-1}) and *Klebsiella pneumonia* (10.02 μmol L^{-1}). In comparison with tetracycline and gentamicin, C_nQNOH surfactants exhibited better or comparable antimicrobial activities especially against multidrug resistant Gram-negative pathogens.

Unlike the log-linear relationship between increasing alkyl chain length and cmc as well as t_{Krafft} (Fig. S2), biological activities of surfactants often show a nonlinear dependence on the chain length as seen from Figs. 3a-b. In a homologous series of surfactants antimicrobial activity often increases progressively with increasing chain length up to optimal point after which the activity decreases. A described phenomenon is referred to as the cut-off effect [48]. Figs. 3a-b clearly showed that surfactant with the most efficient antimicrobial activity in investigated series of alkyl homologues was C_{12}QNOH.

Due to large difference between experimental conditions the assessments how adsorption and aggregation properties of surfactants influence their antimicrobial efficiently is not straightforward. Nevertheless, some conclusions could be made. The C_{12}QNOH and C_{14}QNOH surfactants demonstrated high adsorption efficiency and tightly packed monolayer at air/solution interface. Considering that comparison can be made between the air/aqueous solution interface and the nonpolar cell membrane of bacteria [9], high antimicrobial efficacy is not unexpected.

It is known that surfactants with 12 carbon atoms in alkyl tail often display the optimal biological effects [8,9]. This can be attributed to the combination of several physicochemical properties: surfactant hydrophobicity and aqueous solubility, adsorption efficiency, cmc, transport in the test medium etc. [9]. The solubility is the limiting factor for the transport of surfactants monomers to bacteria membrane. For surfactants containing longer alkyl tail as in the case of C_{16}QNOH antimicrobial efficiently decreased due to their reduced solubility. The cooperative interaction of these variables determines that the homologue with 12 carbon atoms in alkyl hydrophobic tail have the largest tendency to disturb the cell membrane ultimately leading to bacteria death.

In summary, the results of disc diffusion bioassay and MIC values showed that C_nQNOH surfactants have more potent broad spectrum antimicrobial activity, especially against clinically relevant antibiotic resistant Gram-negative strains, than conventional antimicrobial agents tetracycline and gentamicin. The change in the alkyl chain length of

these surfactants resulted in differences in both potency and spectrum of antimicrobial activity.

4. Conclusions

Motivated by diverse biological and pharmacological activity of quinuclidine and oxime compounds we have designed, synthesized and characterized novel class of cationic surfactants, 3-hydroxyimino quinuclidinium bromides with different alkyl chains lengths (C_nQNOH; n = 12, 14 and 16). The variation in alkyl chain length affects hydrophilic-hydrophobic balance of surfactants and thereby physicochemical properties important for their application. Comprehensive investigation of their structure and solution behavior enabled us insight into the structure-property relationship in this nonconventional surfactant systems.

The overall results obtained for C_nQNOH surfactants point out that bulky bicyclic headgroup with oxime moiety which affects both hydrophilicity and hydrophobicity of molecule has dominant effect on their physicochemical properties. Furthermore, presence of hydroxyimino group in molecular structure of investigated surfactants which enables hydrogen bonding with available proton acceptors has a major effect on their packing in solid state, *i.e.* crystal structure as well as at both the air/solution and micelle/solution interface.

The results of disc diffusion bioassay and MIC values showed that all C_nQNOH surfactants have more potent broad spectrum antimicrobial activity, especially against clinically relevant antibiotic resistant Gram-negative strains, than conventional antimicrobial agents tetracycline and gentamicin (Table 4 and 5). Due to their reduced solubility efficiency of C_nQNOH surfactants as antimicrobial agents decreased with the increase in alkyl chain length, C_{12}QNOH being the most active homologue. However, it is important to emphasize that C_nQNOH surfactants displayed potent antimicrobial activity against *Pseudomonas aeruginosa*. This bacterium is a leading nosocomial pathogen and has the distinctive capacity *via* multiple mechanisms to become resistant to virtually all the antibiotics available commercially [47].

In conclusion, the newly synthesized C_nQNOH surfactants demonstrated high adsorption efficiency and relatively low cmc compared with conventional straight-chain cationic surfactants. Their largest drawback is high Krafft temperature which can be circumvented by applying the surfactant mixture. The unique structural features of cationic surfactants with quinuclidine headgroup together with diverse biological activities have made them promising structures in novel antimicrobial drug discovery. The particularity of amphiphilic molecules reflects in the fact that they can be at the same time efficient

therapeutics as well as nano carriers. The global emergence of resistance to multiple antibiotic classes by pathogenic bacteria has become a serious threat to public health and is considered to be one of the greatest challenges for medicine today [49]. Therefore, design and development of antibacterial pharmacophore, especially those with novel molecular structures and modes of action on membrane targeting which provides advantages over conventional antibiotics are imperative to prevent the emergence of multidrug resistant pathogens. Obtained fundamental understanding how combination of different functionalities in a single surfactant molecule affects their physicochemical properties represents a good starting point for further biological research.

Appendix A. Supplementary material

Acknowledgment

We are indebted to dr. N. Filipović-Vinceković for helpful discussions and reading of the manuscript. Financial support from Croatian Ministry of Science, Grant 098-0982915-2949, Croatian Science Foundation Grant HRZZ-5055 and University of Zagreb short-term financial scientific research support is greatly acknowledged.

References

[1] M.J. Rosen, Surfactants and interfacial phenomena, 3th ed., John Wiley & Sons, Hoboken, New Jersey, 2004. doi:10.1016/0166-6622(89)80030-7.

[2] Q. Jin, Y. Chen, Y. Wang, J. Ji, Zwitterionic drug nanocarriers: A biomimetic strategy for drug delivery, Colloids Surfaces B Biointerfaces. 124 (2014) 80–86. doi:10.1016/j.colsurfb.2014.07.013.

[3] L. Tavano, A. Pinazo, M. Abo-Riya, M.R. Infante, M.A. Manresa, R. Muzzalupo, L. Pérez, Cationic vesicles based on biocompatible diacyl glycerol-arginine surfactants: Physicochemical properties, antimicrobial activity, encapsulation efficiency and drug release, Colloids Surfaces B Biointerfaces. 120 (2014) 160–167. doi:10.1016/j.colsurfb.2014.04.009.

[4] C. Aiello, P. Andreozzi, C. La Mesa, G. Risuleo, Biological activity of SDS-CTAB cat-anionic vesicles in cultured cells and assessment of their cytotoxicity ending in apoptosis, Colloids Surfaces B Biointerfaces. 78 (2010) 149–154. doi:10.1016/j.colsurfb.2010.02.013.

[5] D. Jurašin, I. Habuš, N. Filipović-Vinceković, Role of the alkyl chain number and head groups location on surfactants self-assembly in aqueous solutions, Colloids Surfaces A Physicochem. Eng. Asp. 368 (2010) 119–128. doi:10.1016/j.colsurfa.2010.07.025.

[6] M. Sikirić, I. Primožič, Y. Talmon, N. Filipović-Vinceković, Effect of the spacer length on the association and adsorption behavior of dissymmetric gemini surfactants, J. Colloid Interface Sci. 281 (2005) 473–481. doi:10.1016/j.jcis.2004.08.140.

[7] P. Quagliotto, N. Barbero, C. Barolo, E. Artuso, C. Compari, E. Fisicaro, G. Viscardi, Synthesis and properties of cationic surfactants with tuned hydrophylicity, J. Colloid Interface Sci. 340 (2009) 269–275. doi:10.1016/j.jcis.2009.09.009.

[8] D. Malinak, R. Dolezal, J. Marek, S. Salajkova, O. Soukup, M. Vejsova, J. Korabecny, J. Honegr, M. Penhaker, K. Musilek, K. Kuca, 6-Hydroxyquinolinium salts differing in the length of alkyl side-chain: synthesis and antimicrobial activity, Bioorg. Med. Chem. Lett. 24 (2014) 5238–5241. doi:10.1016/j.bmcl.2014.09.060.

[9] C. Morán, P. Clapés, F. Comelles, T. García, L. Pérez, P. Vinardell, M. Mitjans, M. R. Infante, Chemical Structure/Property Relationship in Single-Chain Arginine Surfactants, Langmuir. 17 (2001) 5071–5075. doi:10.1021/la010375d.

[10] M. Lukáč, M. Mrva, M. Garajová, G. Mojžišová, L. Varinská, J. Mojžiš, M. Sabol, J. Kubincová, H. Haragová, F. Ondriska, F. Devínsky, Synthesis, self-aggregation and biological properties of alkylphosphocholine and alkylphosphohomocholine derivatives of cetyltrimethylammonium bromide, cetylpyridinium bromide, benzalkonium bromide (C16) and benzethonium chloride, Eur. J. Med. Chem. 66 (2013) 46–55. doi:10.1016/j.ejmech.2013.05.033.

[11] M. Pisárčik, M. Polakovičová, M. Pupák, F. Devínsky, I. Lacko, Biodegradable gemini surfactants. Correlation of area per surfactant molecule with surfactant structure, J. Colloid Interface Sci. 329 (2009) 153–159. doi:10.1016/j.jcis.2008.10.016.

[12] Y. Bansal, O. Silakari, Multifunctional compounds: Smart molecules for multifactorial diseases, Eur. J. Med. Chem. 76 (2014) 31–42. doi:10.1016/j.ejmech.2014.01.060.

[13] J.L. Goldstein, M.S. Brown, The Cholesterol Quartet, Science (80-.). 292 (2001) 1310–1312. doi: 10.1126/science.1061815

[14] R.K. Elsayed, J.D. Evans, Emerging lipid-lowering drugs: squalene synthase inhibitors, Expert Opin. Emerg. Drugs. 13 (2008) 309–322. doi:10.1517/14728214.13.2.309.

[15] A. Venkateswaran, Y.T. Reddy, V.N. Sonar, V. Muthusamy, P.A. Crooks, M.L. Freeman, K. R. Sekhar, Antiangiogenic properties of substituted (Z)-(±)-2-(N-benzylindol-3-ylmethylene)quinuclidin-3-ol/one analogs and their derivatives, Bioorg. Med. Chem. Lett. 20 (2010) 7323–7326. doi:10.1016/j.bmcl.2010.10.060.

[16] S. Orenes Lorente, R. Gómez, C. Jiménez, S. Cammerer, V. Yardley, K. De Luca-Fradley, S. L. Croft, L. M. Ruiz Perez, J. Urbina, D. G. Pacanowska, I. H. Gilbert, Biphenylquinuclidines as inhibitors of squalene synthase and growth of parasitic protozoa, Bioorganic Med. Chem. 13 (2005) 3519–3529. doi:10.1016/j.bmc.2005.02.060.

[17] J.C.F. Rodrigues, J.L. Concepcion, C. Rodrigues, A. Caldera, J.A. Urbina, W. De Souza, In Vitro Activities of ER-119884 and E5700, Two Potent Squalene Synthase Inhibitors, against Leishmania amazonensis: Antiproliferative,Biochemical, and Ultrastructural Effects, Antimicrob. Agents Chemother. 52 (2008) 4098–4114. doi:10.1128/AAC.01616-07.

[18] M. Sealey-Cardona, S. Cammerer, S. Jones, L.M. Ruiz-Pérez, R. Brun, I.H. Gilbert, J. A. Urbina, D. González-Pacanowska, Kinetic characterization of squalene synthase from Trypanosoma cruzi: Selective inhibition by quinuclidine derivatives, Antimicrob. Agents Chemother. 51 (2007) 2123–2129. doi:10.1128/AAC.01454-06.

[19] J.A. García-Rodríguez, J.L. Muñoz Bellido, J.E. García Sánchez, Oral cephalosporins: current perspectives., Int. J. Antimicrob. Agents. 5 (1995) 231–243. doi:10.1016/0924-8579(95)00015-Z.

[20] R. Odžak, M. Skočibušić, A. Maravić, Synthesis and antimicrobial profile of N-substituted imidazolium oximes and their monoquaternary salts against multidrug resistant bacteria, Bioorg. Med. Chem. 21 (2013) 7499–7506. doi:10.1016/j.bmc.2013.09.041.

[21] N. Singh, Y. Karpichev, A.K. Tiwari, K. Kuca, K.K. Ghosh, Oxime functionality in surfactant self-assembly: An overview on combating toxicity of organophosphates, J. Mol. Liq. 208 (2015) 237–252. doi:10.1016/j.molliq.2015.04.010.

[22] R.M. Dawson, Review of Oximes Available for Treatment of Nerve Agent Poisoning, J. Appl. Toxicol. 14 (1994) 317–331. doi: 10.1002/jat.2550140502

[23] A. Lucić, B. Radić, M. Peraica, M. Mesić, I. Primožič, Z. Binenfeld, Antidotal efficacy of quinuclidinium oximes against soman poisoning, Arch. Toxicol. 71 (1997) 467–470. doi:10.1007/s002040050413.

[24] E. Reiner, M. Škrinjarić-Špoljar, S. Dunaj, V. Simeon-Rudolf, I. Primožič, S. Tomić, 3-Hydroxyquinuclidinium derivatives: synthesis of compounds and inhibition of acetylcholinesterase, Chem. Biol. Interact. 119-120 (1999) 173–181. doi:10.1016/S0009-2797(99)00026-5.

[25] N. Singh, Y. Karpichev, B. Gupta, M.L. Satnami, J. Marek, K. Kuca, K.K. Ghosh, Physicochemical properties and supernucleophilicity of oxime-functionalized surfactants: Hydrolytic catalysts toward dephosphorylation of Di- and triphosphate esters, J. Phys. Chem. B. 117 (2013) 3806–3817. doi:10.1021/jp310010q.

[26] L.Y. Zakharova, T.N. Pashirova, R.R. Kashapov, E.P. Zhil'tsova, N.K. Gaisin, O.I. Gnezdilov, A.B. Konov, S.S. Lukashenko, I. M. Magdeev, Catalytic Properties of Micellar Systems Based on 4-aza-1-alkyl-1-azoniabicyclo[2.2.2]octane Bromides, Kinet. Catal. 52 (2011) 179–185. doi:10.1134/S0023158411020236.

[27] M.K. Dymond, G.S. Attard, Cationic Type I Amphiphiles as Modulators of Membrane Curvature Elastic Stress In Vivo, Langmuir. 24 (2008) 11743–11751. doi:10.1021/la8017612.

[28] K. Imamura, T. Nogami, Y. Shirota, The phase transitions of 1-alkyl-1-azoniabicyclo[2.2.2]octane bromides, Cn-ABCO-Br, Bull. Chem. Soc. Jpn. 60 (1987) 3499–3504. doi:10.1246/bcsj.60.3499.

[29] A. Bhadani, T. Endo, S. Koura, K. Sakai, M. Abe, H. Sakai, Self-Aggregation and Liquid Crystalline Behavior of New Ester-Functionalized Quinuclidinolium Surfactants, Langmuir. 30 (2014) 9036–9044. doi: 10.1021/la502098h

[30] C.A. Grob, E. Renk, Untersuchungen in der Chinuclidin-Reihe. 3. Mitteilung. 3-Chinuclidin-carbonsäure, Helv. Chim. Acta. 37 (1954) 1689–1698. doi: 10.1002/hlca.19540370612

[31] I. Primožič, T. Hrenar, K. Baumann, L. Krišto, I. Križić, S. Tomić, Mechanochemical and Conformational Study of N -heterocyclic Carbonyl-Oxime Transformations, Croat. Chem. Acta. 87 (2014) 153–160.

[32] Y. Moroi, T. Oyama, R. Matuura, Phase Equilibria of Anionic Surfactant Mixtures in Aqueous Solution, J. Colloid Interface Sci. 60 (1977) 103–111. doi:10.1016/0021-9797(77)90260-0.

[33] S. Kamitori, Y. Sumimoto, K. Vongbupnimit, K. Noguchi, K. Okuyama, Molecular and Crystal Structures of Dodeyltrimethylammonium Bromide and its Complex with p-Phenylphenol, Mol. Cryst. Liq. Cryst. 300 (1997) 31–43.

[34] M.R. Silva, A.M. Beja, J.A. Paixão, Pseudosymmetry in tetradecyltrimethyl-ammonium bromide, Acta Crystallogr. Sect. E. E59 (2003) 1151–1152. doi:10.1107/S1600536803015319.

[35] A.R. Campanelli, L. Scaramuzza, Hexadecyltrimethylammonium bromide, Acta Crystallogr. Sect. C. C42 (1986) 1380–1383. doi:10.1107/S0108270186092193.

[36] Z. Rappoport, J.F. Liebman, eds., The Chemistry of Hydroxylamines, Oximes and Hydroxamic Acids, 1st ed., Wiley-Blackwell, 2011. doi: 10.1002/9780470741962

[37] T.W. Davey, W.A. Ducker, A.R. Hayman, J. Simpson, Krafft Temperature Depression in Quaternary Ammonium Bromide Surfactants, Langmuir. 14 (1998) 3210–3213. doi:10.1021/la9711894.

[38] M. Islam, M.R. Rahman, N. Islam, Micellization Behavior and Thermodynamic Properties of N-Alkyl Trimethylammonium Bromide Surfactants in aqueous media at different temperatures, Int. J. Sci. Eng. Res. 6 (2015) 1508–1516.

[39] B. Kronberg, K. Holmberg, B. Lindman, Surface Chemistry of Surfactants and Polymers, 1st ed., John Wiley and Sons, Ltd., West Sussex, UK, 2014. doi: 10.1002/9781118695968

[40] S.P. Moulik, M.E. Haque, P.K. Jana, A.R. Das, Micellar Properties of Cationic Surfactants in Pure and Mixed States, J. Phys. Chem. 100 (1996) 701–708. doi:10.1021/jp9506494.

[41] M. Rosen, J. Mathias, L. Davenport, Aberrant aggregation behavior in cationic gemini surfactants investigated by surface tension, interfacial tension, and fluorescence methods, Langmuir. 25 (1999) 7340–7346. doi:10.1021/la9904096.

[42] R. Zana, Ionization of Cationic Micelles: Effect of the Detergent Structure, J. Colloid Interface Sci. 78 (1980) 330–337. doi:10.1016/0021-9797(80)90571-8.

[43] R. Zana, Alkanediyl-α,ω-bis(dimethylalkylammonium bromide) surfactants 10. Behavior in Aqueous Solution at Concentrations below the Critical Micellization Concentration: An Electrical Conductivity Study, J. Colloid Interface Sci. 246 (2002) 182–190. doi:10.1006/jcis.2001.7921.

[44] R. Robinson, R.H. Stokes, Electrolyte Solutions, Butterworths, London, 1955.

[45] P. Mukerjee, K. Mysels, Critical micelle concentrations of aqueous surfactant systems, National Bureau of Standards NSRDS-NBS 36, Washington, United States, 1971. doi:10.1002/jps.2600610254.

[46] J.N. Israelachvili, Intermolecular and Surface Forces, 2nd ed., Academic Press Inc., San Diego, 1990. doi: 10.1016/B978-0-12-375182-9.10025-9

[47] V. Aloush, S. Navon-Venezia, Y. Seigman-Igra, S. Cabili, Y. Carmeli, Multidrug-Resistant Pseudomonas aeruginosa: Risk Factors and Clinical Impact, Antimicrob. Agents Chemother. 50 (2006) 43–48. doi:10.1128/AAC.50.1.43.

[48] P. Balgavý, F. Devínsky, Cut-off effects in biological activities of surfactants, Adv. Colloid Interface Sci. 66 (1996) 23–63. doi:10.1016/0001-8686(96)00295-3.

[49] G.L. French, The continuing crisis in antibiotic resistance, Int. J. Antimicrob. Agents. 36 (2010) S3–S7. doi:10.1016/S0924-8579(10)70003-0.

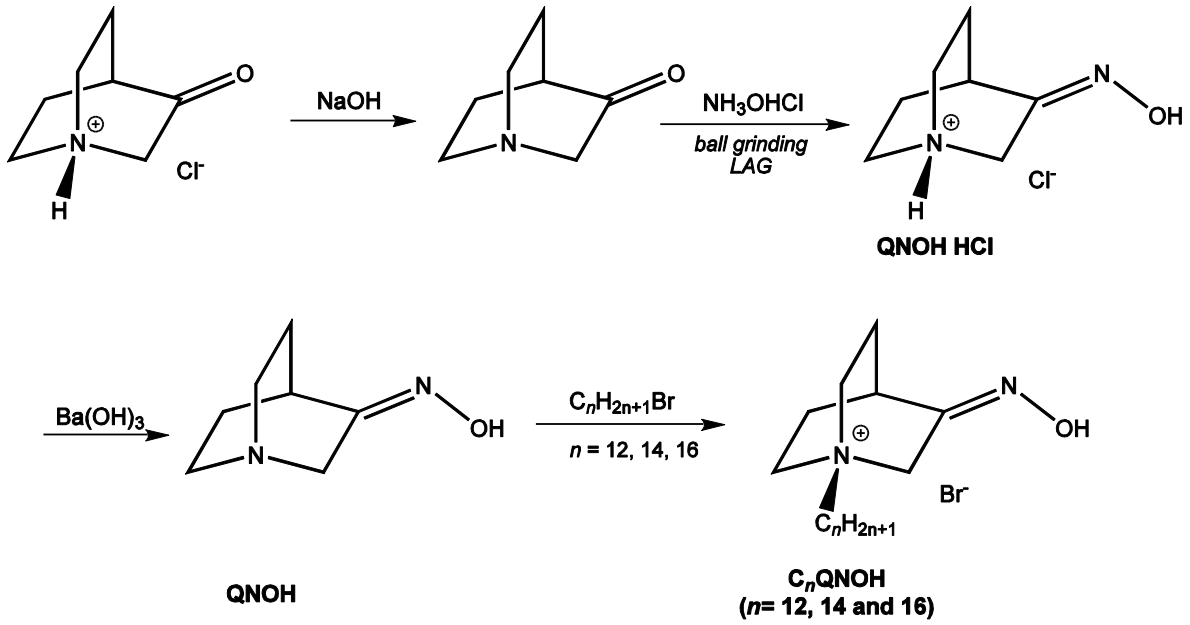
Figure Captions

Scheme 1. Schematic representation of synthesis route for 3-hydroxyimino quinuclidinium surfactants with increasing alkyl chain length (C_nQNOH).

Fig. 1. a) Molecular structure of 3-hydroxyimino quinuclidinium surfactants (C_nQNOH, n = 12, 14 and 16) with the atomic numbering and the torsion angles of the alkyl chains. Displacement ellipsoids are drawn at the 50 % probability level. The hydrogen bonds O– H···Br^- are also shown. **b)** View of the crystal packing of $C_{12}QNOH$ along crystallographic *b* axis. The bilayers thickness is equal to the dimension of the unit cell *a* axis. The carbon atoms in quinuclidinium headgroup and alkyl chains are in gray, nitrogen are in blue, oxygen are in red and bromides are in brown. The crystallographic *a* axis is red and the *c* axis is blue.

Fig. 2. a) Variation of the surface tension (γ) with $C_{12}QNOH$ and $C_{14}QNOH$ concentration (c). Variation of the electrical conductivity (κ) with the concentration (c) for **b)** $C_{12}QNOH$, **c)** $C_{14}QNOH$ and $C_{16}QNOH$. The experimental temperatures are indicated.

Fig. 3. Variation of log reciprocal minimum inhibitory concentrations (1/MIC) for 3-hydroxyimino quinuclidinium surfactants (C_nQNOH) with number of carbon atoms in the alkyl chains (n) for **a)** Gram-positive bacteria and **b)** Gram-negative bacteria.



Scheme 1.

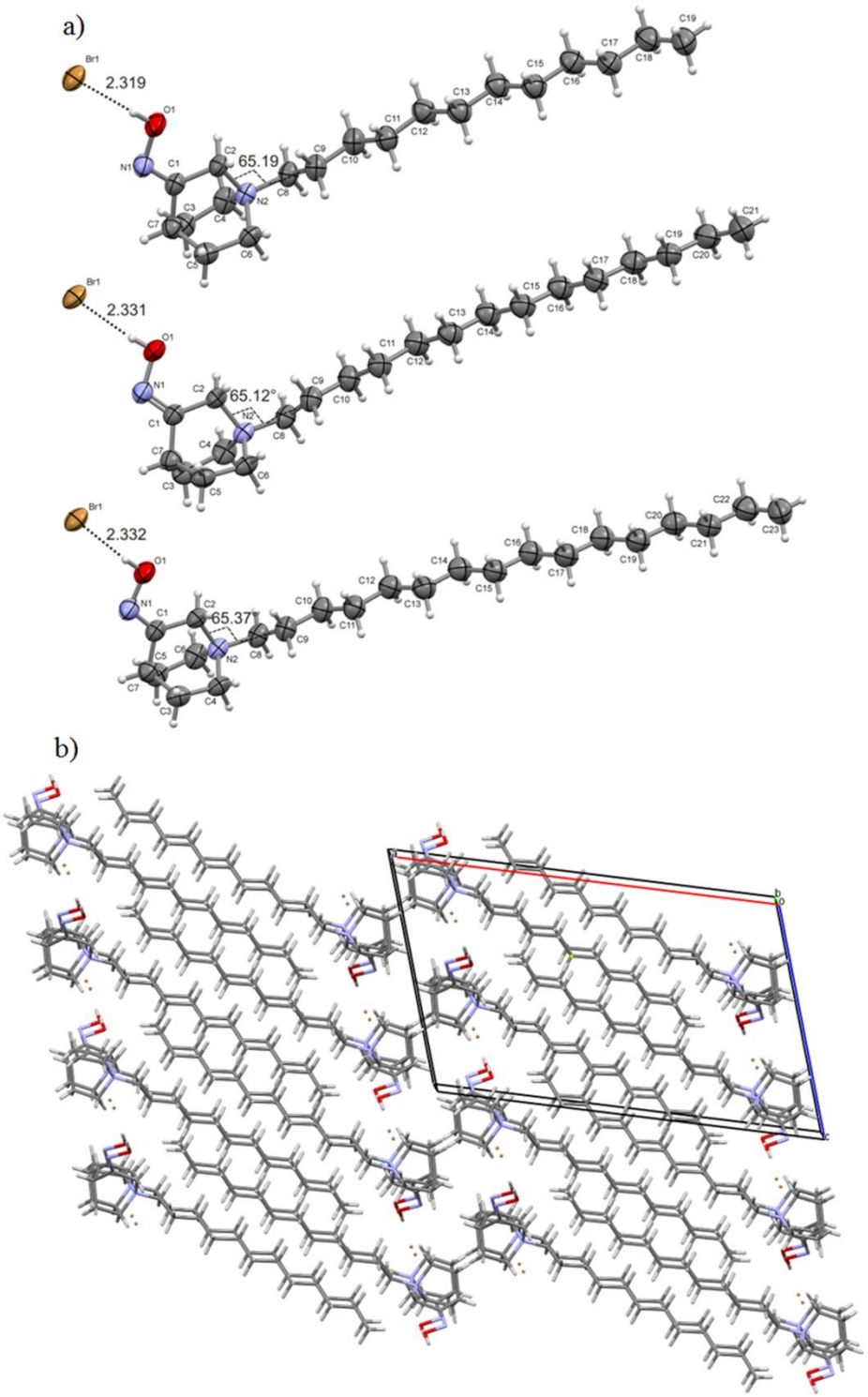


Fig. 1.

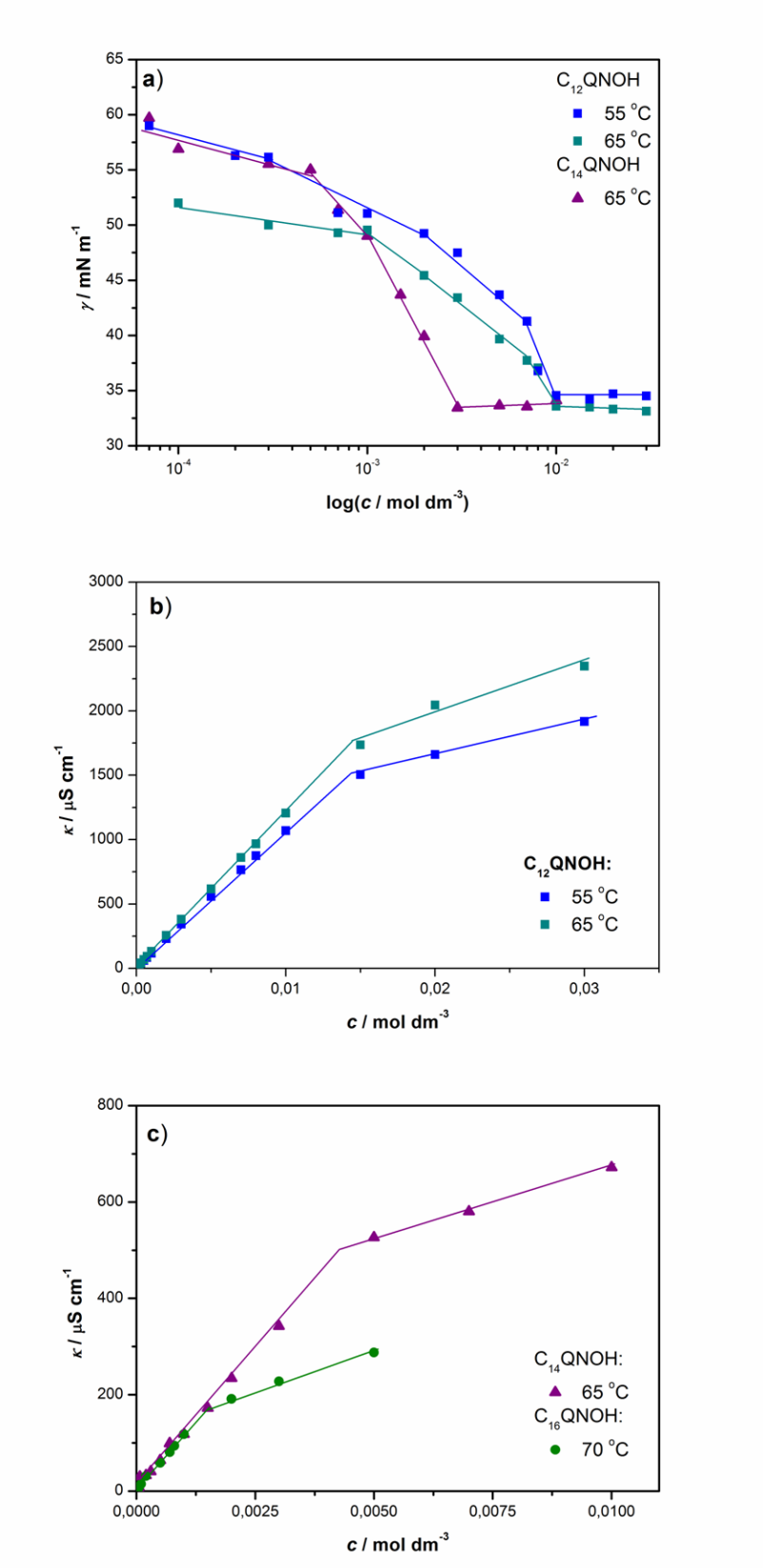


Fig. 2.

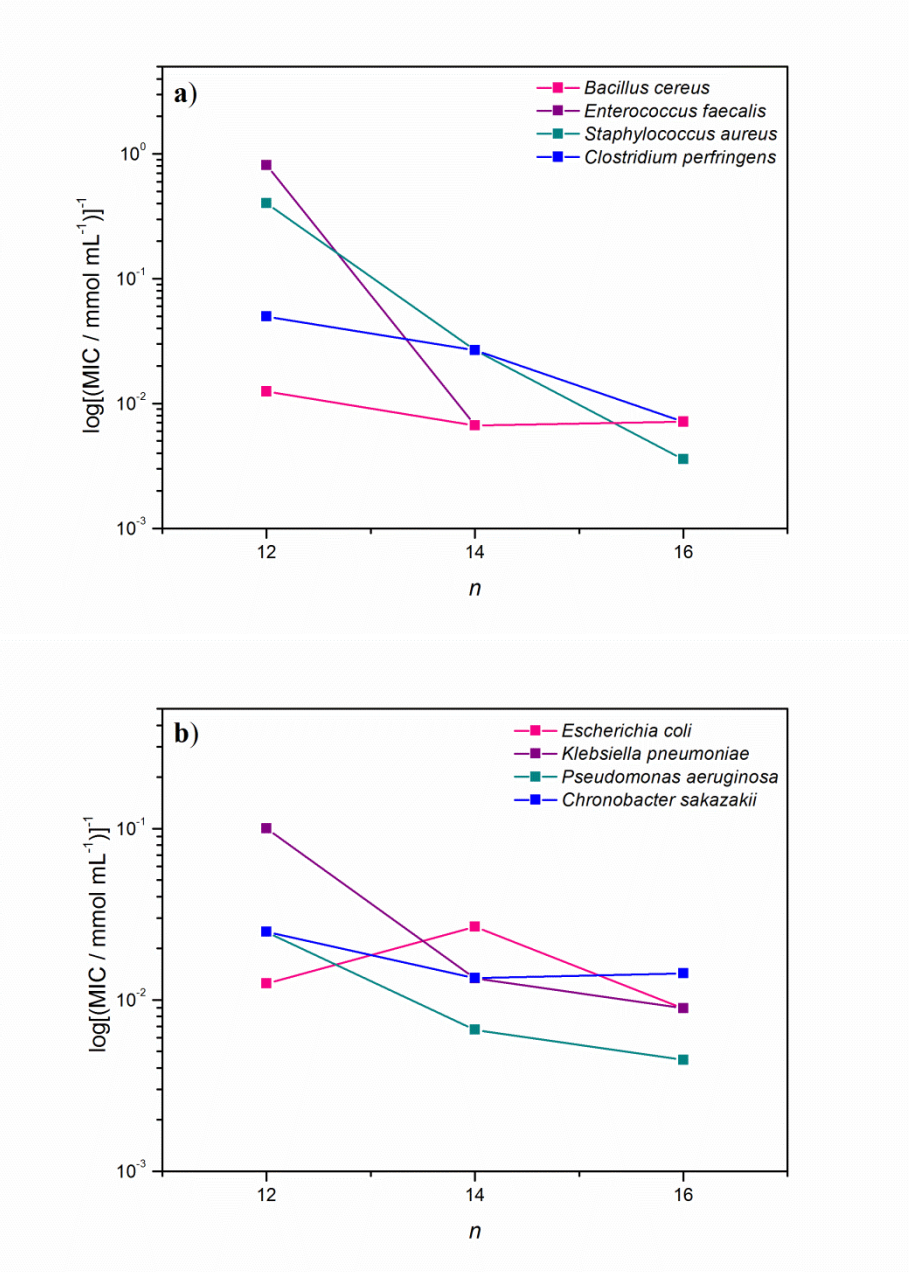


Fig. 3.

Tables

Table 1. Crystallographic data, structure solution, and refinement for the 3-hydroxyimino quinuclidinium surfactants (C_nQNOH) with increasing chain length.

Compound	**C_{12}QNOH**	**C_{14}QNOH**	**C_{16}QNOH**
Empirical formula	C_{19} H_{37} Br N_2 O	C_{21} H_{41} Br N_2 O	C_{23} H_{45} Br N_2 O
Formula weight	389.42	417.47	445.52
Temperature (° K)	293(2)	293(2)	293(2)
Wavelength (Å)	1.54184	1.54184	1.54184
Crystal system	monoclinic	monoclinic	monoclinic
Space group	*P* $2_1/c$	*P* $2_1/c$	*P* $2_1/c$
Unit cell dimensions (Å, °)	*a* = 22.0586(8) *b* = 7.4259(2) *c* = 13.5532(3) β = 108.168(3)°	*a* = 23.3187(2) *b* = 7.42120(10) *c* = 13.57870(10) β = 102.2430(10)°	*a* = 26.7086(6) *b* = 7.42340(10) *c* = 13.6243(2) β = 112.817(2)°
Volume ($Å^3$)	2109.40(11)	2296.39(4)	2489.89(8)
Z	4	4	4
Density (calculated) (g cm^{-3})	1.226	1.207	1.188
Absorption coefficient (mm^{-1})	2.689	2.502	2.336
F(000)	832	896	960
Crystal size (mm)	0.3 x 0.2 x 0.1	0.3 x 0.3 x 0.2	0.4 x 0.3 x 0.2
Theta range for data collection (°)	4.219 to 73.305	3.880 to 73.722	3.591 to 73.886
Index ranges	$-25 \leq h \leq 27$ $-9 \leq k \leq 6$ $-15 \leq l \leq 16$	$-27 \leq h \leq 28$ $-8 \leq k \leq 8$ $-16 \leq l \leq 15$	$-31 \leq h \leq 33$ $-9 \leq k \leq 6$ $-16 \leq l \leq 15$
Reflections collected	7748	24871	9350
Independent reflections	4102 [R_{int}= 0.0178]	4532 [R_{int}= 0.0231]	4866 [R_{int}= 0.0218]
Completeness	99.8 %	99.6 %	99.8 %
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on	Full-matrix least-squares on F^2
Data / restraints / parameters	4102 / 0 / 212	4532 / 0 / 230	4866 / 0 / 248
Goodness-of-fit on F^2	1.063	1.022	1.021
Final *R* indices [$I > 2\sigma(I)$]	R_1 = 0.0322, wR_2 = 0.0882	R_1= 0.0284, wR_2= 0.0760	R_1= 0.0321, wR_2= 0.0824
R indices (all data)	R_1 = 0.0354, wR_2= 0.0917	R_1= 0.0303, wR_2= 0.0777	R_1 = 0.0390, wR_2= 0.0905
Largest diff. peak and hole (e $Å^{-3}$)	0.487 and -0.658	0.561 and -0.402	0.488 and -0.322

Table 2. The Krafft temperatures (t_{Krafft}) for 3-hydroxyimino quinuclidinium surfactants (C_nQNOH) with increasing chain length and corresponding *n*-alkyltrimethylammonium bromides (C_nTAB).

compound	t_{Krafft} / °C	**compound**	t_{Krafft} / °C
C_{12}QNOH	45.0	C_{12}TAB	< 0[a]
C_{14}QNOH	56.0	C_{14}TAB	12.4[b]
C_{16}QNOH	64.0	C_{16}TAB	25.0[a], 24.7[b]

[a] = ref.[37], [b] = ref.[38]

Table 3. Critical micellization concentrations obtained from surface tension (cmc_γ), and conductivity measurements (cmc_κ), surface tension at the cmc (γ_{cmc}), maximum surface excess (Γ_{max}), minimum surface area per headgroup (a_{min}) and degree of counterion binding (β) obtained from conductivity measurements as function of temperature (t) for 3-hydroxyimino quinuclidinium surfactants (C_nQNOH) with increasing chain length and corresponding *n*-alkyltrimethylammonium bromides (C_nTAB) in water.

compound	t (^{o}C)	cmc_γ (10^3 mol dm^{-3})	cmc_κ (10^3 mol dm^{-3})	γ_{cmc} (mN m^{-1})	Γ_{max} (10^6 mol m^{-2})	a_{min} (nm^2)	β
C_{12}QNOH	55	11.0	13.0	34.4	1.95	0.85	0.75
C_{12}QNOH	65	11.0	14.0	33.6	1.48	1.12	0.70
C_{14}QNOH	65	3.10	4.20	33.7	2.88	0.58	0.74
C_{16}QNOH	70	-	1.50	-	-	-	0.72
C_{12}TAB	30	14.0[a], 16.0[b]	15.0[a], 15.8[c]	38.9[a], 39.0[b]	2.70[a], 1.40[c]	0.62[a], 1.18[c]	0.71[a], 0.77[c,d]
C_{14}TAB	30	3.60[b], 2.34[c]	3.60[c]	38.0[b]	2.70[b], 2.32[c]	0.61[b], 0.72[c]	0.73[c], 0.80[d]
C_{16}TAB	30	0.92[b], 0.80[c]	0.90[c]	< 40.0[b]	1.12[c]	1.48[c]	0.70[c], 0.84[d]

[a] = ref. [5], [b] = ref. [41] at 25 ^{o}C, [c] = ref. [40], [d] = ref. [42]

Table 4. Antimicrobial activity of the 3-hydroxyimino quinuclidinium surfactants (C_nQNOH) with increasing chain length as well as antibiotics, tetracycline and gentamicin, by disc diffusion assay.

Microorganisms	Diameters of the inhibition zone (mm)[a]							
	Compounds						Antibiotic[b]	
	C_{12}QNOH		**C_{14}QNOH**		**C_{16}QNOH**		**TET**	**GEN**
Gram-positive bacteria	250	500	250	500	250	500		
Bacillus cereus	29.4 ± 0.3	38.4 ± 0.5	25.0 ± 0.5	37.3 ± 1.8	21.6 ± 0.3	31.8 ± 0.9	13.6 ± 0.1	18.2 ± 0.7
Enterococcus faecalis	37.7 ± 1.2	41.2 ± 0.4	24.2 ± 0.6	36.7 ± 1.6	21.7 ± 0.5	28.7 ± 1.4	14.5 ± 0.7	14.6 ± 1.4
Staphylococcus aureus	32.7 ± 0.7	36.1 ± 0.2	22.4 ± 0.2	29.3 ± 0.5	11.7 ± 0.7	28.1 ± 0.2	12.1 ± 0.8	23.9 ± 0.9
Clostridium perfringens	29.5 ± 0.2	35.7 ± 0.6	23.6 ±1.4	29.2 ± 1.8	14.4 ± 1.1	23.9 ± 1.5	15.9 ± 0.2	21.7 ± 0.4
Gram-negative bacteria								
Escherichia coli	17.8 ± 0.3	23.9 ± 0.2	19.8 ± 0.5	24.3 ± 1.2	17.3 ± 0.4	21.7 ± 1.5	13.4 ± 0.7	11.5 ± 0.9
Klebsiella pneumoniae	18.2 ± 1.5	21.3 ± 0.1	19.3 ± 0.9	25.8 ± 0.3	21.5 ± 0.1	24.3 ± 0.7	16.8 ± 0.5	18.8 ± 0.6
Pseudomonas aeruginosa	16.1 ± 1.5	21.9 ± 0.4	16.8 ± 0.3	24.9 ± 0.9	13.9 ± 1.3	23.2 ± 0.4	12.2 ± 1.1	9.7 ± 1.4
Chronobacter sakazakii	17.7 ± 1.5	22.7 ± 1.9	19.3 ± 0.1	23.1 ± 0.8	20.3 ± 0.5	25.6 ± 0.9	12.1 ± 0.6	13.6 ± 0.9

[a] Diameter of inhibition zone (values in mm) around the disc: 250 µg/disc and 500 µg/disc.
[b] Standard antibiotic disc: TET, tetracycline (30 µg/disc), GEN, gentamicin (15 µg/disc).
Values are expressed as mean ± SE

Table 5. Minimum inhibitory concentration (MIC) of the 3-hydroxyimino quinuclidinium surfactants (C_nQNOH) with increasing chain length as well as antibiotics, tetracycline and gentamicin.

	MIC (μmol L^{-1})							
	Gram-positive bacteria				**Gram-negative bacteria**			
Compounds	*Bacillus cereus*	*Enterococcus faecalis*	*Staphylococcus aureus*	*Clostridium perfringens*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Chronobacter sakazakii*
C_{12}QNOH	80.25	1.23	2.49	20.06	80.25	10.02	40.11	40.11
C_{14}QNOH	149.71	149.71	37.42	37.42	37.42	74.86	149.71	74.86
C_{16}QNOH	140.29	140.29	280.57	140.29	112.23	112.23	224.46	70.14
Gentamicin	8.38	8.38	2.09	1.05	67.00	16.75	134.00	16.75
Tetracycline	4.50	1.13	1.13	4.50	144.00	288.00	576.00	18.00