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Quaternary Ammonium Surfmers: Synthesis, Characterization and Antibacterial Performance

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Abstract

Quaternary ammonium surfmers not only possess antibacterial activity, but also provide new active monomers for the synthesis of polymer cationic surfactants. In this study, four quaternary ammonium surfmers (nQAS) have been synthesized by the quaternization process of dimethylaminoethyl acrylate and brominated alkanes with different carbon chain lengths(n). Their structures were confirmed by ^{1}H nuclear magnetic resonance spectroscopy and high resolution mass spectrometer. The surface activities are measured including surface tension curves and micellar microenvironment of QAS in pure water and solution containing electrolytes. With *Staphylococcus aureus* (*S. aureus)* and *Escherichia coli* (*E. coli*) as the representatives of gram-positive and gram-negative bacteria, respectively, the minimal inhibit concentration (MIC) and minimum bactericidal concentration (MBC) were investigated by the classical plate counting method. 18QAS surfmer showed a very low MIC and MBC against *S. aureus*, at 0.937 and 3.75 μmol/L, respectively, which could illustrate the efficiency of 4.23 Log CFU to *S. aureus*, and 16QAS showed the superior killing efficiency of 2.61 Log CFU to *E. coli.* at the concentration of 32.25 μmol/L. These quaternary ammonium surfmers would provide functional monomers for long-term antibacterial surface coating and other biological applications.

Keywords: Cationic surfmers; Quaternary ammonium salt; Surface activity; Antibacterial; MIC and MBC

1. Introduction

The surfaces of solid materials, such as medical equipment, devices, and environmental surfaces, play a very important role in the transmission of pathogenic microorganisms. In clinical practice, if reused medical devices are not properly cleaned and sterilized, residual bacteria and biofilms can not only cause corrosion of the devices, but also easily lead to surgical infections. Meanwhile, interventional therapy has become a commonly used surgical treatment method in clinical practice, and the implant biomaterials are prone to be contaminated by bacterial or biofilm due to their strong adsorption characteristics. Therefore, the antibacterial design of the surface of such materials is a hot topic in the field of medical device research.[1] According to antibacterial mechanisms, current antibacterial strategies for biomaterials could be categorized into passive or active protocols. The passive antibacterial surfaces utilize micro/nanostructured topographies to inhibit bacterial adhesion and colonization.[2] In contrast, the active antibacterial strategies could be achieved through antibacterial agents release or contact-killing surface. Especially, contact-killing surface could achieve sustained antibacterial effects through delicate surface-bound amphiphilic chemistries. For a long time, cationic surfactants, with amines or tetraalkylammonium as functional groups, are often employed as disinfectants, antiseptics, preservative agents, bactericides and antistatic agents for decades due to the well-known antimicrobial activity and corrosion inhibiting properties.[3,4] Generally, these cationic surfactants accomplish antibactierial function through three possbile mechanism, including bacterial membranes disruption via electrostatic interactions between surface cationic groups and negative bacterial surface[5], hydrophobic alkyl chains insertion lipid envelop,[6] and zwitterionic interfaces design[7]. In order to decrease the cytotoxicity of cationic surfactants to mammalian cells, researchers have performed various effective explorations, including the modulation of host-guest supramolecular conformation[8], cyclodextrin/cationic trimeric surfactant complexes[9], non-covalent assembly of plant gallic acid and quaternary ammonium surfactants[10], and so on. These new methods have effectively prolonged the bactericidal effect time of cationic surfactants to a certain

extent. On the other hand, in order to obtain a long-lasting contact-killing surface, these cationic surfactants must be derivatized to achieve reactive properties, which could be bonded to the surface of materials through chemical bonds. In total, these polymerizable amphiphilic substances are the core materials for achieving sustained antibacterial functions.

Polymerizable surfactants, surface active monomers (surfmers) have polymerizable fragment, which could be incorporated to the polymer materials. Surfmers are amphiphilic compounds consisting of a polymerizable group (usually hydrophobic), and a hydrophilic head group (neutral or charged), in which the head group is a functional group are referred to as functional surfmers.[11] Many literature results have reported that the length of the hydrophobic chain length of cationic surfactants greatly affects its antimicrobial activity.[12-14] In the synthesis process of polymerizable surfactants, both the adoption of reactive functional group structure and the grafting position would affect the interfacial properties of the product molecules. Gao *et. al.* introduced acrylamide group to the branched position of alkyl sulfonic acid. The experiment results showed that branched polymerizable surfactants have characteristic micellar behaviors, including increased solubility, decreased surface adsorption amounts, and improved critical micellar concentration comparing with common anionic surfactant with the same hydrocarbon chain and anionic head group as them.[15]

Raffaella Mancuso prepared four kinds of reactive quaternary ammonium surfactants, and all the newly synthesized reactive surfactants were tested for their antimicrobial activity. The results showed that reactive surfactants bearing an alkyl chain of 11 and particularly 12 carbon atoms possessed significant activity against Gram positive bacteria and yeast strains, which was higher antibacterial activity compared with dodecyltrimethylammonium bromide with the same carbon chain length.[16] In addition to the position of reactive groups, the types of reactive groups also have a significant impact on the properties of the products. For example, ester and amide bonds often appear as connecting groups in the product structures, which will also have a significant impact on the surface and interface properties of the products.
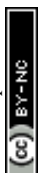
Garcia *et. al.* reported that the insertion of a functional amide group into a surfactant side chain generates significant changes in their surface activity and micellization.[17] Hoque *et. al.* studied the solution assembly and interface aggregation behavior of gemini surfactants with and without amide group, and the results showed that amide functionality increased the surfactant aggregation tendencies as compared to the surfactants having no amide bond.[18] Totally, surfmer composition and molecular structure not only play an extremely important role in the synthesis process, but also essentially determine the final surface properties of the functional coatings. The research of surface active monomers is interdisciplinary fields involving physics, chemistry, biology and materials, and these surfmers with novel structure could serve wide application such as medicine, pharmacy, and biology.[19]

Reactive quaternary ammonium salts can be polymerized or copolymerized to obtain surfaces with long-lasting contact-killing function. However, it is equally important to understand the chain length, grafting position, and functional groups of reactive quaternary ammonium salts on their solution assembly，interface behavior, as well as their impact on bactericidal performance. In this work, four polymerizable quaternary ammonium surfmers with different hydrophobic chain lengths have been synthesized with dimethylaminoethyl acrylate and bromoalkane as raw materials through quaternization process. The structure of as-prepared surfmers have been confirmed through spectroscopic methods. Meanwhile, the effect of hydrophobic chain length and electrolytes on surface activity and micellar environment of surfmers has been studied. Lastly, with *Staphylococcus aureus* and *Escherichia coli* as representative strains, bactericidal and inhibitory efficiency of four polymerizable quaternary ammonium surfmers have been compared to the control group of cetyltrimethylammonium bromide (CTAB). These results will provide a good material basis for the construction of long-lasting antibacterial surfaces.

## 2. Materials and Methods

2.1 Materials
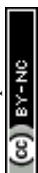
Dimethylaminoethyl acrylate (DMAEA), 1-bromododecane (C_{12}-Br), 1-bromotetradecane (C_{14}-Br), 1-bromohexadecane (C_{16}-Br), 1-bromooctadecane (C_{18}-Br), cetyltrimethylammonium bromide (CTAB), tetrahydrofuran and methyl orange (MO) were purchased from Shanghai Aladdin Reagent Co., Ltd. Methanol, methylene chloride, ethyl acetate and petroleum ether were purchased from Wuxi Yasheng Chemical Co., Ltd. The bacterial medium was purchased from Shanghai Shenggong Biotechnology Co., Ltd. All chemicals are supplied in analytic purity and was used directly without purification. All standard solutions and samples are prepared using ultra-pure water (resistance 18.2 MΩ·cm^{-1}) purified by the laboratory water purification system (Nanjing Yiou Yida). The bacterial strains, *E. coli* (CCTCC No.: 23429) and *S. aureus* (CICC No.: 21600), used in this study were the same as those reported in our previous literature.[20]

2.2 Methods

2.2.1 Preparation of polymerizable quaternary ammonium surfmers

Four polymerizable quaternary ammonium surfmers were synthesized through quaternization process reported by Chen *et. al.* [21] with minor change, and the synthesis diagram has been shown in Fig. 1. C_{12}-Br, C_{14}-Br, C_{16}-Br and C_{18}-Br were used as raw materials to react with DMAEA, and several polymerizable quaternary ammonium surfmers with different hydrophobic chain length were obtained. Typically, 1-bromododecane (0.02 mol), DMAEA (0.01 mol) and tetrahydrofuran (10 mL) were added to a 50 mL three necked flask equipped with a condenser, a thermometer and a mechanical stirrer. The mixture was stirring for 24 h at 60 °C. The reaction process was monitored by thin layer chromatography until DMAEA disappeared. After the reaction, the solvent was removed by a rotary evaporator. The unreacted brominated alkanes were washed repeatedly with a mixed solvent of ethyl acetate and petroleum ether (5:1) for three times, and the acquired products was labeled as 12QAS. Similarly, 14QAS, 16QAS and 18QAS were prepared by the same method.

DMAEA   n=11, 13, 15, 17   QAS(n=12,14,16,18)
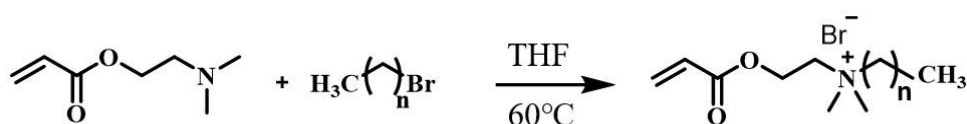
Fig. 1. The synthesis diagram of four polymerizable quaternary ammonium surfmers.

2..2.2 Characterization methods

^{1}H NMR spectra were recorded by the nuclear magnetic resonance spectrometer of AvanceNEO400 of Bruker, Germany, operating at 400 MHz with deuterated chloroform as solvents.

High resolution mass spectra(HRMS) were acquired by the Bruker Autoflexmax MALDI-TOF instrument, the ionization mode is electric spray ionization, and the mass spectrometer operates in positive ion mode (ESI+)

2.2.3 Surface tension

The platinum ring method (duNouyRing method)[22] was employed to measure the surface tension curves of four polymerizable quaternary ammonium surfmers (12QAS, 14QAS, 16QAS, 18QAS) at room temperature using an automatic interfacial tensiometer (BZY-100, Shanghai Fangrui Instrument Co., Ltd.) Before the experiment, the automatic surface tensiometer was calibrated with pure water (surface tension 72.2 mN/m). Before each measurement, the platinum rings were cleaned with pure water and burned until red with an alcohol lamp to remove possible organic residues. The surface tension value was measured three times and averaged. For each surfactant, the surface tension value (γ) and its corresponding concentration (C) were plotted as a γ-ln C curve. Their thermodynamic parameters such as minimum surface tension, critical micelle concentration(CMC), minimum molecular cross-sectional area (A_{min}) and maximum surface excess concentration (Γ) were calculated from the surface tension curves. The surface tension curve of hexadecyl trimethyl ammonium bromide (CTAB) solution was used as a control to analyze the influence of hydrophobic groups on the aggregation behavior of surfactants in water.

In order to analyze the effect of electrolyte on the aggregation behavior of surfactants, 16QAS surfmers were selected and dissolved in deionized water and NaCl solutions at 0.05 M, 0.1 M and 0.2 M, respectively. In order to further study the effects of cations and anion valence, 16QAS surfmers were dissolved into 0.05 mol/L solution

of NaCl, MgCl$_2$, FeCl$_3$, and Na$_2$SO$_4$ and Na$_3$PO$_4$, respectively. The surface tension curve was measured at room temperature using the platinum ring method on an automatic surface tensiometer.

2.2.4 Microenvironment characterization

Methyl orange (MO) can be used as a probe molecule to explore changes in the microenvironment after surfactant assembly.[23] For each surfactant studied, the ultraviolet-visible absorption spectra of different concentrations of surfactant and methyl orange were recorded using the UH5300 ultraviolet-visible spectrophotometer (Hitachi, Japan) equipped with a quartz cell. The final concentration of MO solution was 50 μmol/L by dissolving MO in different concentration of surfactant solution. At a scanning rate of 400 nm/min, the UV absorption spectra were recorded in the wavelength range of 330~580 nm.

2.2.5 *In vitro* antibacterial activity

According to our previous report[24], we used Gram positive *Staphylococcus aureus* and Gram negative *Escherichia coli* as model strains *in vitro*. Both strains were inoculated into LB broth medium and cultured at 37 ℃ with shaking at a speed of 200 rpm. After 12 hours, the bacterial concentrations of *E. coli* and *S. aureus* were approximately 10^9 CFU/mL and 10^8 CFU/mL, respectively.

To ensure sufficient absorption of QAS surfmers by bacteria, 100 μL of appropriate concentration bacterial solution was mixed with 5 μM QAS solution in a sterile centrifuge tube at an equal volume, and incubated for 30 minutes under dark conditions at 37 ℃ and 300 rpm. Then 100 μL of the mixed solution was taken and evenly spread on LB broth agar medium. Finally, the plate was transferred to a incubator at 37 ℃ for 24 hours. After continuous dilution in PBS, cell viability was evaluated by counting colony-forming unit (CFU) on agar plates. With PBS as the blank control, the same process was performed to evaluate the antibacterial effect of several QAS surfmers. The above operations were carried out in an ultra-clean workbench, and each test was repeated three times.

2.2.6 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration determination of as-prepared QAS

surfmers was performed using the two-fold microdilution broth method according to the operating procedures recommended by the Clinical and Laboratory Standards Institute (CLSI) in the United States (CLSI-M07-A8). The antibacterial effect of several QAS surfmers was recorded through visible growth of microorganisms in meat broth.[25] During the MIC determination process of QAS surfmers, bacterial concentration was set at 10^6 CFU/mL.

The surfmers stock solution of 1 mM was prepared and diluted by a double dilution method to obtain a solution having a concentration of 0.5 mM, 0.25 mM, 0.125 mM, 0.0625 mM, 0.03125 mM, 0.015 mM, etc., for future use. 100 μL of bacterial solution was added to 10 mL of surfmers solution with different concentrations, and was mixed thoroughly. With bacterial solution without surfmers as control group, three parallel antibacterial measurements were performed for each surfmers concentration. Finally, the mixture test tubes were placed in a incubator at 37 ℃ for 12 hours and the turbidity were recorded. MIC of each surfmers corresponds to the observed lowest concentration at which visible growth was inhibited. Sub-MICs are concentrations below the MIC.

2.2.7 Minimum bactericidal concentration (MBC)

Based on the MIC results, 100 μL surfmers solution with different concentration such as one, two, four, eight times of the MIC were coated on a solid LB plate, and incubated at 37 ℃ for 24 h, respectively. The minimum bactericidal concentration (MBC) of QAS was recorded when the incubated bacteria amount with colony growth number less than 0.1% corresponded to the concentration of antibacterial agent in the medium tube.

3 Results and discussion

3.1 Synthesis and structural characterization

In order to construct polymerizable cationic amphiphilic surfmers, dimethylaminoethyl acrylate was used as the functional reactants. As we know, the structure and the length of the hydrocarbon chain of the surfactants hydrophobic tail have a significant impact on the interfacial and application properties of surfactants. In order to disclose the effect of hydrophobic tail length of cationic reactive surfactants on

interfacial properties and antibacterial performance, we selected brominated alkanes

with different chain lengths to construct hydrophobic tails of surfactants. As illustrated

in Fig.1, with several brominated alkanes as the alkylating agents, dimethylaminoethyl

acrylate has been transformed into series of polymerizable quaternary ammonium

surfmers (QASs) compounds through quaternization process. In order to confirm these

products structures, ^{1}H nuclear magnetic resonance spectroscopy(^{1}H NMR) and high

resolution mass spectrometry (HRMS) techniques have been used to study the

structures of synthesized compounds, and the corresponding results have been shown
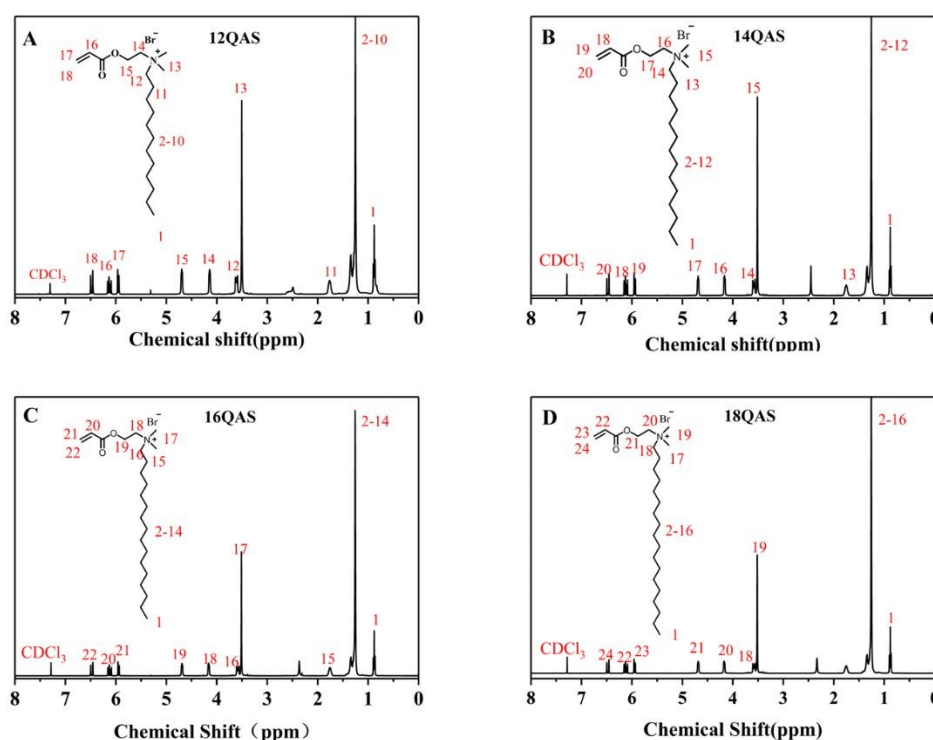
in Fig.2 and Fig.3.

Fig. 2 ^{1}H NMR spectra of four reactive QAS. (A) 12QAS; (B) 14QAS; (C) 16QAS and (D) 18QAS.

The ^{1}H NMR spectra of four polymerizable cationic surfmers have been shown in

Fig.2. According to Nie *et. al.*' results[26] and general ^{1}H NMR spectra analysis protocols,

the corresponding peaks have been ascribed to labeled hydrogen atoms of the products.

As shown in Fig.1, the structural difference among the four cationic surfmers lies in the

different number of carbon atoms in the hydrophobic chain. Therefore, it can be

foreseen that their ^{1}H NMR spectra are similar, and the obvious difference should be

found at the characteristic location corresponding to the hydrogen atoms of methylene groups. The peaks at 0.8-0.9 ppm is the typical characteristics of hydrogen atoms in the methyl -CH_3 groups. The multiple peaks from 1.3-1.4 ppm could be ascribed to the typical hydrogen atoms in the methylene groups of C_{12}-C_{18} hydrocarbon chains in four QAS surfmers. The peaks at 3.6 and 1.7 ppm could be indexed to the hydrogen atoms in the two methylene groups directly connected to N atoms, respectively. Obviously, the peaks at 3.5 ppm is the hydrogen atoms of the two methyl groups (-CH_3) connected to the quaternary ammonium nitrogen. The peaks at 4.2 ppm could be ascribed to the hydrogen atoms in methylene groups directly connected to N atoms in the N,N-dimethyl aminoethyl fragments, and the hydrogen atoms in methylene groups connected to oxygen atomes in the N,N-dimethyl aminoethyl fragments contribute to the peaks at 4.7 ppm directly connected to N atoms. Obviously, the several peaks from 5.9 to 6.5 ppm could be attributed to the hydrogen atoms located in the unsaturated carbon chemical environment in the acrylate alkene (CH_2=CH-) groups. Totally, comparing the ^{1}H NMR spectra of four different products in Fig.2, it can be seen that the number of hydrogen atoms in the methylene group of different hydrophobic chain length is significantly different. According to the peaks area integration results, the number of hydrogen atoms is consistent with the theoretical value. Moreover, the presence of hydrogen atoms in the unsaturated carbon atoms proves that preservation of acrylate polymerizable groups. The above results have indicated that the four polymerizable cation surfmers have been successfully synthesized through the synthesis route shown in Fig. 1.

The specific ^{1}H NMR data for the four compounds are as follows:

12QAS: ^{1}H NMR (400 MHz, Chloroform-d) δ 6.48 (d, J = 17.3 Hz, 1H), 6.13 (dd, J = 17.2, 10.5 Hz, 1H), 5.95 (d, J = 10.5 Hz, 1H), 4.76 – 4.61 (m, 2H), 4.22 – 4.10 (m, 2H), 3.66 – 3.56 (m, 2H), 3.51 (s, 6H), 1.87 – 1.71 (m, 2H), 1.41 – 1.18 (m, 18H), 0.88 (t, J = 6.7 Hz, 3H).

14QAS: ^{1}H NMR (400 MHz, Chloroform-d) δ 6.48 (dd, J = 17.3, 1.2 Hz, 1H), 6.12 (dd, J = 17.3, 10.5 Hz, 1H), 5.94 (dd, J = 10.5, 1.2 Hz, 1H), 4.76 – 4.63 (m, 2H), 4.22

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– 4.11 (m, 2H), 3.65 – 3.56 (m, 2H), 3.51 (s, 6H), 1.82 – 1.70 (m, 2H), 1.42 – 1.19 (m, 20H), 0.88 (t, J = 6.8 Hz, 3H).

16QAS: ^{1}H NMR (400 MHz, Chloroform-d) δ 6.53 – 6.43 (m, 1H), 6.12 (dd, J = 17.3, 10.5 Hz, 1H), 5.98 – 5.91 (m, 1H), 4.73 – 4.65 (m, 2H), 4.20 – 4.12 (m, 2H), 3.63 – 3.50 (m, 2H), 3.51 (s, 6H), 1.82 – 1.69 (m, 2H), 1.39 – 1.20 (m, 22H), 0.88 (t, J = 6.7 Hz, 3H).

18QAS: ^{1}H NMR (400 MHz, Chloroform-d) δ 6.48 (dd, J = 17.3, 1.2 Hz, 1H), 6.12 (dd, J = 17.3, 10.4 Hz, 1H), 5.95 (dd, J = 10.4, 1.1 Hz, 1H), 4.72 – 4.65 (m, 2H), 4.21 – 4.13 (m, 2H), 3.63 – 3.50 (m, 2H), 3.52 (s, 6H), 1.82 – 1.68 (m, 2H), 1.39 – 1.17 (m, 24H), 0.88 (t, J = 6.7 Hz, 3H).

Furthermore, the as-synthesized four polymerizable cationic surfmers have been analyzed by high-resolution mass spectroscopy, and the corresponding results are shown in Fig. 3. Because bromine atoms do not show signals in the mass spectrum, the HRMS results demonstrates the $C_nH_{2n}O_2N^+$ fragments of the four polymerizable cationic surfmers. From Fig.3, the signals m/z=312.28939, 340.31989, 368.35111 and 396.38230 correspond to the molecular ion peaks of four polymerizable cationic surfmers. The HRMS results in Fig.3 further confirm that four polymerizable cationic surfmers have been successfully synthesized.
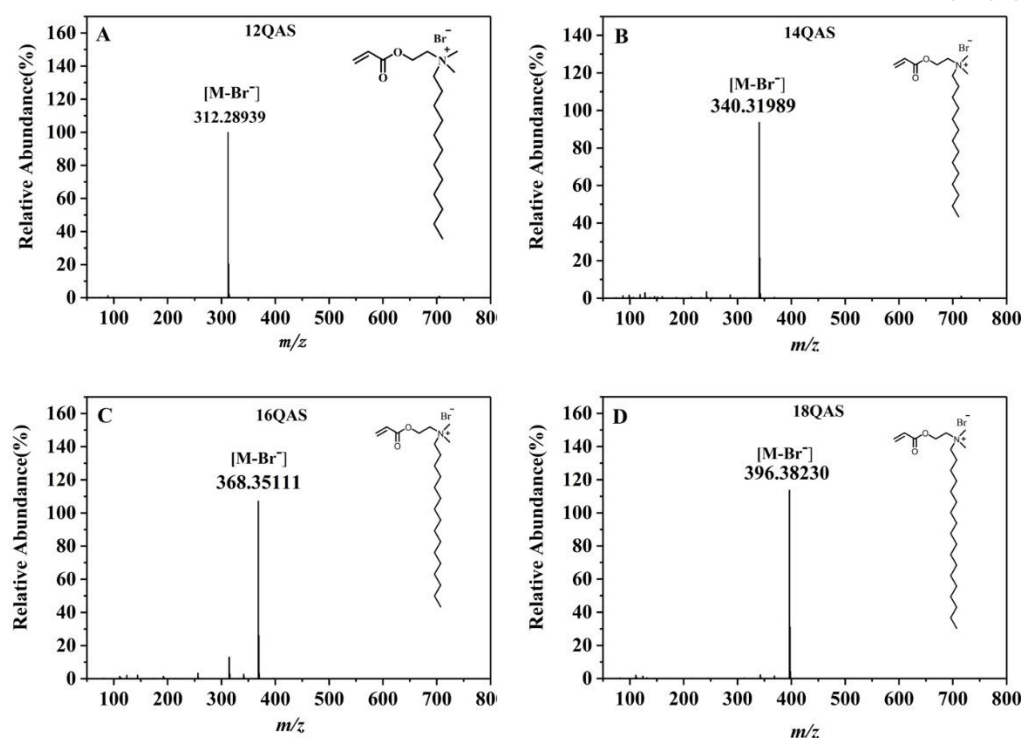
Fig. 3 The mass spectra of four reactive QAS. (A) 12QAS; (B) 14QAS; (C) 16QAS and (D) 18QAS.

3.2 Physical and chemical characterization

3.2.1 Surface tension curves

In order to compare the structure effect of polymerizable quaternary ammonium surfmers on surface tension, hexadecyl trimethyl ammonium bromide (CTAB) with similar amphiphilic quaternary ammonium structure has been used as the control group. Different with hydrophilic quaternary ammonium at the end of CTAB molecules, the as-synthesized polymerizable quaternary ammonium surfmers have the characteristics of hydrophilic quaternary ammonium head located inside the molecules as shown in Fig.1. Using pure water as the medium, the air-water surface tension curves of four polymerizable quaternary ammonium surfmers were studied, and the results have been shown in Fig. 4.
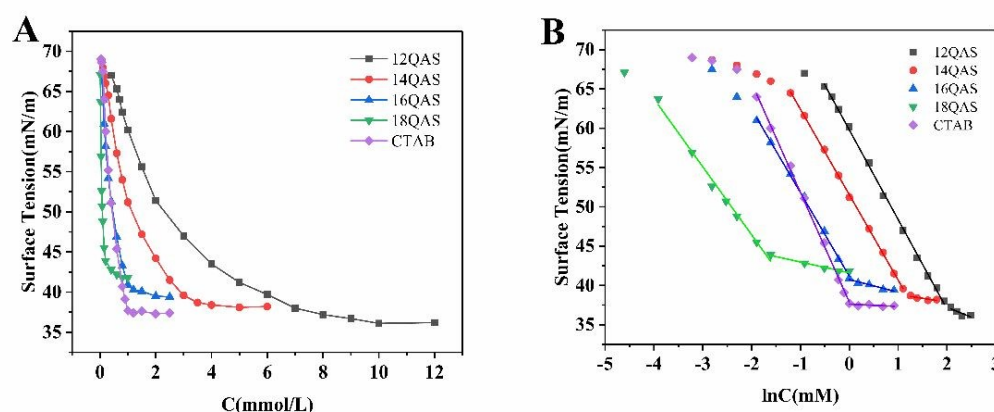
Fig. 4 The surface tension curves of polymerizable quaternary ammonium surfmers (12QAS, 14QAS, 16QAS and 18QAS). (A) The surface tension curve. (B) Fitted surface tension-ln C results corresponding to surface tension curves.

As shown in Fig. 4(A), with the increase of concentration, the surface tension of the five amphiphilic quaternary ammonium salts first decreased rapidly and then stabilized, which are the typical characteristic of surfactants. Generally, the quick decrease of surface tension could be induced by the packing increase at the air-water interfaces of surfactants molecules with concentration increasing due to their amphiphilic characteristics. Specifically, 12QAS reduced the surface tension of pure water from 72 mN/m to 36.2 mN/m (the minimum surface tension), and 14QAS, 16QAS and 18QAS reduced the surface tension of pure water to 38.1 mN/m, 39.4 mN/m, and 41.8 mN/m, respectively. On the other hand, comparing the surface tension curves of surfmers with different carbon chain lengths, it can be found that the decay rate of surface tension accelerates as the hydrophobic chain length increases, indicating the enhanced surface activity. In order to analyze the surface tension curve more accurately, logarithmic processing was performed on the surface tension curves in Fig.4(A), and the corresponding results have been shown in Fig.4 (B). Specifically, two significant features can be observed from Fig.4 (B). The first one is the negative slope of the rapidly decreasing part increases with the carbon chain length increasing. According to Márquez-Beltrán's theory,[27] the slope of surface tension curve could be associated with the ability of surfactants to reduce the surface tension of water. These

results further confirm that the surface activity of four polymerizable surfmers increases with the increase of carbon chain length. The other phenomenon is the change law of surface tension in low concentration range. 12QAS, 14QAS and CTAB show all the slow decline tendencies of surface tension in the low concentration range, while 16QAS, 18QAS show a rapid decline of surface tension. The relationship between surface tension and surfactants concentration reflects the aggregate states of amphiphilic molecules. Micellization is a multistep process, which includes that surfactants monomers are assembled into dimers, trimers and so on.[28]

3.2.2 Thermodynamic parameter

According to the classical Gibbs adsorption isotherm equation[27], a series of thermodynamic parameters such as the critical micelle concentration (*CMC*), the surface tension at the *CMC* (γ_{cmc}), the maximum surface excess concentration (Γ_{max}) and the minimum molecular cross-sectional area (A_{min}) of different surfactants at the air-water interface are calculated. The formula is as follows:

$$\Gamma = -\frac{1}{nRT}\left[\frac{\partial\sigma}{\partial\ln(Cs)}\right]$$

$$A = \frac{1}{(\Gamma \times NA)}$$

In the above equation, σ represents the surface tension in mN/m, R is the gas constant with a value of 8.31 J/(mol×K), and T is the absolute temperature in Kelvin. Cs represents the concentration of the surfactant in mol/L, while n is a constant that depends on the amount of substance adsorbed by the surfactant at the interface. According to previous literature reports, take n=2.[29] Finally, NA represents Avogadro's constant, which has a value of 6.02×10^{23} mol^{-1}.

Table 1. Adsorption parameters of polymerizable quaternary ammonium surfmers with different carbon chain lengths and CTAB at 298 K

Samples	γ_{cmc}	CMC（mM）	$\Gamma(\mu mol/m^2)$	$A_{min}(\text{Å}^2)$	PC$_{20}$
12QAS	36.2	7.19	2.30	72.22	-0.840
14QAS	38.1	3.27	2.19	75.85	0.077

16QAS	39.4	1.05	2.14	77.48	1.019
18QAS	41.8	0.18	1.75	95.08	2.643
CTAB	37.4	1.02	2.82	58.99	1.013

According to surface tension-ln C curves in Fig. 4(B), the critical micelle concentration and the minimum surface tension of five quaternary ammonium surfactants have been calculated and the corresponding results have been listed in Table 1. As shown in Table 1, the *CMC* of 12QAS was determined to be 7.19 mmol/L. Meanwhile, 14QAS, 16QAS, and 18QAS had lower *CMC* values of 3.27 mmol/L, 1.05 mmol/L, and 0.18 mmol/L, respectively. These results demonstrated that the *CMC* of four polymerizable quaternary ammonium surfmers gradually decreases with chain length of hydrophobic tails increasing. As expected, *CMC* values decreased as the alkyl chain length of the surfmers increased, which can be explained by an increase in the hydrophobic chain, where the molecules migrate easily to the surface and form micelles at low concentrations[30]. Compared with 12QAS, 14QAS, 16QAS, and 18QAS have a higher tendency to migrate to the air/water interface. It is worth noting that the *CMC* values of CTAB and 16QAS are not much different, because they have the same number of carbon atoms.

Furthermore, the corresponding adsorption parameters of five polymerizable quaternary ammonium surfactants, including surface excess concentration (Γ) and cross-sectional area (A), have also been calculated according to the above equation, and the results have been listed in Table 1. Obviously, A_{min} is related to the Γ_{max} value. Comparing with 58.99 Å² of CTAB, four polymerizable quaternary ammonium surfmers showed larger minimum molecular cross-sectional area, that is 72.22, 75.85, 77.48 and 95.08 Å², respectively. The larger occupied area results indicates the existence of branched structures of acrylate polymerizable hydrophobic group has induced a larger occupied molecular area when packing at the air-water interface.

In addition, the surfactant efficiency of decreasing surface tension refers to the concentration required to achieve a given surface tension, and the lower concentration

implies the higher efficiency. Generally, it is expressed as the negative logarithm pC_{20} of the concentration required to reduce the surface tension by 20 mN/m as following:

$$pC20 = -lgC20$$

According to the above equation, pC_{20} values of 12QAS, 14QAS, 16QAS, 18QAS and CTAB have been calculated based the surface tension curves in Fig. 4, and the results have been listed in Table 1. The corresponding pC_{20} values of 12QAS, 14QAS, 16QAS, and 18QAS are -0.84, 0.077, 1.019 and 2.643, respectively. When the hydrophobic chain length increase, the corresponding pC_{20} value gradually increases, which indicated that the efficiency of reducing the surface tension of surfmers increases with the increase of the length of hydrophobic chain.

3.2.3 Effect of electrolytes on surfactants

The synergistic effect of electrolytes and surfactants has always been a hot topic in the scientific research and industrial application. When ionic surfactants were dissolved in water, they will become ionized into cations and anions. Therefore, the type and concentration of electrolytes present in the solution will greatly affect the micro processes such as interfacial adsorption, micelle formation, and dissociation of surfactants.[31] In many applications, salts are present or added as an extra additive to enhance the performance of surfactants. Salts can significantly affect the equilibrium surfactant behavior by changing the critical micellar concentrations (*CMC*), equilibrium surface tensions (γ_{eq}), aggregation number, or Krafft temperature.[32]

With 16QAS as the typical surfmers, the influence of electrolyte type and concentration on the surface tension curve of surfactants have been studied and the results have been shown in Fig.5. Furthermore, the interfacial adsorption parameters were calculated, and the results are shown in Table 2. Firstly, the surface tension curves of 16QAS in NaCl solutions of different concentrations were studied. Obviously, the critical micelle concentration of 16QAS in pure water was 1.05 mmol/L, and the *CMC* in solutions of 0.05, 0.1 and 0.2 mol/L NaCl were 0.37, 0.33 and 0.16 mmol/L, respectively. The lowest surface tension (γ_{cmc}) of 16QAS in pure water was 39.4 mN/m,

and the γ_{cmc} in 0.05 M, 0.1M and 0.2M NaCl solutions were 36.1, 35.6 and 34.9 mmol/L, respectively. Lower CMC values and lower surface tension indicate that the solution has showed improved surface activity due to the electrolytes introduction. With the increase of electrolyte concentration, the *CMC* value of the surfactant decreases gradually, and the minimum surface tension of the solution decreases even lower. This indicates that the introduction of electrolytes can promote the formation of cationic surfmers micelles.

Secondly, metal ion with different valence states were used to study the effect of metal ion valence states on surface tension curves. It is generally believed that the effect of electrolytes on the stacking of surfactant molecules and the formation of micelles is achieved through metal ion bridging, so it is necessary to study the influence of different valence state metal ions. The surface tension curves of 16QAS in 0.05 mol/L solution of NaCl, $MgCl_2$, and $FeCl_3$ were determined and the results have been shown in Fig.5 (C) and (D). Comparing with the minimum surface tension of 39.4 mN/m and *CMC* of 1.05 mmol/L, three kind of electrolytes could all reduce the minimum surface tension of 16QAS and CMC to 36.1, 35.7 and 34.9 mN/m, and the *CMC* could be decreased to 0.37, 0.28 and 0.21 mmol/L, respectively. Moreover, as the valence state of metal ions increases, the degree of reduction in surface tension and critical micelle concentration increases, indicating that the higher the valence state of metal ions, the more significant their bridging degree.

Thirdly, in order to further investigate the effect of anionic valence states on the surface tension of 16QAS, the surface tension curves of 16QAS in 0.05 mol/L solution of NaCl, Na_2SO_4, and Na_3PO_4 were determined and the results have been shown in Fig.5 (E) and (F). From Table 2, as the anionic valence state increases, the minimum surface tension gradually decreases to 36.1, 35.5 and 34.9 mN/m. More significantly, the decrease in *CMC* is more pronounced and decreased from 0.37 to 0.13 and 0.18 mmol/L when the anionic valence state ranges from -1 to -3, which indicated that the anions of electrolytes also have a significant impact on the interfacial packing of cationic surfactants, particularly affecting their critical micelle concentration.[33]
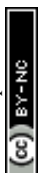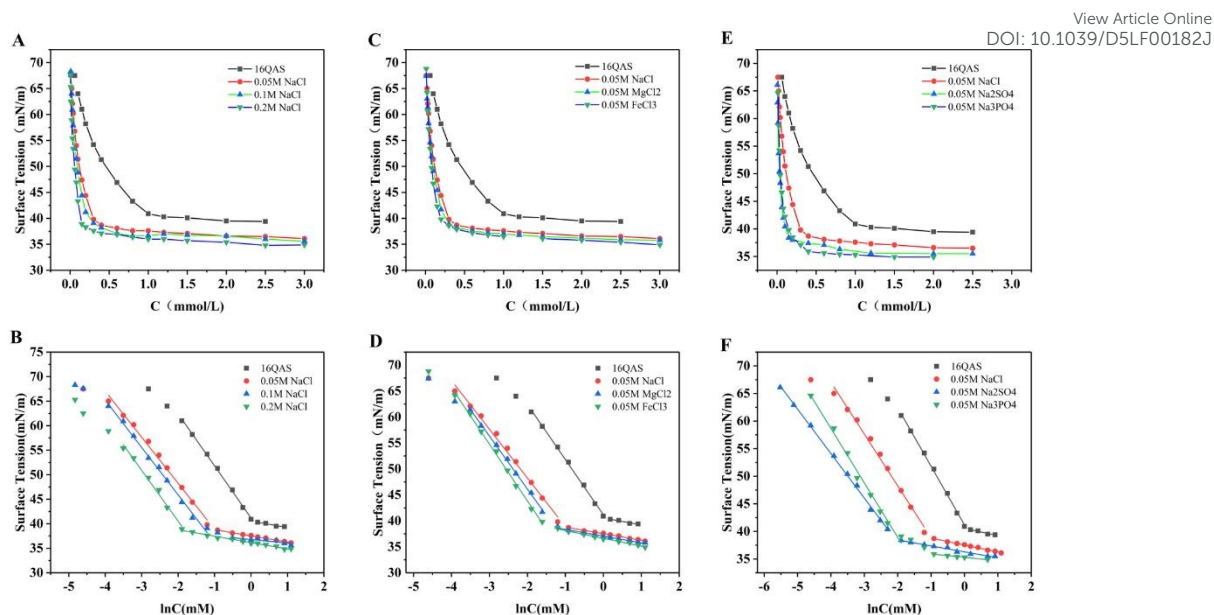
Fig. 5 Effect of electrolytes type and concentration on surface tension of 16QAS. (A) and (B) show the effect of concentrations of NaCl solution. (C) and (D) show the effect of cations valence states at the same concentration. (E) and (F) show the effect of counterions of different valence states at the same concentration.

Table 2. The effect of electrolyte type and concentration on interfacial adsorption parameters of 16QAS

Solution	γ_{cmc}	CMC（mM）	$\Gamma(\mu mol/m^2)$	$A_{min}(Å^2)$	pC20
Pure water	39.4	1.05	2.14	77.48	1.019
0.05M NaCl	36.1	0.37	1.90	87.66	2.399
0.1M NaCl	35.6	0.32	1.95	85.20	2.647
0.2M NaCl	34.9	0.16	2.09	79.33	3.108
0.05M MgCl$_2$	35.7	0.28	2.06	80.64	2.576
0.05M FeCl$_3$	34.9	0.21	2.20	75.40	2.747
0.05M Na$_2$SO$_4$	35.5	0.13	1.63	102.00	3.740
0.05M Na$_3$PO$_4$	34.9	0.18	1.99	83.38	3.326

3.3 Solution micropolarity

For a long time, it has been found that surfactants can affect the spectral properties

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of dyes in solution, which could demonstrate that the absorption and fluorescence emission spectra of dye molecules have been changed due to the complex interaction between surfactants and dye molecules. Based on the spectral changes of dye molecules, the assembly structure of surfactant solution could be disclosed, such as pyrene fluorescence spectroscopy[24] and dye solubilization method to determine the critical micelle concentration of surfactants.[34] Methyl Orange (MO) is an example of an amphiphilic anionic azo dye, which could produce interactions with several cationic surfactants. Especially, the short wavelength absorption band of methyl orange, as induced by low concentrations of surfactant, has been used to infer the surfactants assembly. Probe molecules MO was dissolved into four polymerizable quaternary ammonium surfmers with different concentration, and the UV-Vis absorption spectra were recorded and illustrated in Fig.6. These obvious absorption spectra profiles indicated that surfmers with different chain length have also exerted a dramatic influence on the absorption spectra of methyl orange. From Fig. 6(A)-(E), the maximum absorbance (λ_{max}) of MO in pure water could be found at 464 nm. According to Engberts *et. al.* 's results[35], the maximum absorption band (λ_{max}) for MO is positioned in water at ca. 466 nm and in ethanol at ca. 417 nm, indicating that a more apolar environment leads to a substantial decrease in λ_{max}. When the surfactant concentration is lower than *CMC*, the absorbance of free methyl orange at 464 nm in solution decreases, while a new absorption band appears at 375 nm with concentration increasing, which is attributed to the ion pair formed by electrostatic interaction of 1:1 MO-surfactant. When the surfmers concentrations were further increased more than *CMC*, maximum absorption of MO shifted to 416 nm, which indicate that methyl orange is incorporated in the micellar environment.
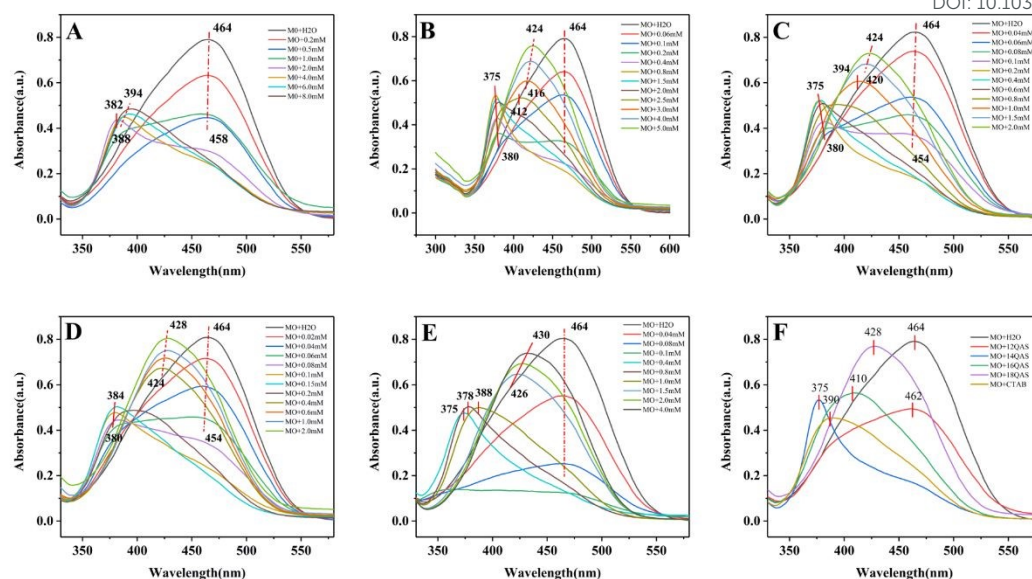
Fig. 6 The microenvironment characterization of surfactant solutions of different concentrations with methyl orange as probes. (A) - (E) are the absorption spectra of methyl orange in different surfactants such as 12QAS, 14QAS, 16QAS, 18QAS, and CTAB; (F) The absorption spectra of methyl orange in the same concentration of surfactant solution. Concentration: 1.0 mM.

In order to study the effect of hydrophobic chain length on micellar environment, the absorption spectra of MO in several cationic surfaction solution at 1 mmol/L were recorded and shown in Fig.6 (F). The MO in 12QAS solution showed the similar absorption profiles to that in pure water, which indicated that 12QAS showed weak micelle formation capability due to shorter hydrophobic chain length. The MO in 14QAS solution at 1 mmol/L showed the strongest absorption at 375 nm, which implied that the complex aggregation of 14QAS and methyl orange through electrostatic interaction. The MO in solution of CTAB, 16QAS and 18QAS showed the similar absorption from 400 to 440 nm, which indicated that micellar structures had formed, and the different vesicle structures with decreasing micro-polarity could contribute to the red-shift of absorption peaks. On the other hand, the *CMC* values from Fig.6 showed that *CMC* decreased with hydrophobic chain length such as 12QAS>14QAS>16QAS>18QAS, which is consistent with the *CMC* measured by the surface tension curves method.

3.4 *In vitro* antibacterial effect

Using the classical colony counting method, the antibacterial effects of several quaternary ammonium salts with different carbon chain lengths on *E. coli* and *S. aureus* were evaluated and the results have been illustrated in Fig. 7. Fig.7 (A) and (B) show the plate plots of *E. coli* and *S. aureus* treated with different quaternary ammonium surfmers under the same conditions, respectively. From the plate images in figures, it can be observed that both *E. coli* and *S. aureus* had a large number of colonies on the 12QAS plates, while the colonies on the 14QAS and 16QAS plates gradually decreased. This indicates that antibacterial efficacy of the QAS surfmers could be enhanced with the carbon chain length increasing.[17] This may be attributed to the longer carbon chains having higher lipid solubility, which can penetrate the cell membrane and enter the cell to play a role, resulting in bacterial inactivation. Interestingly, for 18QAS, there were almost no colonies of *S. aureus* on the agar plate, while the number of *E. coli* colonies increased. It is clear that 18QAS has a strong bactericidal effect on *S. aureus* but a weaker bactericidal effect on *E. coli*, which may be due to structural differences between Gram-positive and Gram-negative bacteria. Since the cell wall of Gram-positive bacteria is thicker, quaternary ammonium salts can penetrate more easily, disrupting cell membrane permeability and leading to leakage of intracellular contents.[36] Generally, there is no efficient antibacterial agent for all pathogenic microorganisms, and antibacterial agents have selectivity for microbial species.

In order to analyze the antibacterial performance of different samples more accurately, colony-forming counting (CFU) method was used to analyze the antibacterial results, and the quantitative $Log_{10}CFU$ results are shown in the Fig.7(C). With the hydrophobic tail increases, the $Log_{10}CFU$ reduction of QAS surfmers against *S. aureus* gradually increases, reaching a max of 4.23 for 18QAS. However, compared to 12QAS, 14QAS and 16QAS surfmers show a greater reduction in $Log_{10}CFU$ for *E. coli*, and 16QAS showed the superior antibacterial performance, that is 2.61 $Log_{10}CFU$. Strangely, 18QAS can only achieve a reduction of 0.43 $Log_{10}CFU$ for *E. coli*, which is consistent with the trend of bacterial colony growth on agar plates as shown in Fig.7 (B). Additionally, 12QAS also exhibits certain antibacterial activity against *S. aureus* and *E. coli*, with $Log_{10}CFU$ reduction rates of 1.2 and 0.67, respectively. This may be
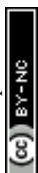
related to the quaternary ammonium structure, as a large number of quaternary ammonium cationic surfactants have been shown to possess antimicrobial activity.[37]
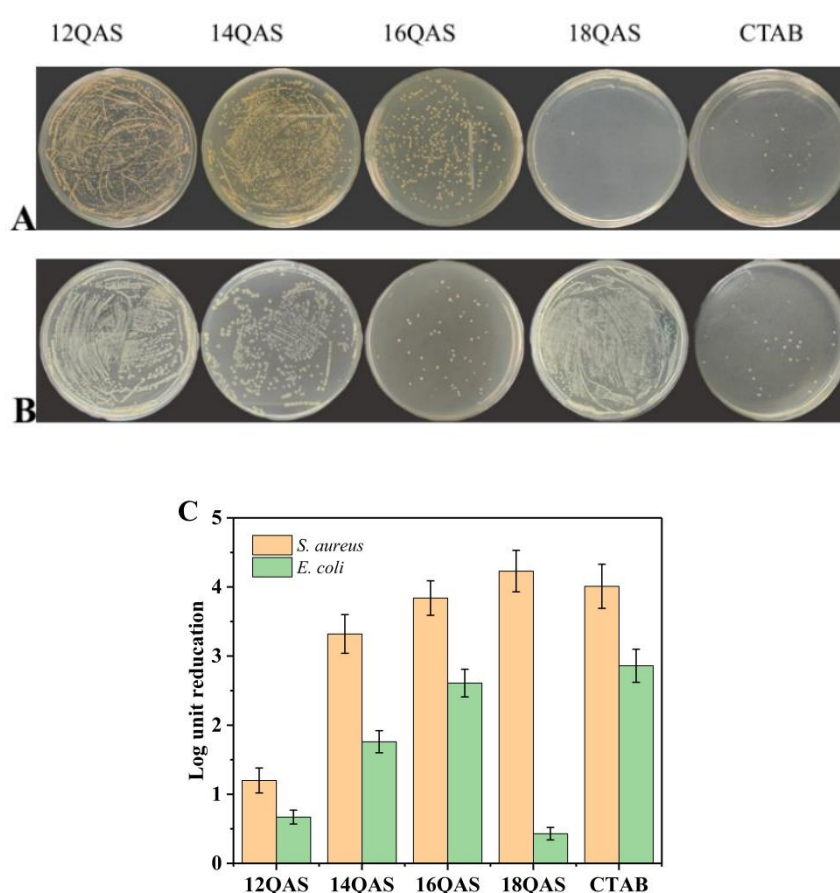

Fig.7 *In vitro* antibacterial activity. Plate images of *Staphylococcus aureus* (A) and *Escherichia coli* (B) treated with QASs. Bacteria were incubated with quaternary ammonium surfmers (5 μM, *S. aureus*; 10 μM, *E. coli*) and CTAB was used as a positive control for both *S. aureus* and *E. coli*. (C) Qualitative colony-forming unit chart of the inhibitory effects on two types of bacteria treated with different QASs.

3.5 MIC and MBC

In order to further compare the bacteria killing efficiency of polymerizable quaternary ammonium surfmers, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration(MBC) were determined. Turbidity is used to distinguish whether there is bacterial growth, and no turbidity indicated an inhibitory effect on bacterial growth. The corresponding MIC and MBC results have been shown in Table 3.

Table 3. MIC and MBC of amphiphilic QASs against *S. aureus* and *E. coli*

QASs	MIC（μmol/L）		MBC（μmol/L）	
	S. aureus	*E. coli*	*S. aureus*	*E. coli*
12QAS	3.75	125	15	125
14QAS	1.875	62.5	7.5	62.5
16QAS	<1.875	32.25	<7.5	32.25
18QAS	0.937	>1000	3.75	>1000
CTAB	<1.875	<32.25	<7.5	<32.25

Except for 18QAS, their MIC values for *E. coli* are mainly concentrated at 32.25-125 μmol/L, and their MIC values for *S. aureus* are 1-4 μmol/L. Strangely, 18QAS surfmer showed very low MIC and MBC against *S. aureus*, at 0.937 and 3.75 μmol/L, respectively, which demonstrated that 18QAS surfmer showed the superior bactericidal and inhibitory efficiency among all polymerizable quaternary ammonium surfmers. However, 18QAS surfmer exhibited unusually low bactericidal and inhibitory efficiency against *E. coli.* at over 1000 μmol/L. For the inhibition efficiency of *E. coli*, the 16QAS showed the best bactericidal and inhibitory efficiency at concentrations 32.25 and 32.25 μmol/L, respectively. Compared with the control sample CTAB, its efficiency was slightly weaker. These results agree with the conclusion from qualitative colony-forming unit protocols. On the other hand, each QAS surfmer has a higher MIC value against *E. coli* than against *S. aureus*. In our previous reports[20,23,24,38], different amphiphilic cationic photosensitizers have been synthetized and used to kill Gram-negative *P. aeruginosa* and Gram-positive *S. aureus* through antibacterial photodynamic therapy process, and all the results demonstrated higher antibacterial efficiencies *to* Gram positive bacteria than that to Gram-negative bacteria, which are agreed with our current results. In our previous researches[20,23,24,38], we have explored the antibacterial mechanism using SEM and confocal fluorescence microscopy. The results showed that Gram positive bacteria are more easily killed by amphiphilic cationic antibacterial agents due to differences in cell wall and membrane structure, and

the specific mechanism may be electrostatic binding, hydrophobic chain insertion into the cell membrane, or involving both processes. Therefore, for the current results, 16QAS shows the best effect on Gram-negative *E. coli*, while 18QAS is highly effective against Gram-positive *S. aureus*, but ineffective against Gram-negative *E. coli*. This 'cross-advantage' targeting different bacterial strains is very interesting. However, the complexity of microbial structure makes it difficult to clearly elucidate this mechanism at present. We think that QAS has stronger antibacrerial activity against Gram positive bacteria, which may be related to differences in cell wall composition. Gram positive bacterial cell walls contain more peptidoglycans and phosphates, but porous peptidoglycans make it easier for foreign molecules to enter. The double-layer structure of Gram negative bacterial cell walls can more effectively prevent the entry of foreign molecules, thus requiring higher concentrations of QAS for sterilization.

4. Conclusion

In conclusion, four polymerizable quaternary ammonium surfmers with different hydrophobic chain length have been synthesized, characterized and used as bacterial inhibitory and killing research. As the length of the hydrophobic chain increases, the minimum surface tension of these polymerizable quaternary ammonium surfmers gradually increases, while *CMC* gradually decreases, resulting in improved efficiency in reducing surface tension. On the other hand, not only does the electrolyte concentration increase the interfacial adsorption activity of surfmers, but the electrolyte composition, such as cation and anion valence states, also greatly enhance the surface activity reduction efficiency of these QAS surfmers. 18QAS surfmer showed very low MIC and MBC against *S. aureus*, at 0.937 and 3.75 μmol/L, respectively, which could illustrate the efficiency of 4.23 Log CFU to *S. aureus*, and 16QAS showed the superior killing efficiency of 2.61 Log CFU to *E. coli.* at concentrations 32.25 μmol/L. The introduction acrylate polymerizable group to amphiphilic quaternary ammonium would provide infinite possibilities for polymerizable cationic functionalized surfaces. These results will provide ideas for the design of long-term antibacterial surface or implanted materials surface, thereby providing a reference for the interface design of biological

functional materials .

Author contributions

Cailing Li: Data curation, Methodology, Writing, review&editing. **Jinlu Zhong**: data curation, Investigation. **Yanan Weng**: Methodology, Investigation. **Sensen Xie**: Conceptualization, Methodology, Review. **Shuang Li**: Microbacterial Data curation. **Dinghua Yu**: Supervision, Data curation, Project administration, Funding acquisition.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

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Data Availability Statement

The authors confirm that the data supporting the findings of
this study are available within the article.

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