Biological properties of sodium alkyl methyl ester sulfonate/alkyltrimethylammonium bromide surfactant mixtures

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A B S T R A C T

Quaternary ammonium compounds (QACs) are commonly used as disinfectant in medical care, food industry, detergent and glue industries. This is due to a small concentration of QACs is sufficient to inhibit the growth of various bacteria strains. In this work, the inhibitory power of cationic surfactants, alkyltrimethylammonium bromide (C n TAB) in the presence of anionic surfactants, sodium alkyl methyl ester α-sulfonate (C n MES) was studied. The growth inhibition test with gram-positive (Staphylococcus aureus) and gram-negative (Escherichia coli and Pseudomonas aeruginosa) bacteria were used to determine the toxicity of single and mixed surfactants. Results from this work showed that certain mixed surfactants have lower minimum inhibition concentration (MIC) as compared to the single C n TAB surfactants. Besides that, it was also found that alkyl chain length and the mixing ratios of the surfactants play a significant role in determining the mixture inhibitive power.

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1. Introduction

Quaternary ammonium compounds (QACs) are cationic surfactants that have been long known for its broad spectra of antimicrobial activities [1]. As such, the use of QAC as disinfectant in the medical care, food industry, detergent and glue industries are very common since its discoveries in the early 1900 [2]. Their antimicrobial actions generally begin with penetration into the cell wall, followed by partitioning of the agents in between the core membrane, solubilizing the membrane and then lyse the cells. Lealthy of the cells occurs through leakage of cytoplasmic materials [3,4]. Beside this, there are also many other different mechanisms employed by QACs to cause lethality to the microbes depending on the nature of the living organisms [4].

However in recent years, surfactants are seldom used in single or pure form in industry applications. This was simply due to the higher processing cost and lower surface activities as compared to mixed surfactant systems. Among different types of binary mixed surfactants, anionic/cationic mixed systems showed exceptionally high surface activities [5]. This is due to the oppositely charged surfactants can act as counterions to each other and thus screen the repulsive forces between each other. When the repulsive forces between the surfactant molecules were greatly reduced, surfactant molecules can arrange closer to each other. Such assemblies attribute to greater reduction in interfacial tension [6–8]. Despite some precipitation problems, the excellent physical properties of anionic/cationic mixed surfactants have sparked great interests to many researchers. Numerous researches have been conducted to study the phase behavior, physical properties and interactions at molecular level of various types of anionic/cationic mixtures [5–8]. However, the effects of adding anionic surfactants to cationic surfactants were very much less explored [2].

In our previous work [9], we have studied the physical properties of mixed alkyltrimethylammonium bromide (C n TAB) and sodium methyl ester α-sulfonate (C n MES) surfactants, where n denotes hydrocarbon chain length of 12–16 carbons and n denotes as 12–18 carbons long. While C n TAB is a conventional cationic surfactant, C n MES is relative new anionic surfactants that exhibit good surface activities as compared to conventional anionic surfactants such as alkyl sulfate (AS) and linear alkylbenzene sulfonates (LAS). The C n MES also exhibits better biodegradability than other anionic surfactants of its grade [10–12]. From the work, we found that C n MES/C n TAB mixed surfactants had lower tendency to form precipitates as compared to C n LAS/C n TAB mixed surfactants. Results also showed that the mixed surfactants have better surface properties as compared to both of its single surfactants [9].

The aim of this work is to explore the antibacterial properties of mixed (C n MES/C n TAB) surfactants. Besides matching different hydrocarbon chain length, mixing different ratios of the anionic/cationic mixtures were also been carried out. Potency of these C n MES/C n TAB mixed surfactants systems were tested against

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common types of gram-positive (Staphylococcus aureus) and gram-negative (Escherichia coli and Pseudomonas aeruginosa) bacteria.

2. Materials and methods

2.1. Materials

Cationic surfactants, namely, dodecyltrimethylammonium bromide (C12TAB), tetradecyltrimethylammonium bromide (C14TAB), hexadecyltrimethylammonium bromide (C16TAB) and octadecyltrimethylammonium bromide (C18TAB) were purchased from Sigma–Aldrich, Switzerland. Sodium dodecyl methyl ester sulfonate (C12MES), sodium tetradecyl methyl ester sulfonate (C14MES) and sodium hexadecyl methyl ester sulfonate (C16MES) were obtained from Malaysian Palm Oil Board (MPOB). All chemicals were purified and recrystallised before use.

2.2. Sample preparation

Stock solutions (25 mM) of all single surfactants were prepared in deionized water. The stock solutions were sterilized by filtered through cellulose acetate filters (0.2 μm pore size; Millipore). Mixed surfactants (CmMES:CnTAB) were then prepared by mixing the stock solution of two surfactants according to the desired molar ratios i.e. 25:75, 50:50 and 75:25, where m = 12, 14, 16 and n = 12, 14, 16, 18 carbon number in the hydrophobic chain.

2.3. Antibacterial activity

In vitro antibacterial activity of the single and mixed surfactants against gram-positive and gram-negative bacteria was expressed as the minimum inhibition concentration (MIC) values, which was defined as the lowest concentration of the surfactants needed to inhibit visible growth after 24 h of incubation at 37 °C. MIC values were determined by broth dilution method against monoculture strain of S. aureus (ATCC 6538), E. coli (ATCC 8739) and P. aeruginosa (ATCC 15442). The final concentrations of surfactants examined in the medium were 12.5, 1.25, 0.125, 0.0125 and 0.00125 mM. The effect on bacteria growth was determined by visual counting and MIC values were determined using scientific research software. IC90 is defined as the lowest concentration of surfactant at which there is a decrease of 90% in bacteria colonies as compared to the positive control.

3. Results

3.1. Antibacterial property of single surfactant system

The effects of CmMES and CnTAB (where m = 12, 14 and 16, and n = 12, 14, 16 and 18) surfactant series against S. aureus was shown in Fig. 1a. Results indicated C10TAB surfactant series were very active against S. aureus, with IC90 values lower than 2 μM. The inhibitory effectiveness towards S. aureus increased with the increase of alkyl chain length of CnTAB surfactants. Meanwhile CmMES surfactants series exhibited IC90 values above 900 μM (which was above 450 times more concentrated than CnTAB surfactant series). Even though CmMES surfactant series showed weak inhibitory activity, their trend on the increase of inhibitory effectiveness with increasing hydrophobic chain length towards S. aureus was similar to CnTAB surfactant series.

Inhibitory effectiveness of both CmMES and CnTAB surfactant series against two gram-negative bacteria (namely E. coli and P. aeruginosa) were shown in Fig. 1b and c. Both CmMES and CnTAB surfactant series were less effective against gram-negative bacteria as compared to gram-positive bacteria. S. aureus. High concentration (above 9 mM) of CmMES surfactants (having different hydrocarbon chain length from m = 12 to 16) were needed to inhibit the growth of both gram-negative bacteria. CnTAB surfactant series needs less than 100 μM and even lesser concentration (below 5 μM) as the hydrocarbon chain length increases from n = 12 to n = 18 when tested on E. coli. However, it was noted that higher concentration (from 10 to 50 times more concentration) of CnTAB surfactant (from n = 12 to n = 18) was needed to inhibit the growth of P. aeruginosa as compared to the former even though both were gram-negative bacteria.

3.2. Antibacterial property of anionic/cationic binary surfactant system

In many instants, mixtures of more than one surfactant (either similar or different type) provide many advantages either in the physical or chemical properties over the use of a single surfactant. Individual surfactant series have been described in the earlier section. In this section, both anionic (CmMES) and cationic (CnTAB) surfactants series were mixed together into three different ratios (representing anionic-rich, equimolar and cationic-rich portions respectively) in the aqueous systems. Interesting inhibitory results exhibited by the anionic/cationic surfactant mixtures against the three types of microbes (S. aureus, E. coli and P. aeruginosa) were observed as indicated in Figs. 2–4.
As indicated in Fig. 2, mixtures between Cm MES and Cn TAB surfactants series generally showed increased in the inhibitory activity against S. aureus with the increase of hydrocarbon chain length (n = 12–18) in the hydrophobic portion of Cn TAB in all three Cm MES (where m = 12–16) molar ratios in mixed anionic/cationic surfactant systems except 0.5 molar ratio of mixed C16 MES and C16 TAB surfactant system. The weakening of inhibitory effectiveness was also been noted with the increase of hydrocarbon chain length in the hydrophobic portion of Cm MES (m = 12–16) respectively, except an anionic-rich molar ratio in the mixtures. Interestingly among all the mixed surfactant systems, both C12 MES/C12 TAB and C16 MES/C12 TAB mixed systems exhibited maximum inhibitory activity (20 μM and 7 μM, respectively) at equimolar ratio followed by cationic-rich and lastly anionic-rich ratio. It was believed that C16 MES/C12 TAB mixed systems also exhibited similar trend as the former even though IC50 values were lower than 1 μM (below the tested concentration).

As noted in the previous section, gram-negative bacteria (both E. coli and P. aeruginosa) were quite resistant to Cm MES and Cn TAB surfactants series. However in general, similar trend as anionic/cationic surfactant mixtures against gram-positive bacteria were observed on the gram-negative bacteria where the inhibitory activity increases along with the increased amount of cationic surfactants in the mixed surfactant systems (except C12 MES/Cn TAB, where n′ = 12–16, exhibited reverse inhibitory activity trend and C16 MES/C14 TAB with no inhibitory change in the mixed systems) as shown in Figs. 3 and 4. Another interesting finding was noted when optimum inhibition power was exhibited against E. coli on 0.5 and 0.75 molar ratios of C12 MES/C14 TAB and C12 MES/C16 TAB; and 0.25 and 0.5 molar ratios of C16 MES/C16 TAB mixed surfactants systems (see Fig. 3). Similarly when tested on P. aeruginosa, where both 0.5 and 0.25 molar ratios of C12 MES/C16 TAB and C16 MES/C16 TAB shows optimum inhibitory concentration.

4. Discussion

In general S. aureus is more susceptible to antibacterial agents as compared to E. coli and P. aeruginosa. This was because S. aureus (gram-positive bacteria) does not have an outer membrane which acts as a barrier against antibacterial agent [13]. Whereas gram-negative bacteria (such as E. coli and P. aeruginosa), contain an outer membrane wall (consist of lipopolysaccharide and protein) which envelope the bacteria. On top of that, P. aeruginosa is able to produce slime which covers itself, thus, exceptionally resistant to chemical agents [14,15]. Previous studies of QAC compounds against these bacteria have indicated that the order of increase resistivity were as followed where S. aureus < E. coli < P. aeruginosa [13,16].

Surfactant molecules, which have both polar and non-polar portions, exhibited the ability to interact with the lipid layer of cell membranes wall of the organism. This interaction may result in changing the membrane orientation which could solubilize,
damage and destruct the membrane that lead to the death of the cell. Results in Figs. 1–4 showed that the biological activity of the tested surfactant (both individual and anionic/cationic mixtures) compounds were depended on both the character of polar head (size and electrical charge distribution) and hydrophobic hydrocarbon chain length. The length of the alkyl chain length of the surfactant substances incorporated into the membranes affects the biological activity. This was clearly demonstrated by the increased of hydrophobic alkyl chain length from dodecyl to octadecyl chain of cationic surfactants, C₆TAB, increases the biological activity against all tested bacteria (S. aureus, E. coli and P. aeruginosa). This was attributed to the increase in adsorption tendency of C₆TAB surfactants onto the bacteria membrane surface (which disrupt the membrane of the bacteria) with the increase hydrophobic chain length as indicated in their surface activity properties. Long alkyl chain length of the hydrophobic portion increases the adsorption of the molecules at the interfaces. On the contrary, surfactants having shorter hydrophobic chains will have lower tendency towards adsorption at the interfaces. Apart from these, MIC values for all C₆TAB surfactants occurred below the critical micelle concentrations (CMC) values. This showed that the surfactant monomers are the species that play the crucial role in interacting with the membrane cells and not the aggregates [17].

Similar to C₆TAB surfactant series, the increases of hydrophobic chain length of C₈MES series, increases the inhibitive power towards gram-positive bacteria, S. aureus. However, a normal bell-shaped curve with a maximum inhibitive power at 14 carbon atoms in the alkyl chain length of the hydrophobic group was observed against gram-negative bacteria, E. coli and P. aeruginosa. Such inhibitive trend (optimum biological effects at a chain length of 14 carbon atoms) were also observed in other studies [18,19]. This phenomenon was attributed by a combination of physico-chemical properties of the surfactants (i.e., CMC, adsorption, aqueous solubility and hydrophobicity) [17,19]. Besides these, morphology of the bilayer biological membrane (i.e. bilayer thickness, stability and hydrophobicity) also plays a crucial role in affecting the inhibitive mechanism of surfactants [19].

In the mixed C₆MES/C₆TAB surfactant systems (as indicated in Figs. 2–4), synergistic inhibitive activity effect against bacteria growth (on both gram-positive and gram-negative types) were noted. The inhibitory potency differences between the individual (of both C₆MES and C₆TAB) surfactant and C₆MES/C₆TAB mixtures on the bacteria growth can be explained through their differences in polar head (size and electrical charge distribution) and hydrocarbon chain length.

Basically, the bacterial inhibition efficacy of C₆MES/C₆TAB mixtures is dedicated to the presence of the positively charged nitrogen atoms [20]. Therefore, the MIC values of the mixtures were more affected by the change of alkyl chain length of the C₆TAB surfactants instead of C₆MES. Though mixed surfactants at the cationic-rich region were expected to have the highest inhibitive power among the different mixing ratios, results from Figs. 2–4 showed otherwise where mixtures at equimolar mixing ratio have the highest potency. This could be explained by the close packing of the mixed surfactant molecules at this mixing ratio [9]. Usually, close packing of surfactant molecules were associated with high adsorption tendency at the interface. Hence, inhibitive power of the C₆MES/C₆TAB mixtures was also raised.

However, a reverse inhibitory activity (where the inhibition strength decreases from anionic-rich to equimolar and lastly cationic-rich ratio) for C₆MES/C₆TAB mixed systems was also observed. This could be due to the size of the aggregations formed were smaller in comparison to cationic-rich and equimolar mixing ratio of the mixed systems. Smaller aggregations allowed the actives to penetrate easily through the cellular membrane and thus disrupt the membrane. Besides that, absorption of the aggregates increases with decreasing size [21].

Besides this, hydrophobicity of the mixed surfactants also played a crucial role in the inhibitive efficacy against the bacteria tested. As the hydrocarbon chain length of C₆MES or C₆TAB surfactant increases, the surface activity of the mixtures also increases. This trend was shown in our previous paper [9]. High surface activity means that the surfactant molecules have higher tendency to adsorb at the interface and therefore more readily to destruct the membrane of the bacteria [20].

Despite the long alkyl chain length of C₈MES/C₁₂TAB and C₊₈MES/C₁₈TAB mixtures, their inhibitive power towards E. coli and P. aeruginosa were relatively low or even no activity. This can be explained by the compatibility of the surfactant molecules length with the membrane thickness. Partitioning of the surfactant into the membrane were favored when the length of the surfactant molecules were similar with the thickness of the membrane. Meanwhile, surfactant molecules that are too big or long would have difficulties to diffuse into the membrane layer. Therefore, its inhibitive power was greatly reduced [19]. On top of this, limited aqueous solubility of the long chain length surfactant mixtures could also be one of the factors that cause the decrease of inhibitive ability [19].
5. Conclusion

Addition of anionic surfactants, CnMES did not directly inactivate the inhibitive activity of QAC in cationic surfactants, CnTAB. For *P. aeruginosa*, certain systems even can enhance the inhibitive power of the cationic surfactants. In view of the toxicity against bacteria, not only the different alkyl chain lengths of the surfactants, but also the different mixing ratios play a crucial role.

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