IDENTIFICATION AND CHARACTERIZATION OF COMPLEXES OF CATIONIC AND ANIONIC SURFACTANTS AT EQUAL CHARGE RATIO

by

YU WANG

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ABSTRACT OF THE DISSERTATION

Identification and characterization of complexes of cationic and anionic surfactants at equal charge ratio

By YU WANG

Dissertation Director:
Edward W. Castner, Jr.

The objective of this study was to investigate the complex structures formed of cationic and anionic surfactants. X-ray diffraction, ^{1}H-NMR, FTIR and LC-MS methods have been applied in the current study. A novel crystalline complex, consisting of chlorhexidine-dodecyl sulfate, was obtained after aging at 60 °C for three weeks. The structure was solved by single crystal X-ray diffraction. The molar ratio of chlorhexidine and dodecyl sulfate was 1:2, indicating a complex between a divalent cation and two nonvalent anions. In

addition, the impact of phosphate, pyrophosphate and triphosphate anions on the stability and solubility of the complex was monitored by observing precipitate formation. The complex was further examined by LC-MS method with a direct injection. For comparison, other surfactants were studied: including anionic surfactant sodium lauroyl methyl taurate (SLMT) and two cationic surfactants, cetylpyridinium chloride (CPC) and benzalkonium chloride (BKC). Electrospray ionization interface of MS enabled us to study both positively and negatively charged clusters.

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Last but not least, I am deeply indebted to my parents for their upbringing and unconditional love, for their encouragement to help me relieve the stress and anxiety, to their dedication and hard work in raising me up to who I am today.

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1. Introduction

1.1 General properties of all surfactants

The word "Surfactant" is a contraction of the three words "Surface Active Agent". Surfactants are materials that can lower the surface tension (or interfacial tension) between two liquids or between a liquid and a solid. Inherent chemical barriers, differences in molecular weight and surface or interfacial tension between two different materials would normally make them difficult or impossible to mix. What makes surfactants special is their ability to mobilize and combine materials - typically water and oils - that otherwise would not mix due to their incompatible molecular properties by lowering the surface tension. In general, any material that lowers the interfacial surface tension can be considered a surfactant. Surfactants may act as wetting agents, emulsifiers, foaming agents, and dispersants. Surfactants have been widely used for cleaning and detergent formulations in home care, personal care and oral care products, cosmetics, pharmaceuticals and find a diverse range of important industrial applications (Schramm, 2003). Many efforts have been given to combine cationic and anionic surfactants together in formulation to develop novel products. However, the biggest challenge for this kind of formulation is that the strong interaction between positive and negative head groups of surfactants could cause the precipitation and reduce both cationic bio-efficacy and anionic foaming function.

Surfactants are usually amphiphilic organic compounds. They contain both hydrophobic groups (the tails) and hydrophilic groups (the heads). The importance of surfactants in our daily life cannot be overemphasized. For example, cationic surfactants can kill a

broad range of microbials due to their affinities to anionic cell membranes. Anionic surfactants have very strong foaming abilities. Surfactants can be classified into four categories based on the polar head composition, namely, anionic, cationic, non-ionic and amphoteric (zwitterionic) (Sakamoto et al., 2017, Kume et al., 2008). Anionic surfactants have anionic functional groups on their heads, such as sulfate, sulfonate, phosphate and carboxylate. Cationic surfactants have positively charged hydrophilic heads, including some protonated primary, secondary and tertiary ammoniums, also some permanently charged quaternary ammoniums. This charge may be either permanent or only exist at certain pH values. Nonionic surfactants have covalently bonded oxygen-containing hydrophilic groups, which are bonded to hydrophobic parent chain structures. There are various types of non-ionic surfactants, such as polyglycerol alkyl ethers, glucosyl dialkyl ethers, crown ethers, ester-linked surfactants, polyoxyethylene alkyl ethers, etc. Amphoteric (Zwitterionic) surfactants have both cationic and anionic centers attached to the same molecule, usually a chain compound. The cationic part is based on primary, secondary, tertiary amines or quaternary ammonium cations. The anionic part can be more variable, such as sulfate, phosphate and carboxylate. In the bulk aqueous solution, surfactants spantaneously form aggregates, such as micelles, in which the hydrophobic tails form the core of the aggregate and the hydrophilic heads are in contact with the surrounding liquid, usually water, as shown in Figure 1. Other types of aggregates may also form, such as spherical or cylindrical micelles or lipid bilayers.

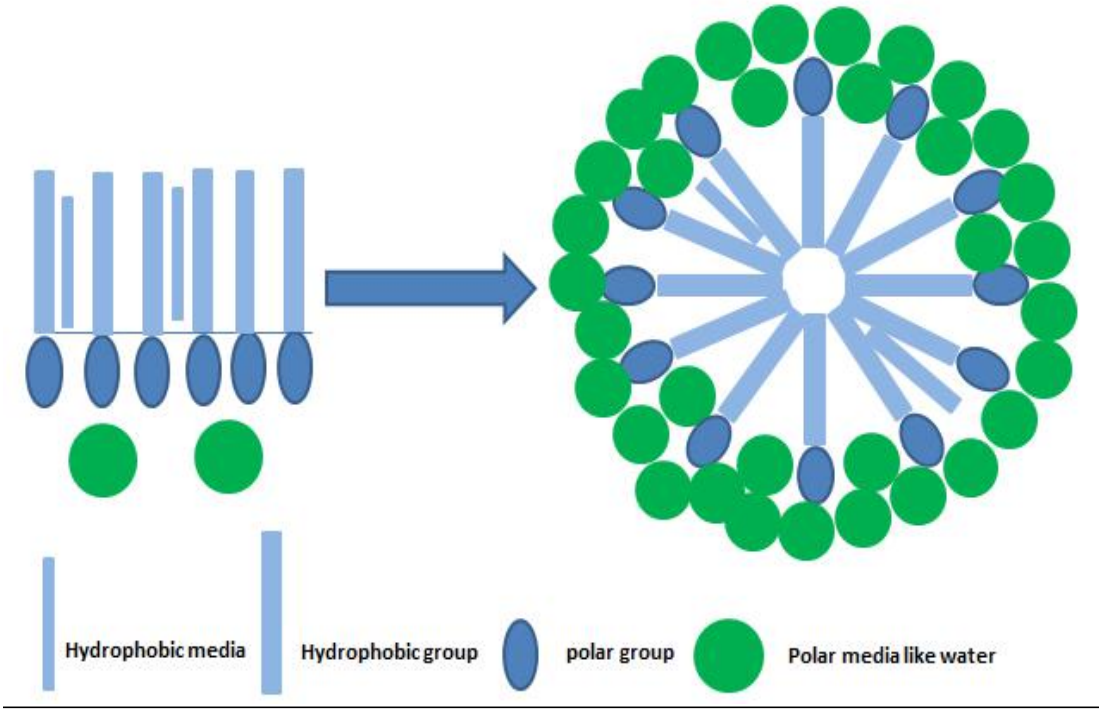


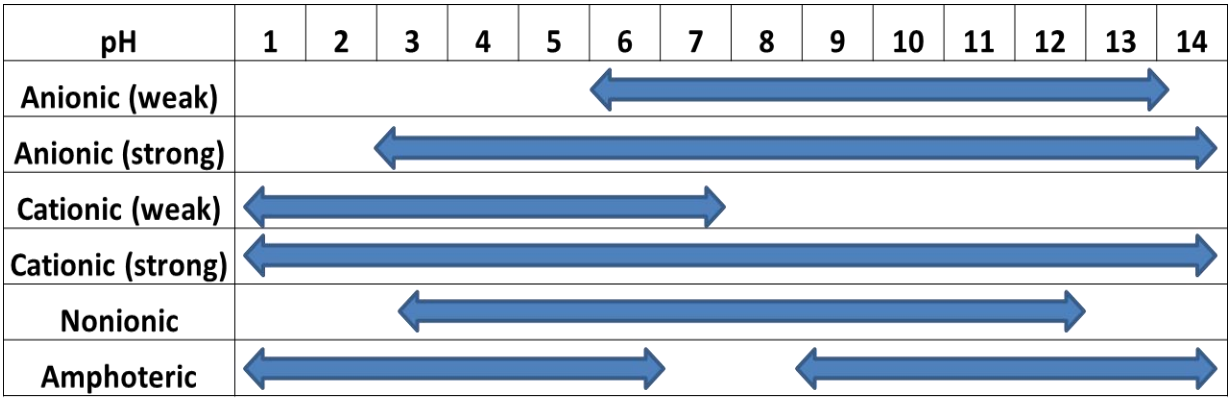
Figure 1. Cartoon illustrating attractions between like molecules, particularly between surfactant hydrophobic groups in oil and surfactant hydrophilic groups in water.

Surfactants can also be classified depending on the solubility, for example hydrophilic surfactants are soluble in water while hydrophobic (lipophilic) surfactants are soluble in lipids (Ontiveros et al., 2014). Ionic surfactants are generally hydrophilic, nonionic surfactants can be either hydrophilic or lipophilic, depending on the balance between the hydrophilic and lipophilic groups. In non-polar media the forces of attraction between molecules are primarily London dispersion type of Van der Waals interaction, which occur even among inert gas atoms. Surfactant lipophiles can be dispersed in non-polar or low-polar media because the nature of intermolecular bonding is very similar to the bonding between the lipophiles themselves. Solubility in polar media, especially water, simply depends on the hydrogen-bonding between surfactant polar heads and water molecules. The surfactants could be either ionic or nonionic but nonionic groups are

much less effective in terms of solubility, for example, ten ethylene oxide units will be needed to give similar water-solubility to that of an ionic group. (Eric Lomax 1996)

Strong ionic groups can be fully dissociated while the dissociation of weak ionic groups is more pH dependent. For example, weak anionic groups such as carboxylates are only dissociated and solubilized in the alkaline pH range. Weak cationic groups containing primary, secondary and tertiary nitrogen are only solubilized in the acidic pH range. Quaternary ammonium groups and sulfates solubilize over almost the whole pH range. Nonionics do not require counter-ions, so they will also be solubilized over a large pH range. The solubility is due to hydrogen-bonding between the hydrophiles and water molecules. Amphoterics have both anionic and cationic groups, and will be soluble over a wide pH range, even broader than nonionics. At high and low pH regions, especially with a high electrolyte concentrations, nonionic surfactants lose their solubility because electrolyte competes with the ethoxylate chain for hydrogen-bonding with water. (Eric Lomax 1996). Table 1 provides a brief solubility summary for the various types of surfactants.

Table 1. Typical solubility ranges of different types of surfactants

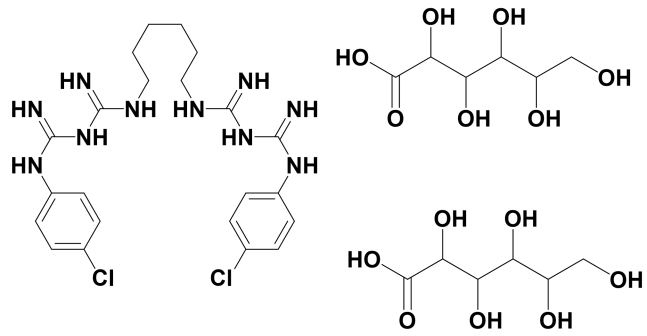


1.2. Cationic surfactants with strong antimicrobial activities

Most surfactants, especially with positive charges, have antimicrobial activities because of their ability to interact with cell membranes. It is suggested that interaction with bacteria occurs by the disruption of membrane, starting from leakage of cytoplasmic material and ultimately the collapse of the intra-cellular equilibrium (Hurdle et al., 2011). Quaternary ammonium compounds are considered low-level disinfectants, as defined by the US Centers for Disease Control and Prevention. They are effective against most vegetative bacteria and enveloped viruses, and some fungi (Somasundaran et al., 2007). They are extensively used in a number of personal care and domestic products such as shampoo, body wash and dish soaps and other industrial applications like cleaning agents and lubricants.

Chlorhexidine (CHX) is a widely used disinfectant of the skin and hands, in the cosmetics industry as additive to creams, toothpaste, deodorants, and antiperspirants. In pharmaceutical industry, CHX is used as preservatives and antiseptics. A review paper from a group of Italian researchers stated that CHX shows a prolonged activity and would

be influenced by the presence of body fluids (Privitera et al. 2017). This gives significant advantages in several surgical procedures. At the same time, no adverse effects were found to be related to the use of chlorhexidine gluconate (CHG), the structure of which is shown in Figure 2. Wang's group carried out experiments in central venous catheter (CVC) disinfection reduction. By comparing the maintenance of CVCs using CHX and povidone, they found CHX aqueous solution greatly reduce the rates of catheter-related bloodstream infection and catheter colonization in comparison to povidone solutions (Shi et al., 2019). Also, the disinfection effect of CHX-alcohol solution is better than that of other solutions. Other evidence show that CHX rinsing reduces plaque accumulation and gingival inflammation after periodontal and implant surgery. It could be used as a valuable chemo-preventive tool when self-performed oral hygiene is compromised (Arora et al., 2014).

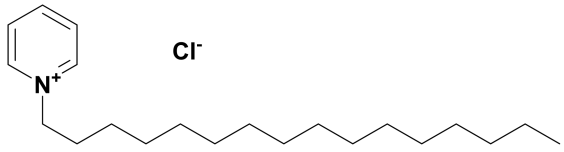


Chlorhexidine gluconate

Figure 2. Chemical structure of chlorhexidine and gluconate at 1:2 ratio

Cetylpyridinium chloride (CPC) is a quaternary ammonium compound, the structure of that depicted in Figure 3. CPC is an active ingredient at 0.01–1% (w/w) of many personal care products such as antiperspirant deodorants, oral hygiene products and skin lotions and surface-disinfecting agents in poultry processing facilities. Many papers have been

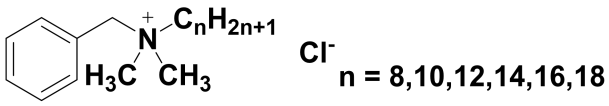
published on the efficacy of CPC mouth rinses. Teng et al has shown that a CPC-containing mouth rinse, when used as the only oral hygiene regimen, provides a significant benefit in reducing gingival inflammation by disturbing the succession of dental plaque maturation and balancing the diversity and composition of the oral microbiota (Teng et al., 2016). Researchers in Andrew McBain's group found that mouth rinse containing CPC gives significant antibacterial efficacy against oral bacteria in planktonic and biofilm modes at various concentrations. And they suggest to combine the use of CPC with sodium fluoride together to control oral bacteria and protect tooth enamel (Latimer et al., 2015).



Cetylpyridinium chloride

Figure 3. Chemical structure of cetylpyridinium chloride (CPC).

Benzalkonium chloride (BKC) is among one of the most common and widely used preservatives in pharmaceutical formulations for the eyes, ears and nose, belonging to the families of cationic surfactants and detergent preservatives. The structure is shown in Figure 4. According to the report of Cosmetic Ingredient Review Expert Panel, BKC was utilized in 83 cosmetic products at a concentration of 0.1%–5% in 1986 and its use increased to 567 cosmeceuticals (0.46% of total cosmetic products) at concentrations ranging from 0.01%–0.5% in 2013 (Halla et al., 2018). It is also used as an antiseptic and disinfectant at higher concentrations. Nonetheless, the use of BKC has been associated with inducing some adverse effects in humans (Baudouin et al., 2008 and Martone et al., 2009).



Benzalkonium chloride

Figure 4. Chemical structure of a typical benzalkonium chloride (BKC) with possible numbers of carbon chain.

1.3. Applications of mixtures of cationic and anionic surfactants

In 1987, Jokela, Jönsson and Khan defined a catanionic surfactant as an equimolar mixture of two oppositely charged surfactants (the parent surfactants) from which the inorganic counterions are completely removed (Jokela et al., 1987, Khan and Marques, 1997). However, over the years, catanionic system sometimes include the mixture containing equal mole of cationic and anionic surfactants with the presence of inorganic counterions. Ion pair amphiphiles (IPA) was defined to specify the catanionic mixtures without counterions. Catanionic surfactants have been under extensive investigations recently because of their bio-efficacy.

Mixtures of anionic and cationic surfactants spontaneously form various microstructures such as micelles, vesicles, lamellae, columnar, and cubic mesophases. The detailed microstructures were dependent on many factors such as the shape of the surfactant molecules, the strength of intra- and intermolecular interactions including electrostatic interactions, hydrophobic associations, hydrogen bonding, and the relative fractions of different groups within the surfactant molecules. When cationic and anionic surfactant solutions are mixed, the strong reduction in area per head group resulting from ion pairing induces formation of molecular bilayers at low concentrations. At the right mixing ratios, thermodynamically stable species vesicles may be established

spontaneously (Norvaisas et al., 2012). The cationic-anionic surfactant systems may produce a precipitate when the stoichiometry between the cationic and anionic surfactants is exactly 1.

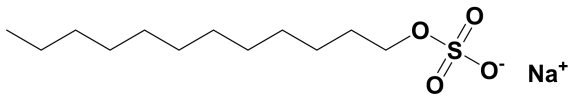
In general, cationic surfactants can't be used together with anionic surfactants due to precipitation caused by the strong electronic interactions between cationic and anionic head groups. However, this precipitation phenomenon did not stop the cationic active ingredients like CPC, CHX and BKC from being widely used with anionic surfactants in consumer products such as toothpaste and shower gel together. For example, hydrophobic counterions are introduced to control micellar growth, by changing the degree of hydrophobicity and the geometry of the counterions. Special attention has been given to surfactant systems that form vesicles, which have been found to be useful agents in many practical applications and also serve as a model for several theoretical investigations (Abdel-Rahem, 2008).

In order to characterize numerous surfactant materials and to properly monitor the commercial products on the market, the development of a more rapid and efficient analysis method is needed. Because many surfactants have alkyl homologues, lack of chromophores and thermal instability, surfactants are often difficult in commercial products. Determination have been reported by different experimental tools in the literature, like titration, FTIR, ion chromatography (IC), gas chromatography (GC), capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC). Researchers from Korea showed that it is possible to simultaneously analyze cationic,

anionic, amphoteric and nonionic mixtures in shampoo and hair conditioner by RP-HPLC/ ELSD and LC-MS (Im et al., 2008). A special emphasis was given to the mass spectrometry elucidation approaches used to identify cocamidopropyl amidoamines, a major class of hydraulic fracturing compounds and also a surfactant. (Ferrer and Thurman 2015).

More efforts have been focused on the 1:1 system with cationic and anionic surfactants mixtures with the stoichiometry close to 1 (catanionic system) in this thesis. Common inorganic counterions such as mono-, di- and tri- phosphates were selected to check the impacts on catanionic systems. FTIR, LC-MS, NMR and X-ray diffraction methods were applied to characterize the targeted surfactant systems. LC-MS is a good characterization tool for 1:1 catanionic system. The findings will be applied to characterize products containing the mixture of cationic and anionic surfactants in the future.

In addition, studies of surfactants crystalline structure have been conducted for both cationic and anionic molecules, respectively (Hirata and Iimura, 1998; Paradies and Clancy, 2000). The counterions were usually inorganic ions. In the study, chlorhexidine as an organic counterion, crystallized with dodecyl sulfate (Figure 5) was a first finding for surfactant crystalline structure.



Sodium dodecyl sulfate

Figure 5. Chemical structure of sodium dodecyl sulfate (SDS).

2. Crystallization of chlorhexidine with its surfactant counterions

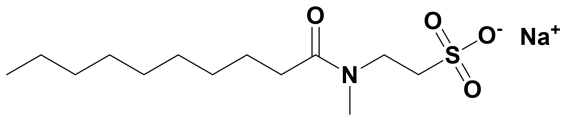
2.1 Introduction

Chlorhexidine (CHX) is a prescription drug. One CHX molecule usually comes with two gluconate molecules in an oral rinse, which is used to treat gingivitis including swelling, redness and bleeding gums. Driven by customer needs, CHX has been used as an active ingredient in toothpaste on the market now. A key aspect for toothpaste is its foaming capability, sodium dodecyl sulfate (SDS) is the most popular surfactant used in toothpaste products today.

The literature for CHX and SDS has been reviewed at the beginning of this study but no chemical structural information on their complex was found. Therefore, the experiment was designed to study CHX-DS complex at 1:2 ratio based on charge status. In the beginning, we examined this complex solubility before further structural characterization. The complex solubility in water is very low but it is significantly improved in glycerin media. Glycerin is a high viscous poly-alcohol that's commonly used as toothpaste and mouth rinse media. However, glycerin is not a good crystallization solvent. Instead, we used methanol-water mixture to study the complex crystallization.

In addition, salts containing phosphates are very common ingredients used in toothpaste and the negatively charged phosphate groups can interact with CHX as counterions as well. We checked not only the crystallization between CHX and sodium monophosphate (SMP) but also the mixture of CHX, SDS with tetrasodium pyrophosphate (TSPP) and sodium tripolyphosphate (STPP).

Even if SDS has been widely used in consumer cleaning products, there is a concern about SDS to cause irritation to the eyes and the skin (de Jongh et al., 2007). Compared to SDS, sodium lauroyl methyl taurate (SLMT) is a much milder, non-irritating, anionic surfactant from the natural amino acids family (Figure 6). It can be used as a substitute of SDS as a foaming agent. At the same time, we also studied the interaction of SLMT with CHX.



Sodium lauroyl methyl taurate

Figure 6. Chemical structure of sodium lauroyl methyl taurate (SLMT).

2.2. Experimental procedures

2.2.1 Materials

Table 2. List of chemicals and suppliers

Name	Supplier
CHX	Sigma-Aldrich
CHG	Colgate Inventory
SDS	Sigma-Aldrich
SLMT	Jarchem Industries
Phosphoric acid	J. T. Baker
Methanol	Fisher Scientific
CD_3OD	Cambridge Isotope Laboratories

$(CD_3)_2SO$	Cambridge Isotope Laboratories
SMP	Sigma-Aldrich
TSPP	Colgate Inventory
STPP	Colgate Inventory

2.2.2 Chlorhexidine-dodecyl sulfate complex crystallization at room temperature

A total of 0.07 mg of CHX and 0.08 mg of SDS was mixed with 5 mL methanol in a 20 mL scintillation vial. The mixture was put into an 80 °C oven for 15min to completely dissolve the solids, then it was left at room temperature. After 6 hours, needle like crystals grew in the solution. On the following day, the crystals were separated from solution by a filter paper. The crystals were dried in air for further experiments. An FTIR spectrum of the crystal was obtained from Perkin Elmer Spectrum 2000 FTIR spectrometer after 16 scans over the range of 550 to 4000 cm^{-1}. The crystals were dissolved in methanol-d_4 and analyzed by ^{1}H-NMR. ^{1}H-NMR was conducted using Bruker spectrometer operated at the proton frequency of 500.13 MHz equipped with a double resonance cryoprobe. The sample temperature was kept at 25°C. The spectrum was collected using a single pulse with a 30° pulse angle, 1 second acquisition time, 5 second recycle delay, and a sweep width of 12 ppm. The number of scans was 32, it required about 10 min for each spectral collection.

2.2.3. Crystallization of chlorhexidine-dodecyl sulfate complex at elevated temperature

The sample was prepared by mixing 2g of 20% CHG plus 0.257g of SDS in a 20 mL scintillation vial. And then the sample was transferred to a 60 °C oven and was kept there for three weeks. After that, the aged solution was taken out and diluted by methanol at 1:4 ratio in room temperature. The cloudy solution was then filtered by a 0.45 μm PTFE filter. Crystal grew out of the clear solution overnight. As small amount of crystals were taken out of the solution and dried on a piece of filtration paper. The FTIR spectrum of the crystal was obtained from Perkin Elmer Spectrum 2000 spectrometer after 4 times scanning over the range of 550 to 4000 cm^{-1}. To further confirm the complex formation, the crystals were fully dissolved in DMSO-d_6 for ^{1}H-NMR analysis because the solubility was much better compared to methanol-d_4.

Crystals of CHX-DS suitable for X-ray crystallography were isolated under a polarity microscope. The X-ray diffraction data were collected using Bruker single crystal instrument D8 VENTURE PHOTON 100 CMOS INCOATEC ImuS system equipped with a Cu Kα (λ = 1.54178 Å) micro-focus source. X-ray diffraction data were collected at 100 K. Crystallographic indexing was performed using APEX3 (Version 2015.9) (Difference Vectors method). Data integration and reduction were performed using SaintPlus 6.01 (Bruker 2016). Absorption correction was performed by multi-scan method implemented in SADABS (Sheldrick, G.M., 2015). The space group was determined using XPREP implemented in APEX3 (Version 2015.9). The structure was solved using direct methods in SHELXT and was refined using SHELXL-2017 (Sheldrick 2008) (Sheldrick 2015) (full-matrix least-squares on F2) through OLEX2

interface program (Dolomanov et al., 2009). All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were placed in geometrically calculated positions and were included in the refinement process using riding model.

2.2.4 Crystallization chlorhexidine-monophosphate complex

70 mg of CHX powder was dissolved in 10 mL methanol to get a clear solution. 86% phosphoric acid was diluted 10 times by deionized water. A total of 23.69 mg 10% diluted phosphoric acid was mixed with the CHX solution based on a 3:2 charging ratio. The FTIR spectrum of the crystal was obtained from Perkin Elmer Spectrum 2000 spectrometer with 4 scans over the range of 550 to 4000 cm^{-1}.

2.2.5 Chlorhexidine-sodium lauroyl methyl taurate complex

Chemicals include 2 g of 20% CHG plus 0.306 g of SLMT were mixed in a 20 mL scintillation vial. Then the sample was transferred to a 60 °C oven and was kept there for three weeks. After that, the aged solution was taken out of the oven and diluted by methanol at 1:4 ratio at room temperature. The precipitate was then filtered by a 0.45 μm PTFE filter. No crystal grew out of the solution over time. The MS spectrum was obtained from a Thermo Q Exactive hybrid Quadrupole-Orbitrap mass spectrometer. The filtered solution was injected to a divert valve and was delivered into the MS detector with the mobile phase containing 50% methanol-water solvent. The MS detector was set in the positive mode for a full scan in the range of 80.0 to 1200.0 m/z. The resolution was set to 35,000. With sheath gas at a speed of 45, aux gas 15 and sweep gas glow at 10, the

spray voltage is 3.30 kV and the capillary temperature is 275 °C. Other parameters including S-lens RF level is at 50.0 and the aux gas heater temperature was at 300 °C.

2.3. Results and Discussion

2.3.1. Temperature impact on crystallization of chlorhexidine-dodecyl sulfate complex

The crystallization of CHX-DS complex with methanol-water solvent started at room temperature. CHX and SDS were separately dissolved in methanol with the calculated amount for the mixing. After mixing CHX and SDS at 1:2 molar ratio, the solution was heated for a period of 15 min at 80 °C and it became clear. Four hours later, a cluster of needle-shaped of crystals were obtained after solvent was removed by filtration. The crystal was analyzed with FTIR and the results are shown in Figure 7.

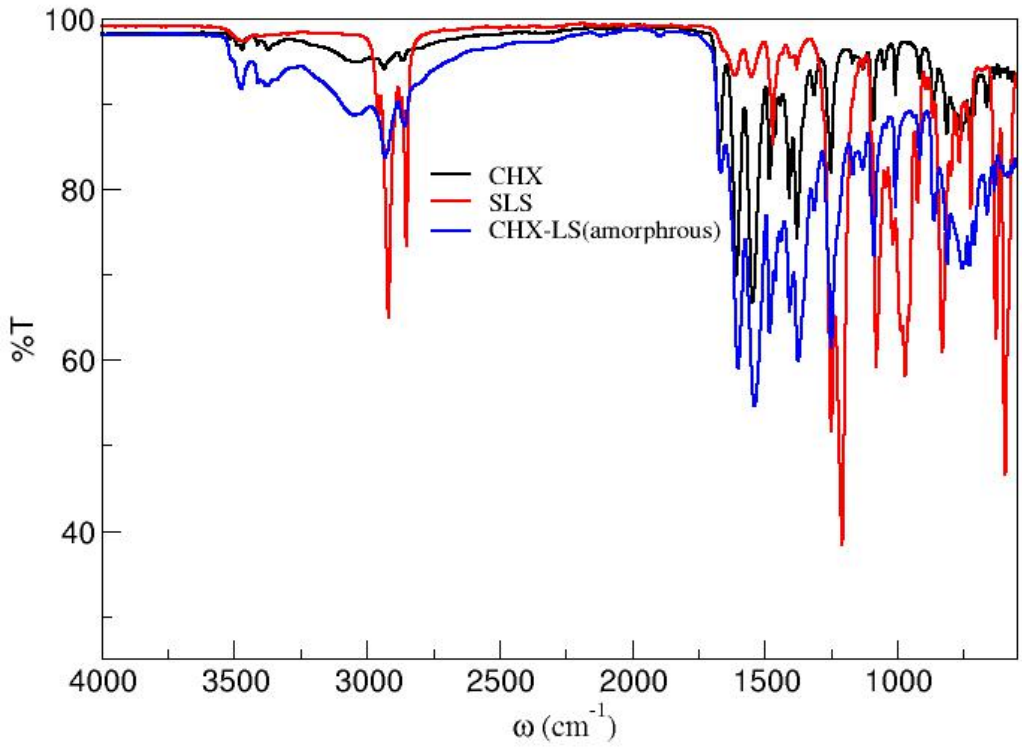


Figure 7. FTIR spectrum of chlorhexidine-dodecyl sulfate adduct

The FTIR of the complex in blue is more similar to the starting material CHX instead of SDS. To further check if a complex was formed, the crystal was dissolved in methanol-d_4 and analyzed by NMR (Figure 8). The broad multiple peaks at 1.4 ppm and 1.5 ppm with the integration 4.05 and 4.12 are corresponding to H-3, 4 and H-2, 5 proton signals from CHX. The triplet peaks at 3.1 ppm represent H-1 and H-6 with two proton signals each. Two doublet peaks at 6.9 ppm and 7.2 ppm represent the benzene ring protons with the two protons closer to chlorine have a lower chemical shield. The solvent peaks were cut from the spectrum for simplicity. Most importantly, no dodecyl chain proton signal in the ^{1}H NMR was detected. The result clearly indicates that no dodecyl sulfate was present and the crystal is composed of only CHX. This in turn clarifies the FTIR results.

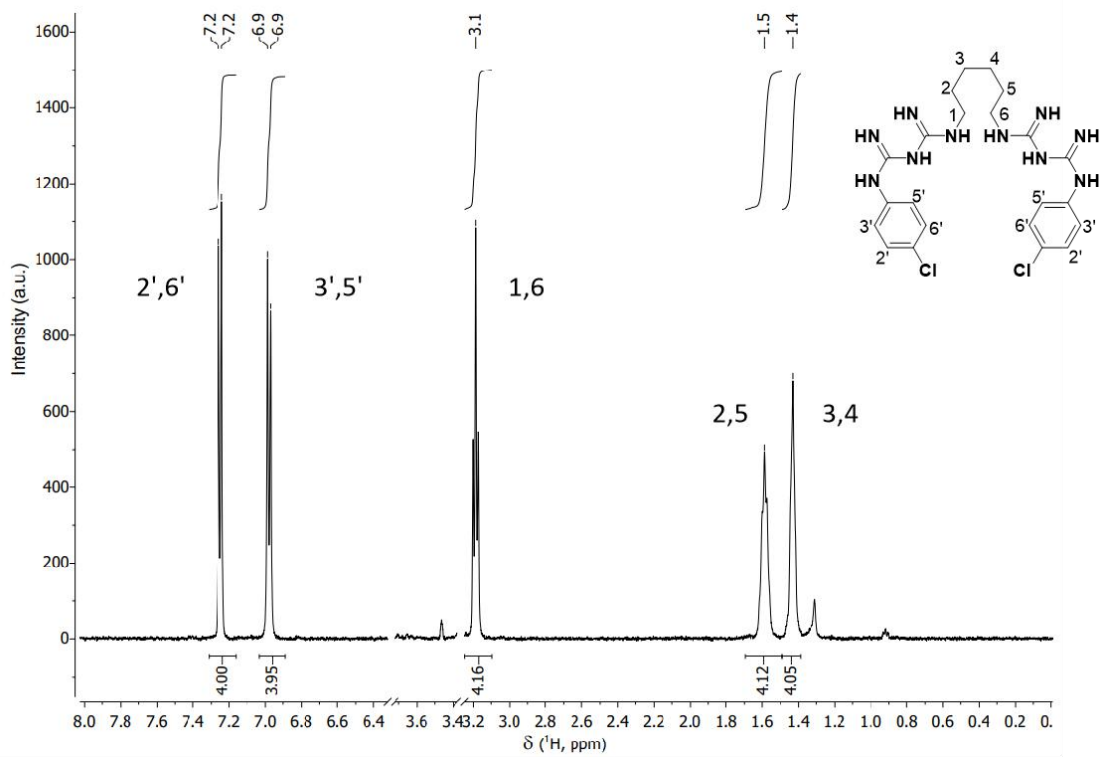


Figure 8. ^{1}H-NMR spectrum of chlorhexidine-dodecyl sulfate in methanol-d_4 solvent.

Based on the reference description (Patist et al., 2001), when the cationic and anionic surfactants were mixed together at the right ratio, a thermodynamically stable system could be obtained. So we mixed CHG with SDS in aqueous solution at 1:2 molar ratio and heated to 60°C for three weeks, which is a common aging study condition. After that, the cloudy solution was filtered with a 0.45 μM PTFE membrane filter. A crystal cluster grew out of the solution overnight. The crystal was then separated from solvent by filter paper.

The crystal FTIR spectrum is depicted in Figure 9, which is different from the previous one obtained at room temperature. The peaks from 950 to 1200 cm^{-1} in blue clearly indicated the presence of dodecyl sulfate. And the peaks from 1400 to 1600 cm^{-1} provided evidence for the existence of CHX, when comparing against CHX standard in black and SDS standard in red. The detailed peak assignments according to literature were as follows. The two peaks at 2917.5 cm^{-1} and 2850.0 cm^{-1} correspond to the C-H stretching. While the adsorption at 1467.8 cm^{-1} is because of the bending of CH_2 (Cabrera, Balbin et al. 2017). The strong doublet at 1248.1 cm^{-1} and 1206.9 cm^{-1} correspond to the asymmetric S-O stretching. In addition, the peaks at 1078.2 cm^{-1} and 969.3 cm^{-1} result from symmetric S-O stretching (Nguyen, Phan et al. 2016). The FTIR spectrum for CHX shows that the peak at 2933.3 cm^{-1} was assigned as the asymmetric and symmetric C-H stretching. The C=N stretching vibration of imine group is at 1672.3 cm^{-1} (Holesova, Valaskova et al. 2014). The band at 1605.6 cm^{-1} corresponded to N-H stretching of CHX. The bands that occured at 1600-1300 cm^{-1} could be explained due to the N-H bending of secondary amine and imine groups. Four bands at 1249.4cm-1, 1087.8 cm^{-1}, 866.4 cm^{-1} and 756.9 cm^{-1} belong to the C-N stretching vibration of secondary aromatic amines, C-

Cl stretching vibration of aromatic halogen compounds, C-H out-of plane deformation vibration of aromatic ring and C-H rocking deformation vibration of methylene group.

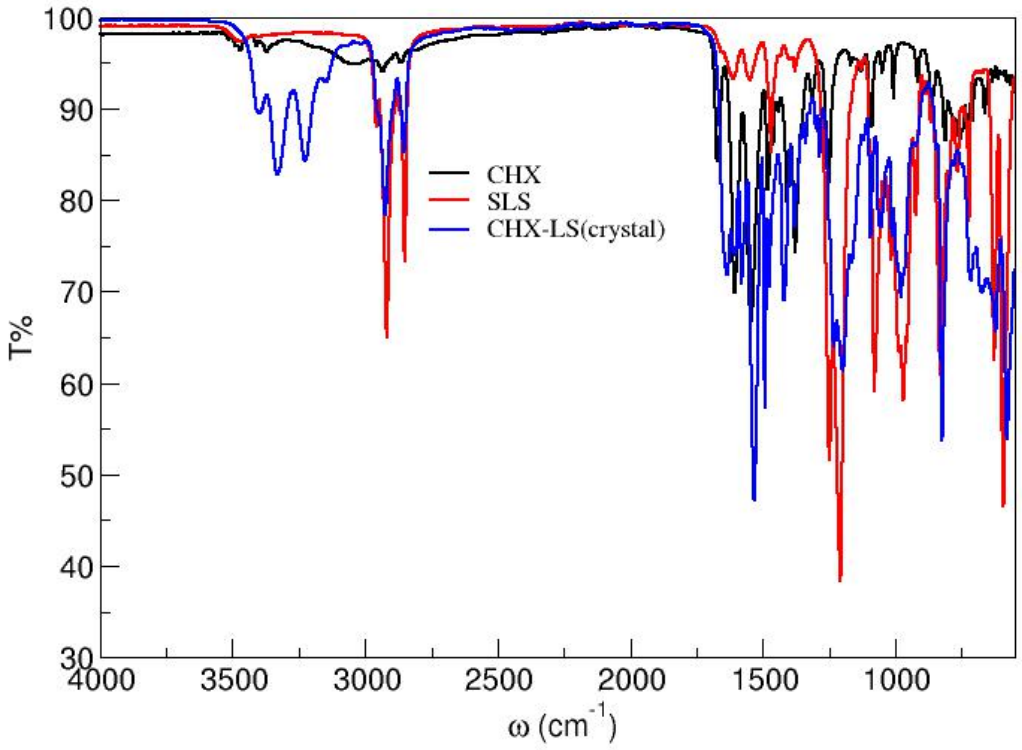


Figure 9. FTIR spectrum of crystal obtained from chlorhexidine-gluconate and sodium dodecyl sulfate after aging.

To confirm the result from FTIR of CHX-DS crystal, ^{1}H-NMR spectroscopy was conducted, as shown in Figure 10. The triplet peaks at 0.8 ppm with an integration value of 6.00 refer to the methyl group of SDS. The multiple peak at 1.2 ppm with an integration value of 40.28 account for the two hydrogen atoms on the carbons of the alkyl chain and the triplet peak at 3.6 ppm with an integration value of 4.00 correspond to H-2,3,4,5 on the middle part of CHX. The ^{1}H integration between DS and CHX molecules

indicated the molar ratio is 2:1 (2 moles of DS and 1 mole of CHX), which balanced the total charge of CHX and DS to zero. NMR result is consistent with that of FTIR.

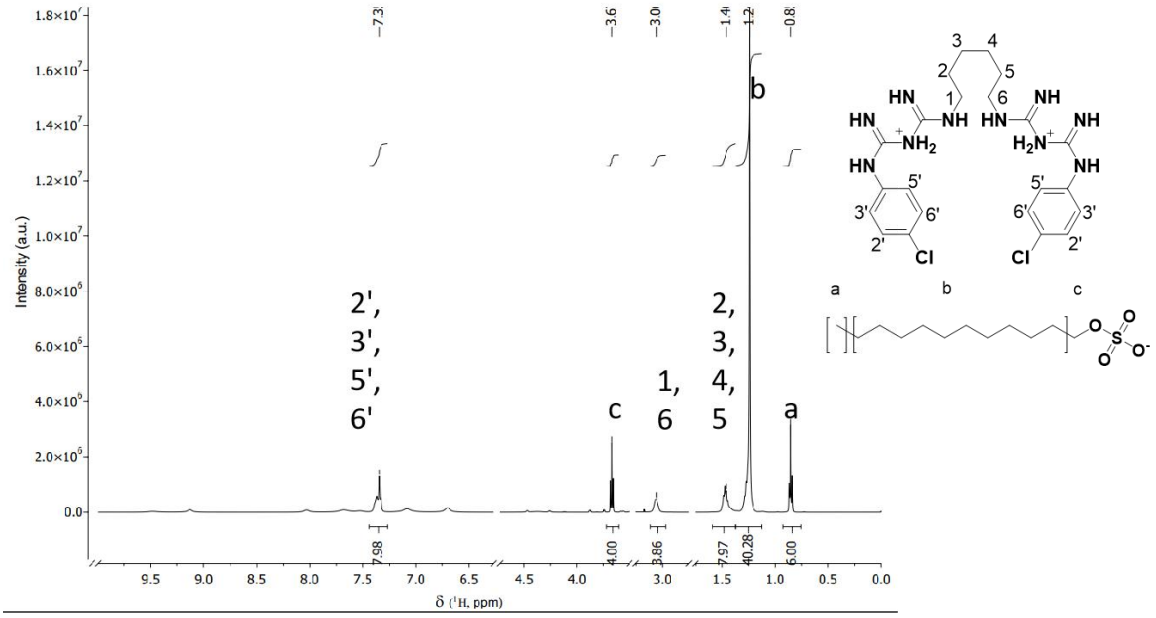


Figure 10. ^{1}H-NMR spectrum of chlorhexidine-dodecyl sulfate crystal obtained after aging.

To further characterize the crystal structure in more detail, an X-ray diffraction experiment was performed by our collaborators at the University of South Florida. The result is shown in Figure 11.

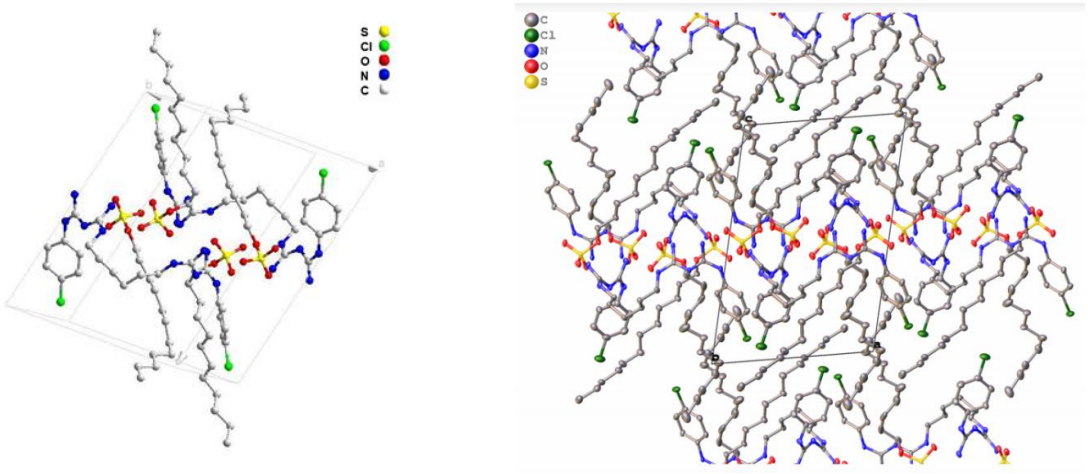


Figure 11. Chlorhexidine-dodecyl sulfate crystal structure from X-ray diffraction.

From the structure in Figure 11, it is consistent that each CHX molecule is holding two DS molecules (see left) and no other counterions such as sodium cation or gluconate anion from the original solution were involved in the crystal structure. In addition, polar guanidine functional groups from CHX and sulfate groups from DS stay together. The polar areas are separated by non-polar CH_2 chains in DS and benzene rings in CHX. The result further reinforced FTIR and ^{1}H-NMR results.

2.3.2. Phosphate groups impact on chlorhexidine-dodecyl sulfate complex

Phosphates, including monophosphate, diphosphate and triphosphate are often used in toothpaste for both pH adjustment and benefits to teeth. Negatively charged phosphate groups could be actively integrated with CHX polar head due to the cationic and anionic affinity. The mixture of CHG-SDS in a simple mouth rinse formula is cloudy. When phosphates such as sodium tripolyphosphate (STPP) were added into this mixture, the

solution turned clear. This phenomenon can be clearly seen in Figure 12.

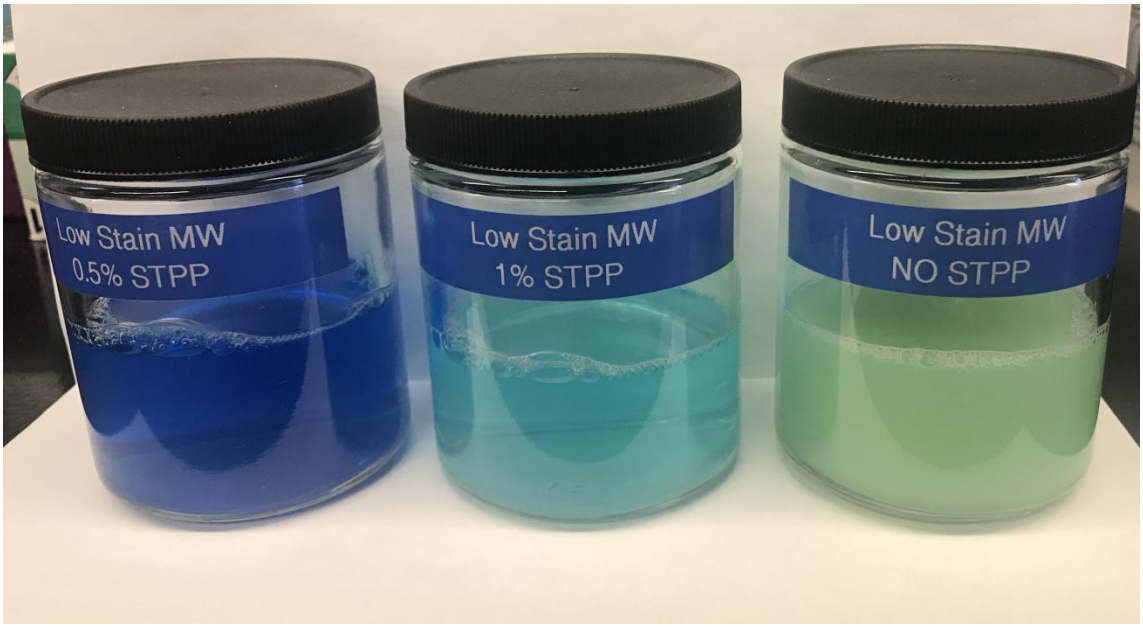


Figure 12. Mouth-rinse solutions using chlorhexidine gluconate as active ingredient and sodium dodecyl sulfate for foaming agent with and without tripolyphosphate at 0.5 or 1% wt concentration.

To better understand this phenomenon, equal amounts of CHG-SDS solutions were mixed with Na_3PO_4 (SMP), $Na_4P_2O_7$ (TSPP) and $Na_5P_3O_{10}$ (STPP) respectively and analyzed by LC-MS. Due to chlorine's isotopic pattern caused by the molecular weights at 35 and 37, CHX and its complex peaks can be very easily identified in the mass spectrum. In the mixture, even if it formed precipitate but soluble complex peaks with one CHX and one DS can be found at the mass cluster of 771-776 Daltons in positive mode (Figure 13). When phosphates were present, those complex cluster peaks intensities show very significant decrease, especially with TSPP and STPP.

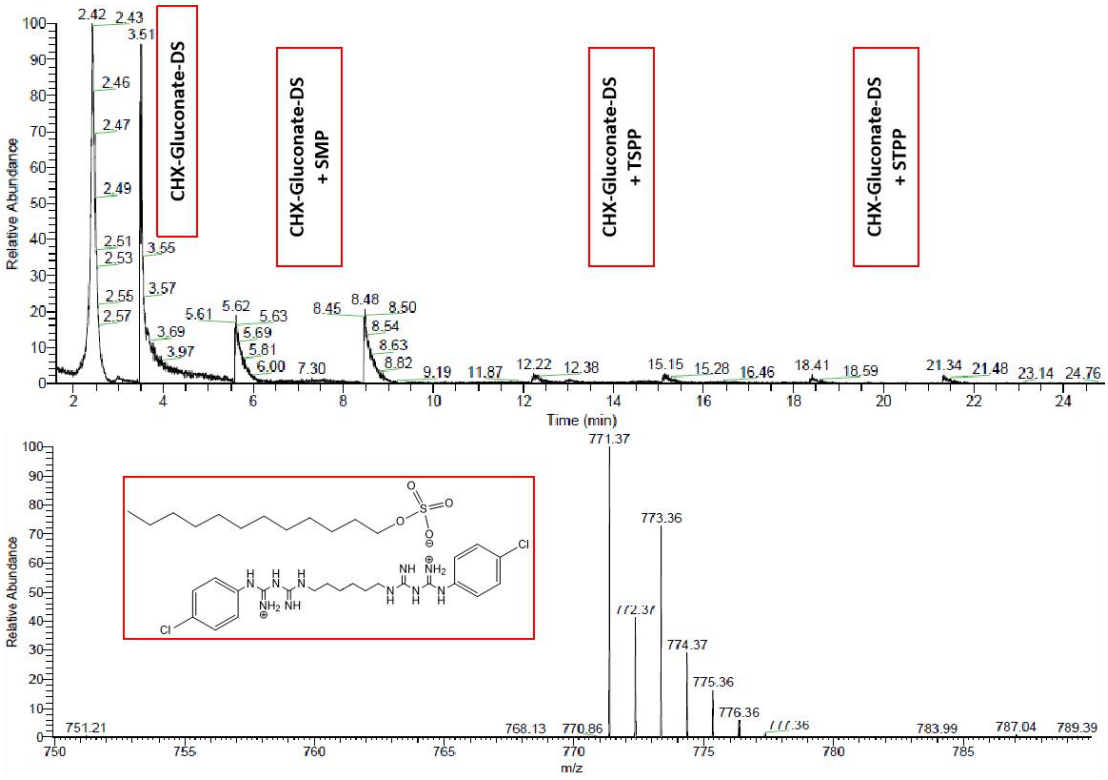


Figure 13. MS chromatogram and spectra of the mixture of chlorhexidine-dodecyl sulfate with sodium monophosphate, tetra-sodium polyphosphate and tripolyphosphate.

Crystallization of CHX with phosphate salts were tried out but only CHX-monophosphate crystal was obtained, the FTIR of which is shown in Figure 14. The peaks from 950 to 1150 cm^{-1} in blue clearly indicated the presence of phosphate group and the peaks from 1250 to 1750 cm^{-1} confirmed the existence of CHX, especially when comparing it with CHX standard in black and SMP standard in red.

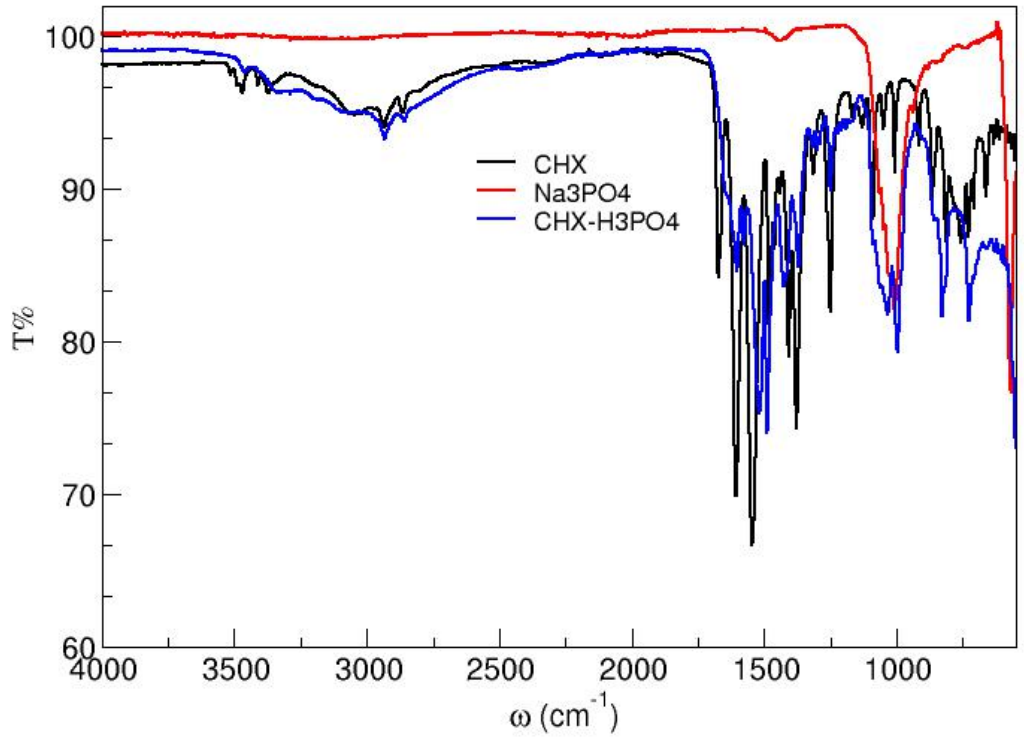


Figure 14. FTIR spectrum of chlorhexidine-phosphoric acid crystal.

The crystal was sent to collaborators at the University of South Florida for X-ray diffraction experiment. Due to the crystal instability, the complete crystal structure has not been successfully solved at this moment.

2.3.3. Substitution of sodium dodecyl sulfate by sodium lauroyl methyl taurate

SLMT is another surfactant in consumer products, the interaction between CHX and lauroyl methyl taurate (LMT) was examined by mass spectrometry as well. According to the degree of transparency of the solution, the complex solubility was significantly better than that of CHX-DS. Again, CHX-LMT complex in solution was mixed with three different phosphate salts, respectively. The complex cluster signals from 826 to 832 Daltons containing one CHX and one LMT was significantly suppressed due to the

presence of phosphates, as shown in Figure 15. The results are very similar to that of CHX-DS complex. It is clear that phosphate groups can interfere the complex formation between positive CHX and negative LMT.

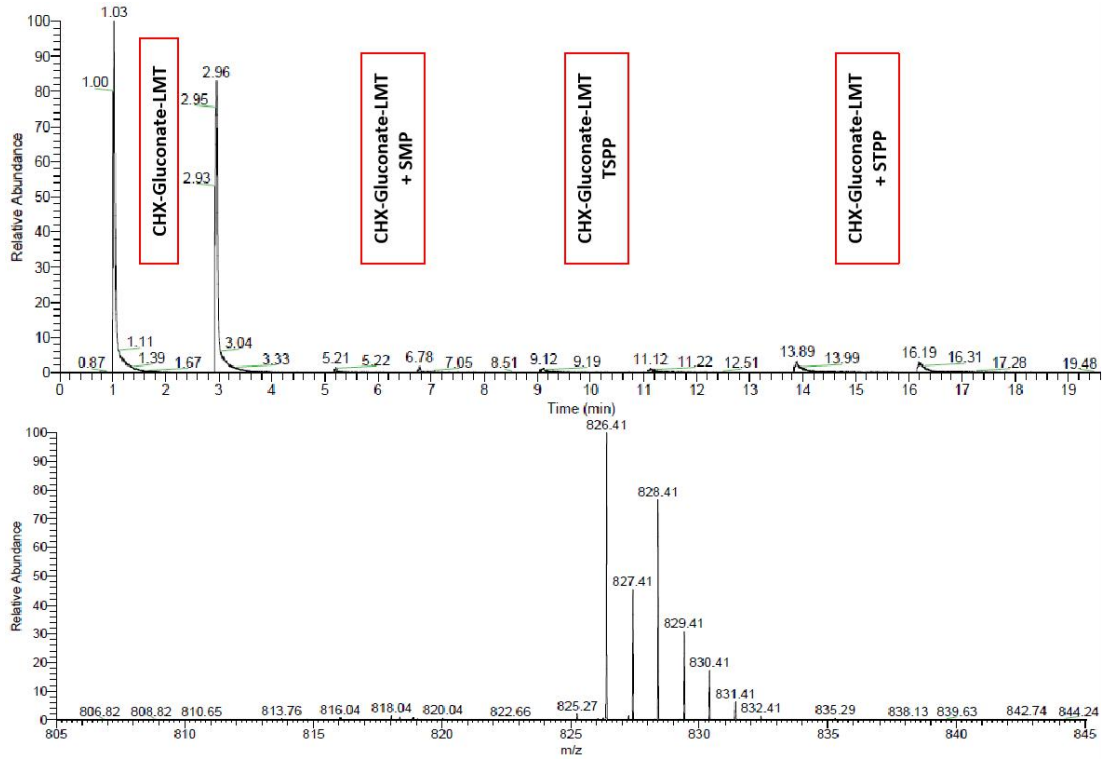


Figure 15. MS spectrum of the mixture of chlorhexidine gluconate- sodium lauroyl methyl taurate with sodium monophosphate, tetra-sodium polyphosphate and tripolyphosphate.

We also tried to grow the crystal for CHX-LMT under the same conditions as CHX-DS. However, no crystal was grown. The FTIR of solid obtained after evaporating the solvent is shown in Figure 16, where the blue spectrum is very similar to the black spectrum from CHX but no matching between red and blue peaks. In conclusion, CHX doesn't crystallize with SLMT under this experimental condition.

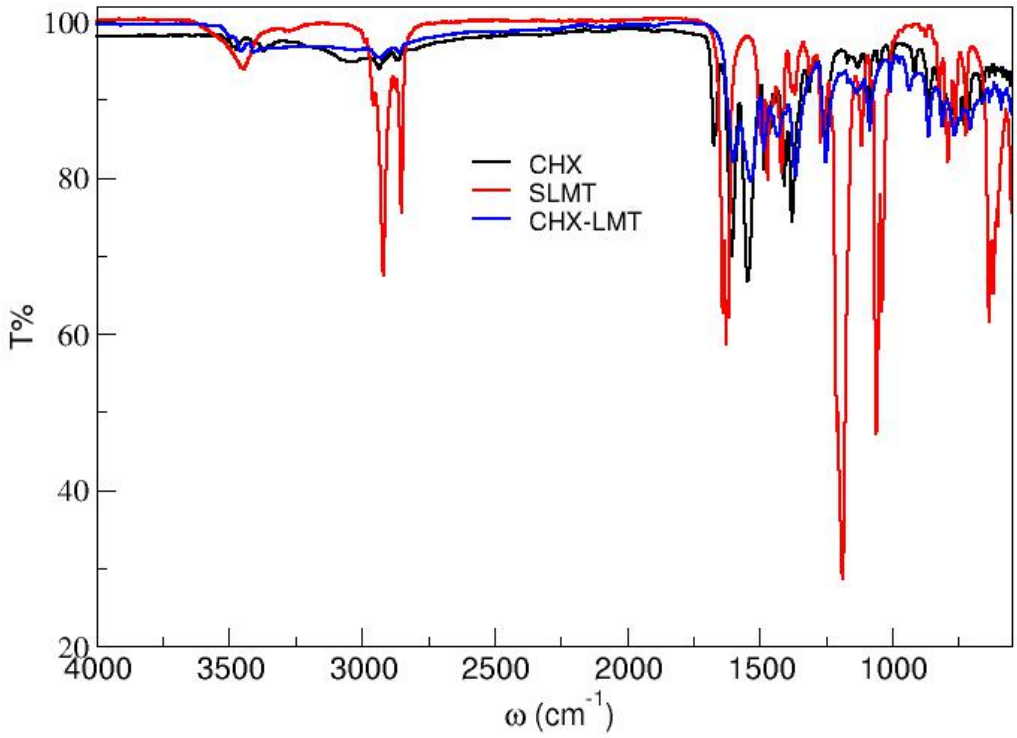


Figure 16. FTIR spectrum of crystal obtained from chlorhexidine-sodium lauroyl methyl taurate mixture.

3. LC-MS study of the ionic interactions between cetylpyridinium chloride and anionic surfactants

3.1. Introduction

Cetylpyridinium chloride (CPC) is a cationic surfactant used in consumer products including mouthwashes and toothpastes. CPC is not a prescription drug like CHX and it can be used in our daily life. CPC is an antiseptic that can kill a variety of microorganisms. More importantly, it has been shown to be effective in preventing dental plaques and reducing gingivitis. Though one study seems to indicate CPC does not cause brown teeth stains (Rahman et al., 2014), at least one mouthwash containing CPC as an active ingredient bears the warning label. In some cases, antimicrobial rinses may even cause surface staining to teeth (Wintonyk et al., 2012).

3.2. Experiments

3.2.1 Materials

Table 3. List of chemicals and suppliers for LCMS behavior study

Name	Supplier
CPC	Sigma-Aldrich
SDS	Sigma-Aldrich
BKC	Sigma-Aldrich
Methanol	Fisher Scientific

3.2.2 The sample preparation of CPC and SDS solution for MS analysis

Half a milliliter of 22% CPC was mixed with 0.5 mL 18 wt% SDS in 9 mL deionized water. White precipitate formed as soon as the two solutions were mixed. The precipitate was then filtered by a 0.45 μm PTFE filter and the supernatant was used for LC-MS analysis.

3.3. Results and discussion

In LC-MS experiments, the charging status is necessary at the electrospray ionization interface for ion transfer and detection. As a result, extra charged ions such as sodium ions or protons from the solution media are usually needed for neutral components when positive detection mode is used. The extra charged surfactant ions can act as an adduct for MS signals. This phenomenon occurred in both positive and negative detection modes. More details of the results will be discussed in the following section. For each system, both positive and negative modes were used to verify the structures. In addition, the spectrum simulation function in the Qual Browser of Thermo Scientific Xcalibur 4.1 program can generate mass spectrum according to the given chemical formula.

When CPC was mixed with SDS, one positively charged CP will complex with one negatively charged DS, but this cannot be detected in LC-MS because the net charge is zero. So one more DS act as an adduct to allow ion transfer and detection in MS. The top high resolution MS peak at 843.5951 in Figure 17 corresponds to the complex consisted of one positive CP and two negative DS. The middle mass spectra was simulated from one CP ($C_{21}H_{38}N$) with a C_{21} chain and two DS (2 * $C_{12}H_{25}SO_4$) each having a twelve carbon chain. The simulated mass spectra matches the high resolution MS obtained from

samples on top. The high resolution $R = \frac{M}{\Delta M} = \frac{834.5951}{(843.5951-834.5946)} = 1669K$. The bottom mass spectrum peaks were simulated from one CP with twenty-one carbons plus one DS with twelve carbons and another one DS with thirteen carbons. This simulated mass spectrum doesn't overlap with the one on top because the material used in this experiment is pure SDS purchased from Sigma and it contains no odd number C_{13} chain in it. In general, natural fatty compounds always have even-numbered carbon chain distribution. If odd number of carbon like C_{13} is present, the material should come from a synthetic resource. This combination of experimental with simulated spectrum can easily characterize the surfactant mixtures.

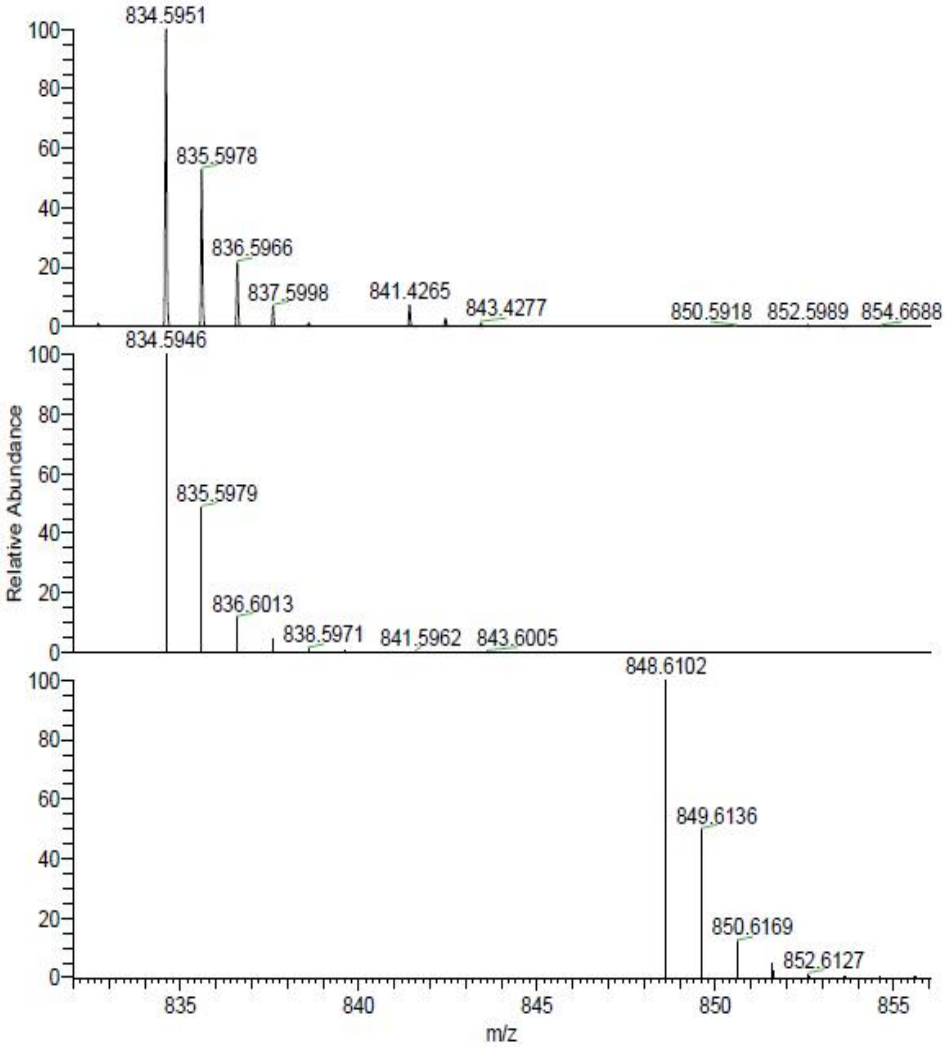


Figure 17. MS spectrum in negative mode of cetylpyridinium chloride-dodecyl sulfate complex (top). Simulated MS spectra given the molecular formula of $C_{45}H_{88}O_8NS_2$ (middle) and $C_{46}H_{90}O_8NS_2$ (bottom).

Figure 18 is the mass spectrum of CPC and SDS mixture in positive MS detection mode. Similar to the negative mode, extra charge is needed for detection. The top mass spectrum peak at 873.7408 corresponds to the complex consisted of two positive CP (2 * $C_{21}H_{38}N$) and one negative DS ($C_{12}H_{25}SO_4$). Within this complex, one cationic surfactant CP ion was combined with one anionic surfactant DS and another CP acted as an adduct

to give an extra positive charge in order to be detected in positive MS mode. The middle mass spectrum peaks were simulated from two CP with forty-two carbons and one DS with twelve carbons, which matches very well the sample high resolution MS on top. The resolution equals 126k. ($R = \frac{M}{\Delta M} = \frac{873.7477}{873.7477 - 873.7408} = 126{,}000$) The bottom mass spectra was simulated from one CP with twenty-one carbons ($C_{21}H_{38}N$) and one CP with twenty-two carbons ($C_{22}H40N$) plus one DS with twelve carbons ($C_{12}H_{25}SO_4$) or two CP with twenty-one carbons ($C_{21}H_{38}N$) plus one DS with thirteen carbons ($C_{13}H_{27}SO_4$). Both scenarios give the chemical formula of $C_{55}H_{103}O_4N_2S$. However, the resolution calculated from the simulated molecular weight and the actual molecular weight is merely 2,000 in this case. ($R = \frac{M}{\Delta M} = \frac{887.7633}{887.7633 - 887.3944} = 2{,}000$). It is much less than the lowest resolution 35,000 of a high resolution MS instrument, which means the peaks around 887.3944 are not from surfactant complexes. They could be impurities from unknown compounds. The MS data in Figure 18 not only confirmed that the sample of CPC and SDS does not contain odd number chain, but also demonstrated the power of high resolution MS for surfactant material identification.

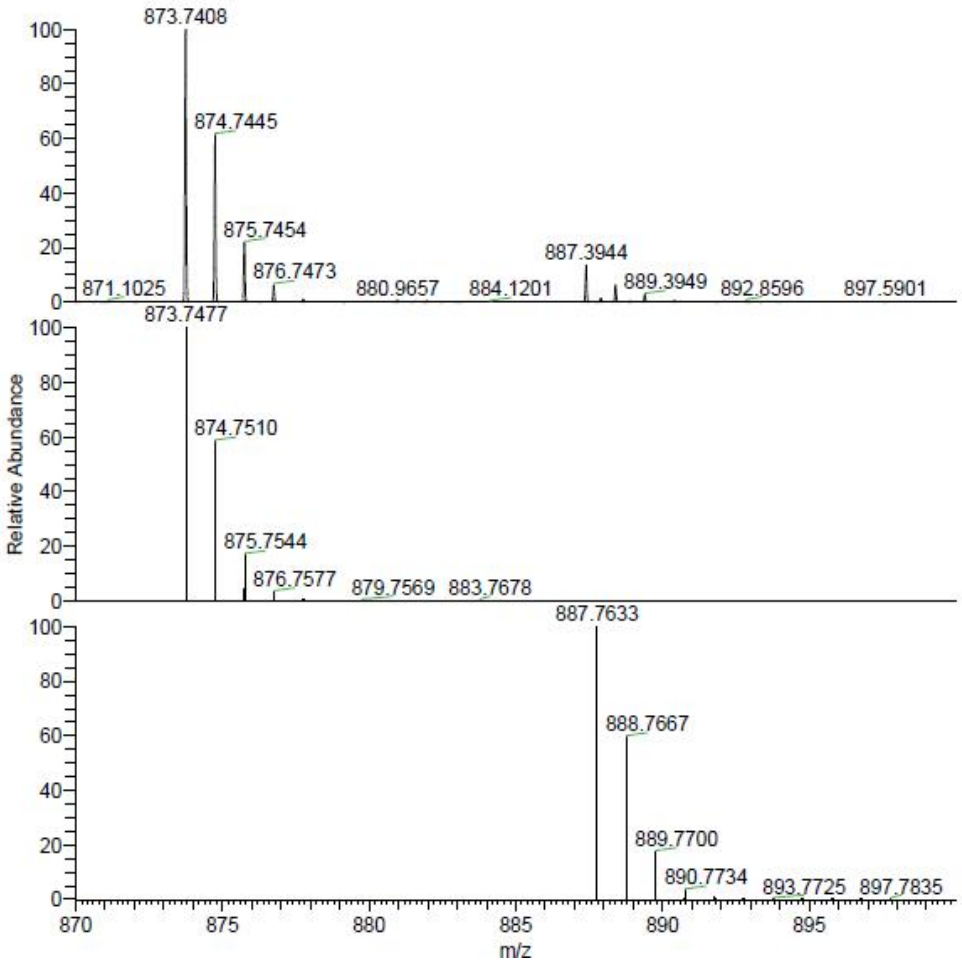


Figure 18. MS spectrum in positive mode of cetylpyridinium chloride-dodecyl sulfate crystal (top). Simulated MS spectra given the molecular formula of $C_{54}H_{101}O_4N_2S$ (middle) and $C_{55}H_{103}O_4N_2S$ (bottom).

Similar but different conclusion was obtained for LCMS identification of CHX and DS complex. One CHX molecule has two positive charges at neutral pH. In negative mode, two DS molecular ions can only balance one CHX. Therefore, a third DS negative ion is required to generate negative signals for the complex. The results are shown in Figure 19 for CHX-DS negative adduct.

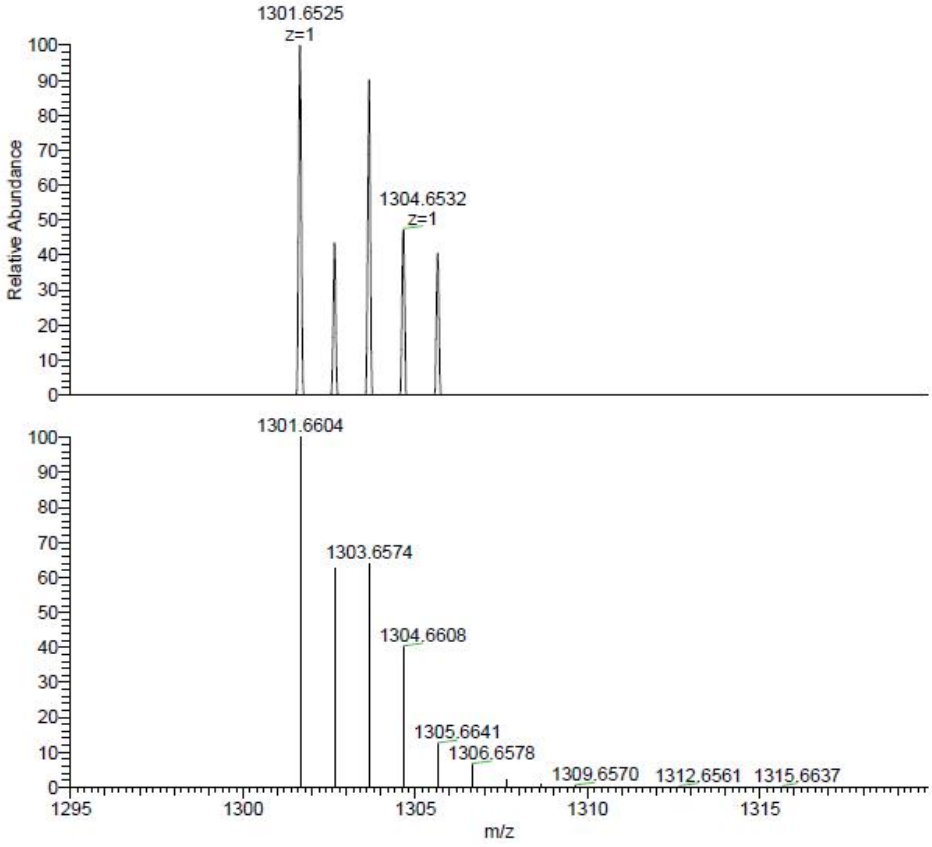


Figure 19. MS spectrum in negative mode of CHX-DS crystal (top), simulated MS spectra given the molecular formula of $C_{58}H_{107}O_{12}N_{10}S_3Cl_2$ (bottom).

The top mass spectrum was obtained from the sample complex and the bottom one was postulated with one CHX and three DS. The resolution between the simulated and actual sample is 165k. ($R = \frac{M}{\Delta M} = \frac{1301.6604}{1301.6604 - 1301.6525} = 165{,}000$) These two spectra show a very good resemblance.

In the positive mode of CHX and DS complex, one CHX has two positive charges and one DS only has one negative charge. The net charge at 1:1 ratio of CHX and DS is one positive charge. When one CHX formed a complex with two DS ions, the whole complex molecule is charge neutral. To get positive signals, one sodium ion can be added

as an adduct to the complex. Their mass spectra can be found in Figure 20, where the top mass spectrum peak at 1059.5007 is shown. The bottom MS peaks are simulated from one positive CHX and two negative DS ions plus one sodium ion, which matches the sample mass spectrum on top.

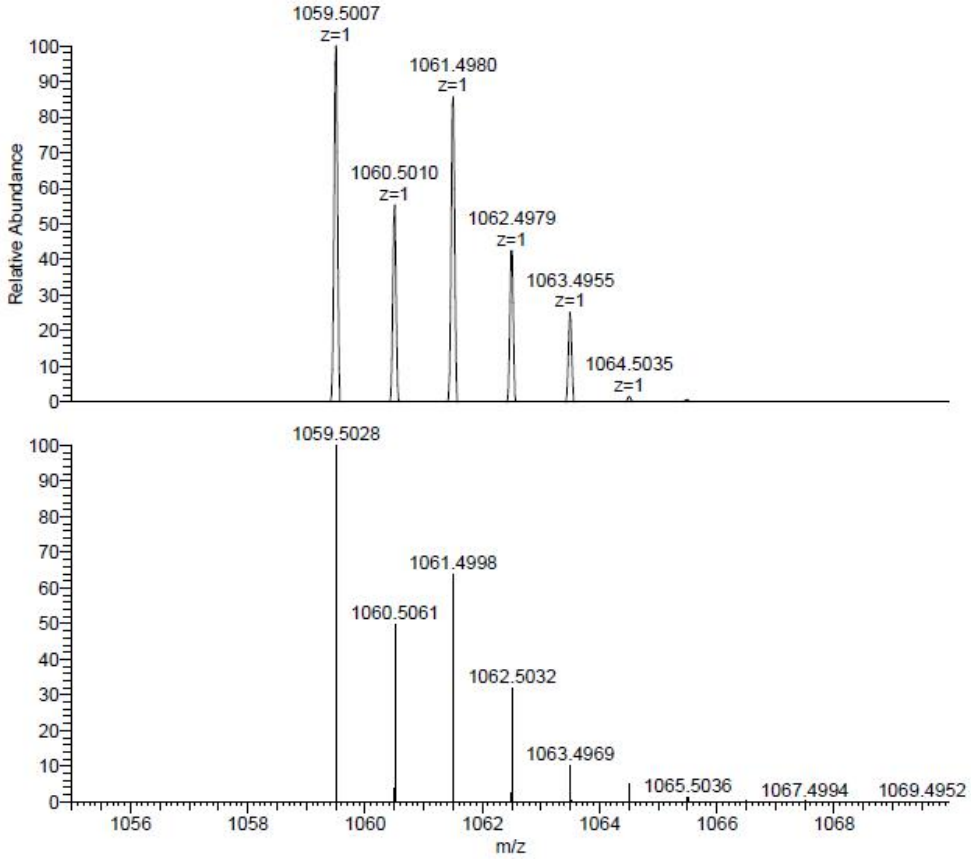


Figure 20. MS spectrum in positive mode of the chlorhexidine-dodecyl sulfate complex (top), simulated MS spectrum given the molecular formula $C_{46}H_{82}O_8N_{10}S_2Cl_2Na$ (bottom).

4. LC-MS study for ionic interaction between benzalkonium chloride and anionic surfactant

4.1. Introduction

Benzalkonium chloride (BKC) is an organic salt classified as a cationic quaternary ammonium compound. It has three main applications: as a biocide, a cationic surfactant, and as a phase transfer agent. ADBKCs are a mixture of alkylbenzyldimethylammonium chlorides, in which the alkyl group has various even-numbered carbons of alkyl chain, ranging from C_{10} to C_{16} in nature. Like CPC, BKC can kill a variety of microorganisms, which could be potentially useful for personal care and home care products.

4.2 Experiments

0.5 mL of 25% BKC was mixed with 0.5 mL 6.5% SDS in 9 mL DI water. White precipitate formed as soon as the two solutions were mixed. The precipitate was then filtered by a 0.45 μm PTFE filter. The supernatant was then diluted 10 times for direct injection into MS detector.

4.3. Results and discussion

The same phenomenon was found for the BK-DS complex as for CP-DS and CHX-DS complexes. However, due to the various chain lengths BKC from C_{12} to C_{16}. The chain distribution can be identified with a series of twenty-eight mass unit differences for C_{12}, C_{14} and C_{16}. In Figure 21, the top MS was obtained from the sample complex BK-DS in a negative detection mode, where three compound clusters each having a 28 Daltons mass unit difference. The second spectrum from the top is simulated from one BK with C_{12}

alkyl chain and two DS ions. The high resolution is 379k. ($R = \frac{M}{\Delta M} = \frac{834.5946}{834.5946 - 834.5924} = 379{,}000$) The results indicate the complex is consisted of one BK with two DS molecular ions. The third spectrum is simulated from one BK with a C_{14} alkyl chain and two DS, which matches the sample mass spectrum on top. The last spectrum on the bottom is simulated from one BK with C_{16} alkyl chain and two DS, which is then compared with the sample spectrum on top. From this high resolution spectra matching results, the alkyl chain lengths from either cationic or anionic surfactant can be identified.

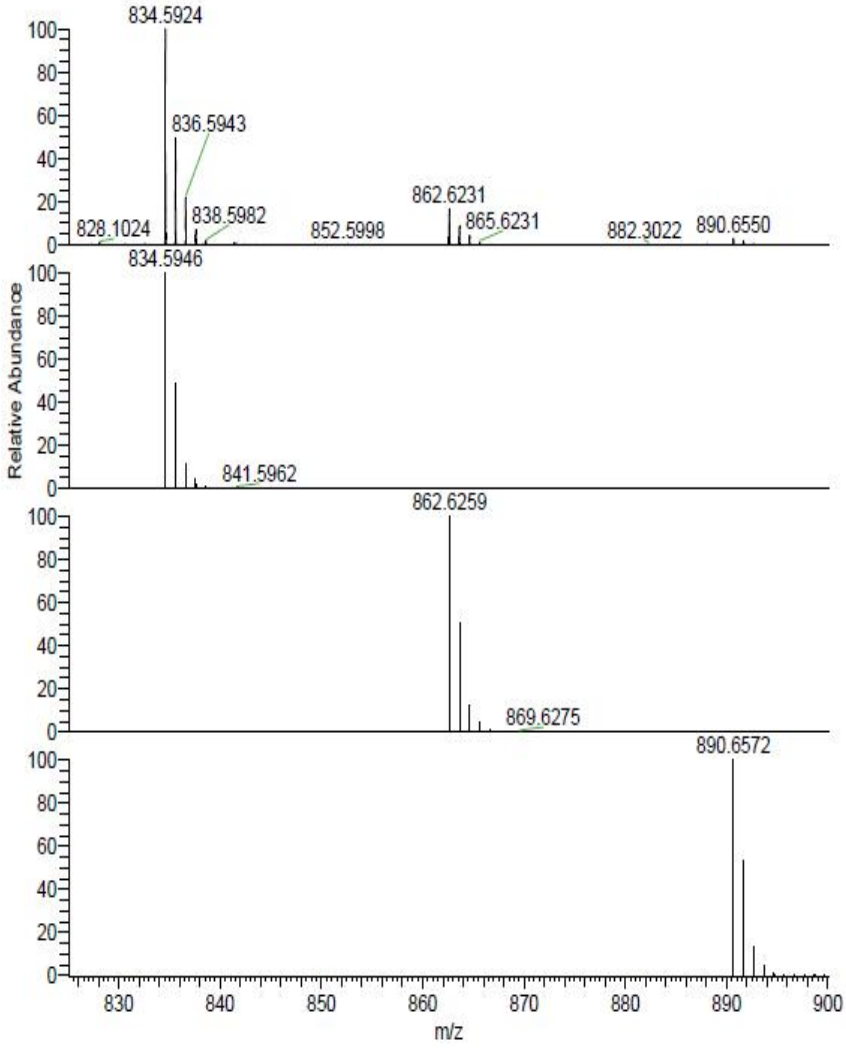


Figure 21. MS spectrum in negative mode of benzalkonium-dodecyl sulfate solution (top), simulated MS spectra given the molecular formula of $C_{45}H_{88}O_8NS_2$ (second one), $C_{47}H_{92}O_8NS_2$ (third one), $C_{49}H_{96}O_8NS_2$ (bottom one).

In the positive mass spectra shown in Figure 22, the top was obtained from sample complex BK-DS. The second MS spectrum from top is simulated from two BK molecular ions with C_{12} alkyl chain and one DS, this is very close to the sample MS on top. The third spectrum from top was simulated from one BK with C_{12} alkyl chain and one BK with C_{13} alkyl chain plus one DS. The difference between the simulated and sample spectrum is too big to match each other, which indicated sample signal at 887.3946 is not from BK-DS complex or in other words, BKC and SDS do not have odd- numbered chain distribution. The bottom MS was simulated from one BK with C_{12}, one BK with C_{14} chain plus one DS with C_{12} chain. Either way, the bottom MS matches the sample MS on top. The results from the positive mode also indicated that the alkyl chain structure in surfactants can be easily identified by high resolution MS.

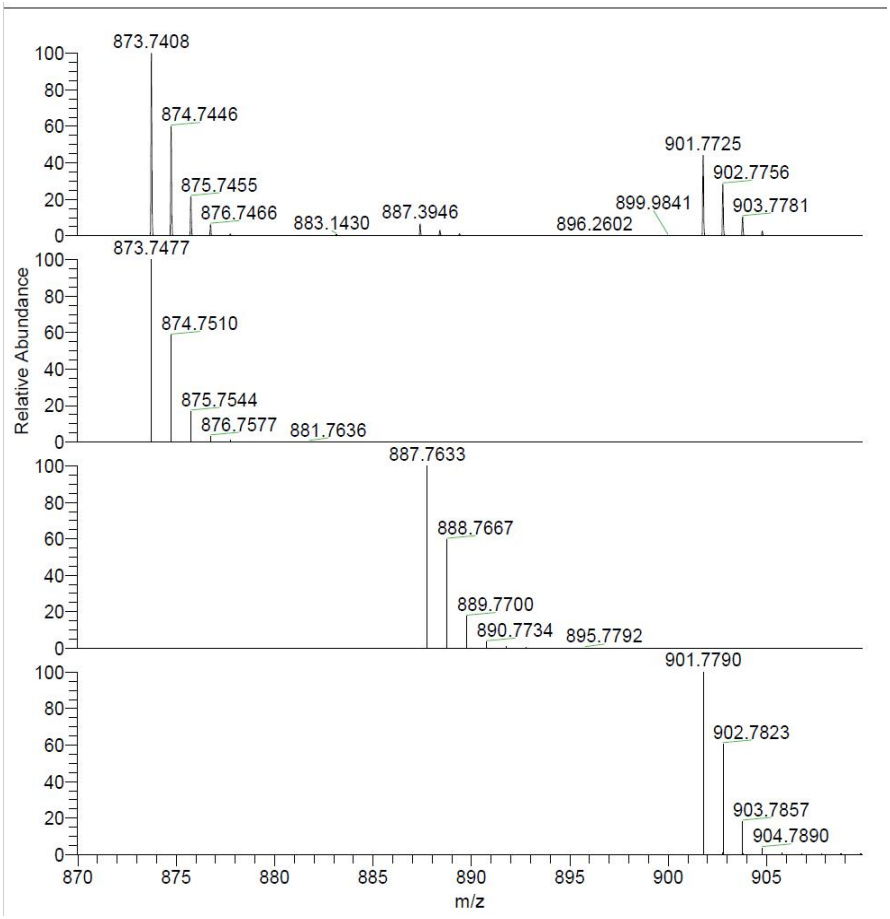


Figure 22. MS spectrum in positive mode of benzalkonium-dodecyl sulfate crystal (top), simulated MS spectra given the molecular formula of $C_{54}H_{101}O_4N_2S$ (second from top), $C_{55}H_{103}O_4N_2S$ (third from top) and $C_{56}H_{105}O_4N_2S$ (bottom).

5. Conclusion and Perspectives

Consumer products with cleaning and antibacterial functions are in high market demand. While anionic surfactants are the major ingredients for foaming and cleaning and cationic surfactants process the antibacterial property. How to formulate these two categories of surfactants into the products is very an interesting but challenging task. Many studies have been conducted to study the mechanism of the combination, such as various microstructure including micelles, vesicles, lamellae, columnar and cubic mesophases based on the shape of the surfactant molecules. Under the availability of the instruments at Cross Category Research & Innovation group at Colgate-Palmolive Company, the interactions among several surfactants were studied.

For the first time, a novel crystalline complex, consisting of chlorhexidine-dodecyl sulfate was obtained after aging at 60°C for three weeks. The crystal structure has been determined by X-ray crystallography. A combination of functional head group signals from FTIR, alkyl chain signals from NMR and molecular weight information from mass spectrometry further elucidate the molecular interaction between cationic and anionic surfactants.

Mass spectrometry equipped with the electrospray ionization (ESI) interface enables the compounds to be ionized without being fragmented. ESI can be applied to various systems, ranging from small inorganic salts to large biomolecules such as peptides and proteins. The experimental results in this thesis provided an excellent example to show ionization and interaction at certain molar ratios. Also, it is found that the complex

formation between cations and anions can be significantly interfered with the presence of phosphate groups. LC-ESI-MS did provide stronger evidence to explain the observation of surfactant mixture solubility when formulated in the products.

6. References

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