

# CHALMERS



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Master Thesis in Chemistry and Bioscience Programme

## **Synthesis and physicochemical study of novel amino acid based surfactants**

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# **Synthesis and physicochemical study of novel amino acid based surfactants**

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# Abstract

Amino acid based surfactants are of great interest in the field of novel surfactants research, mainly because of their environmentally friendly character. The hydrophobic group can be attached to amino acids either by the amine moiety or by carboxylic moiety, leading to four types of different surfactants. In this work, we have focused on two synthesis paths and prepared ester linkage and amino linkage surfactants of four amino acids, one with a single acid function, namely glycine, and three with two carboxylic groups, i.e. amino malonic acid, aspartic acid and glutamic acid. The dicarboxylic amino acids differ in the number of methylene between the acid groups. Variations of chain length were also investigated. Krafft temperature and critical micelle concentration (CMC) of amino linkage surfactants were determined by means of surface tension and conductivity measurements. The linear dependence of the logarithm of CMC on chain length was compared with conventional ionic surfactants. The solution properties in the presence of hydrochloric acid and calcium chloride, referred as acid and lime resistance respectively, were determined by pendant drop tensiometry and  $^1\text{H-NMR}$ . The effects on micellization were discussed in terms of chain length, spacer length, pH and calcium concentration. The overall results were compared with amido linkage surfactants previously studied by our laboratory.

# Acknowledgements

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# **I Introduction**

## **1.1 Background**

Surfactants as a large consuming chemical are closely interrelated to our life, industry and environment. Nowadays energy and environment are two main concerns which need to be solved by science and technology. They are also the triggers of surfactants development. Over the years, efficiency of surfactants has been dramatically increased. For example, dosage of detergents which is required to achieve the best cleaning effect has been reduced to one-half or even one-third compared with ten years ago. Concentrated detergent attained almost 28% market share of usual products in Europe. The amount of energy required for washing is further reduced as well. Today, 50% of household washing is done at 30-40°C. Future formulations will take this energy saving trend into account.[1] Meanwhile surfactants with good biodegradability are keeping replacing the older ones.

Amino acid based surfactants are of great interest as novel surfactants because they use amino acids as sources. In this way surfactants can be prepared from renewable natural feedstock and can undergo degradation that results in harmless products. Furthermore they have a high safety during manufacturing and handling by the end-users.

## **1.2 Aim**

Aim of this work is firstly to manufacture different types of amino acid based surfactants according to four fundamental synthesis paths which were summarized in a previous study of our laboratory. Amino acid based surfactants can be synthesized by attaching the hydrocarbon chain to the functional groups of amino acids. Basically the formed linkage is of three different types, which is determined by the structure of amino acid. They are ester linkage, amino linkage and amido linkage. So the second goal is to explore the effect of the different type of linkage on the solution properties. Moreover, the specific amino acids, which were used as the building blocks of surfactants in this study, have two carboxyl groups. The lengths of the spacer groups between the two carboxyl groups are different. Therefore the third purpose is to investigate how a spacer group plays a role on the aggregation behaviors of the surfactants.

# **II Theoretical background**

## **2.1 Surfactant**

Detergents, washing powders, cosmetics and skin care products play very important roles in our daily lives. Surfactants, as the main ingredients in such products, provide various functions including decontamination, emulsification as well as fabric-softening etc.

### 2.1.1 Definition

The word surfactant results from the contraction of “surface active agent”. [2] Surface active means that they have the tendency to adsorb at the interface between two immiscible phases and reduce the interfacial tension.

A surfactant molecule possesses an amphiphilic structure and is composed of a hydrophobic moiety and a hydrophilic moiety. The hydrophobic moiety is soluble in the oil phase or the air phase while the hydrophilic moiety is soluble in the water phase. The hydrophilic groups which constitute the polar head groups are based on functional groups such as carboxy, sulfonate, ammonium, hydroxyl and amide. Hydrophobic groups are nonpolar tails, such as hydrocarbon chain with eight or more carbon atoms, and can be linear or ramified. Figure 1 illustrates the situation where surfactant molecules align at the air/water interface, and form micelles.

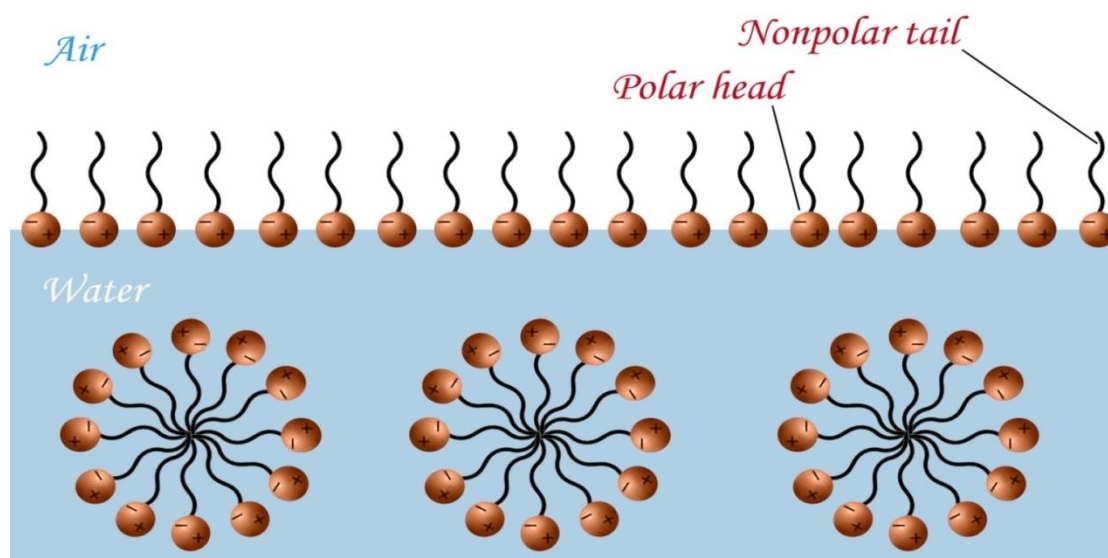


Figure 1 Scheme of surfactant molecules aligning on water/air interface

### 2.1.2 Classification

Surfactants can be classified into four categories according to their polar head groups which are anionic, cationic, amphoteric and nonionic surfactants. Anionic surfactants are the largest category among them. They carry a net negative charge on their polar head and they have good water solubility and foaming ability. Therefore they are often used as cleansing



agent in many detergent formulations. Cationic surfactants carry a net positive charge. There are only a few structures which have the ability to carry a positive charge so their numbers are much less than anionic surfactants. Normally they are ammonium salt, quaternary ammonium salt or heterocyclic compounds. Their decontamination efficiency is much less than anionic surfactants. However, due to the fact that solid surfaces often carry negative charges, cationic surfactants are often used as textiles softener and antistatic agent. Amphoteric surfactants carry both positive and negative charge in a same molecule. They have lower irritation to eyes and skin so they are applied in cosmetic industries. Nonionic surfactants are the second largest category. They have repeated hydroxyl or ether bond as the polar head group and they do not ionize in water. Hydroxyl or ether bond has weak hydrophilicity so large amounts of these kinds of groups are needed to show hydrophilicity. They have better stability in hard water compared with anionic surfactants.

By definition the amino acid based surfactants are either amphoteric or ionic surfactants. Therefore nonionic surfactants will not be discussed further in this study. Amphoteric surfactants can be applied in various pH due to their zwitterionic structures and they are able to change types by varying pH. They are anionics in alkaline condition, cationics in acidic condition and amphoteric in neutral condition. When they carry the same amount of anions and cations by varying pH, they reach the isoelectric point. At this time they have the highest surface activity and lowest solubility. By comparison, some anionic surfactants are soluble under alkaline condition and have narrower pH range.

### 2.1.3 Application

Surfactants are primarily used as detergent and cleaners in daily life. Soap, which is the earliest surfactant, has been used for removing dirt over 2000 years.[3] The industrial production of soap starts from the 19<sup>th</sup>-century. Sodium stearate is the main ingredient of soap at that time. It is the product of the saponification reaction which involves the hydrolysis of fatty acid esters to form sodium salt of carboxylate. This process is illustrated in Figure 2.

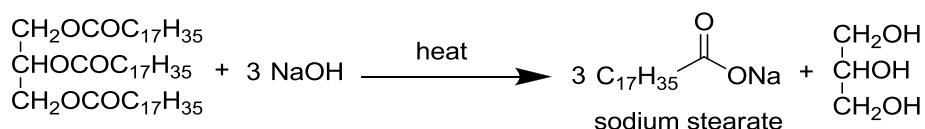


Figure 2 Saponification reaction

In the 1930s, synthesized surfactants and detergents, which used petrol derivatives as feedstock, were employed in industry and gradually replaced soap as the main household

cleansing product on the market. Synthetic detergents are developed to satisfy various requirements as cleaners and to increase performance. They can be used for cleaning clothes, dishes, houses, skins, hairs, etc. Compared with soap products, these surfactants have better foaming ability, lower irritating and lower temperature requirements.

In 1958, huge amounts of foam are built up at weirs in rivers. In 1960s, surfactants with branched alkyl chains, which are environmentally harder to degrade, started to be replaced by biodegradable materials.[4]

Surfactant is consumed in a huge amount every day. Therefore it brings a very heavy burden to the environment. Nowadays, effectiveness of surfactant is not enough to satisfy the environmental requirements, and a better biodegradability along with a renewable feedstock required. And this is the aim of this project: the synthesis of environmental friendly surfactants which meet the modern request and study of their properties.

## 2.2 Amino acid based surfactants

Amino acid based surfactant is of great interest in the field of novel surfactants research because it is a type of environmental friendly and readily biodegradable surfactants. Their raw materials are natural amino acids and they have shown low toxicity and quick biodegradation in previous studies.[5] Therefore they have great potential to be applied in food, pharmacy and cosmetic industries.

### 2.2.1 Characteristics

Amino acid based surfactants use amino acids as starting materials. It is defined as a surfactant having an amino acid or its residue as the hydrophilic group.[5] The basic form of amino acids has two functional groups, which are an amine group and a carboxyl group. They also have a side group, R, as illustrated in Figure 3. This enables a lot of possibility to synthesize new surfactants.

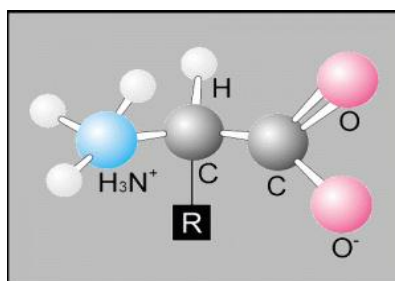


Figure 3 Basic structure of an amino acid [6]

Amino acids come from nature and are important to life. There are 20 amino acids that are called natural amino acids which can be incorporated into polypeptides. They differ by the side group, R, which is indicated in blue in Figure 4. Eight amino acids in orange area are nonpolar and hydrophobic. The other amino acids are polar and hydrophilic. Three amino acids in blue box are referred as basic because their side group is an amine. Two amino acids in the purple box are acidic due to the side group is a carboxylic acid. Amino acids are a renewable source, which reduce their impact to the environment. In a word, surfactants using amino acids as sources are potentially environmental friendly.

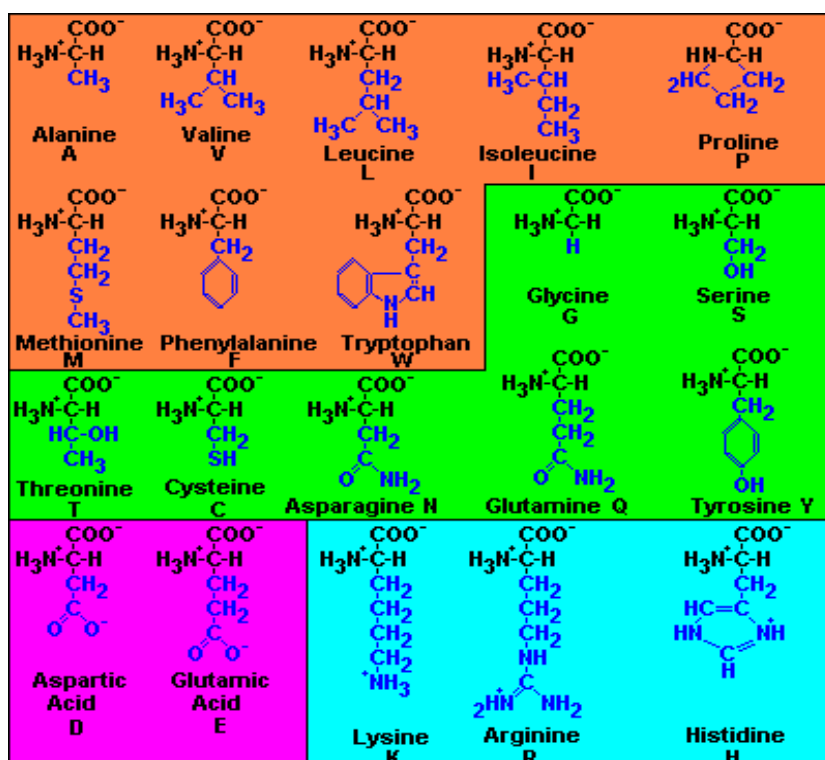


Figure 4 Chemical structures of 20 natural amino acids [7]

### 2.2.2 History

In the beginning of 20<sup>th</sup> century, surfactants using amino acids as raw materials are applied in medical preservation. Later they were found to be effective against various disease-causing bacteria, tumors, and viruses.[5] By the end of 1988, amino acids had been available at relatively low cost. Due to this reason, they were utilized in seasonings, food additives, pharmaceuticals and elsewhere.[8] Their applications in cosmetics industry were extensively investigated.[9] As the development of biotechnology, some of the amino acids are able to be produced in a large scale by yeasts nowadays, which means the production of amino acid based surfactants are developed to be more environmental friendly.

### 2.2.3 Classification

Theoretically the hydrophobic group is attached to the amino acids either at the amine moiety or at the carboxylic moiety. Therefore four fundamental synthesis paths are deduced based on this approach, which are described in Figure 5.[10] By modifying the type of bond the properties of the resulting surfactants will be influenced. This represents one side of this project.

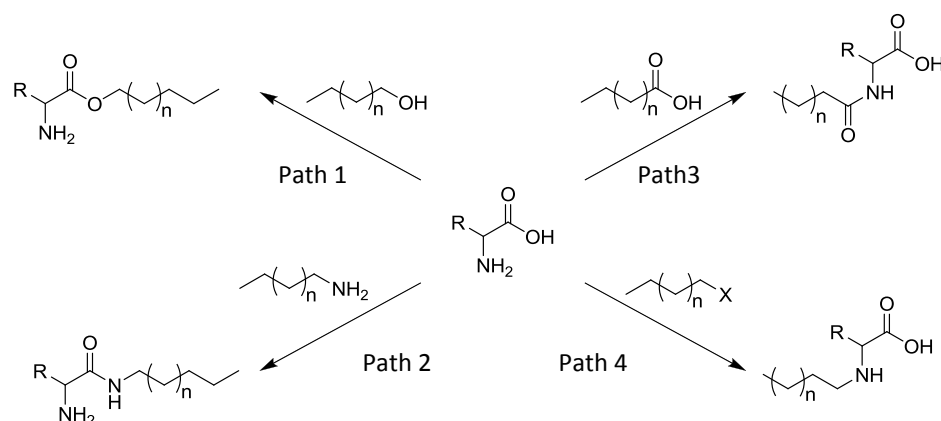


Figure 5 Fundamental synthesis paths of amino acid based surfactants

In path 1, an amphiphilic esteramine is produced by the reaction of fatty alcohol and amino acid. Such reaction is a typical esterification, which is achieved by refluxing the amino acid and fatty alcohol in the presence of acidic catalyst and dehydrating agent. Normally concentrated sulfuric acid is used as both catalyst and dehydrating agent.

In path 2, amino acid reacts with an alkyl amine to create an amide bond and yield an amphiphilic amidoamine. Usually the acid is activated and then reacted with the amine.

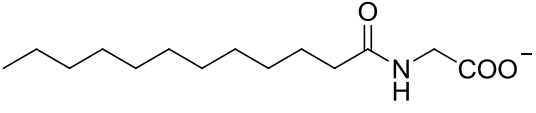
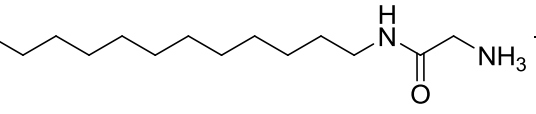
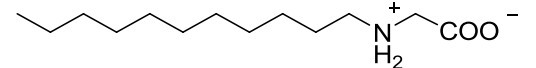
In path 3, the amine group of amino acid reacts with fatty acid and, usually under the form of an acyl chloride, and an amidoacid is produced.

In path 4, the amine group is reacted with an alkyl halogen and the product is a long-chain alkyl amino acid.

A fifth path, not illustrated here, is based on the coupling of the specific function of the side group of the amino acid. This will be discussed further for the specific case of aspartic acid and glutamic acid where the side carboxylic group can react with a fatty alcohol, as in path 1, to form an ester or a diester.

These paths of synthesis can lead to different species which will have specific ionization properties.

Amino acid based surfactants can be classified into two fundamental types, which is illustrated in Table 1.[8]

Type 1	N-substituted		Anionic
	C-substituted		Cationic
Type 2	N-alkyl		Amphoteric

**Table 1 Fundamental structures of amino acid based surfactants (glycine based here)**

There are two varieties of the first type, which are N-substituted compounds and C-substituted compounds. In N-substituted compound, an amino group is substituted with a hydrophobic moiety or a carboxylic group and loses the alkalinity. For C-substituted compound, a carboxylic group is substituted through an amide or an ester bond. N-acyl amino acids surfactants are typical N-substituted compounds. They are fundamentally anionic surfactants. They have an amide linkage between the hydrophobic moiety and the hydrophilic moiety. Amide linkage has a strong hydrogen bonding ability. This linkage can be broken through hydrolysis process under acidic conditions. Therefore they have good biodegradability. C-substituted compound are esters or amides, which are fundamentally cationic surfactants.

With the second type of the surfactants, they have both the amino group and the carboxylic group as the hydrophilic moiety. There are also two varieties in this type, including C-alkyl and N-alkyl amino acids surfactants. They are amphoteric surfactants.

### 2.2.5 Application

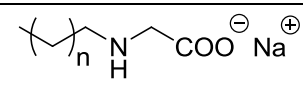
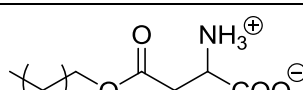
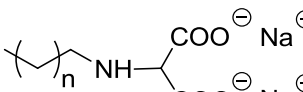
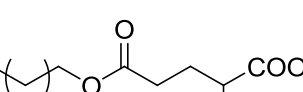
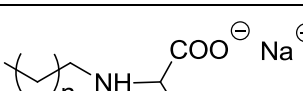
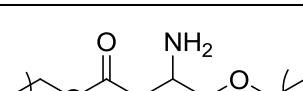
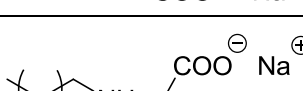

Two types of amino acid based surfactants are employed as disinfectants which are long-chain alkyl amino acids and alkyl betaines. These two types of compounds show significant bactericidal and fungicidal properties at pH values lower than their isoelectric point.[11]

Amino acid based surfactants are used in the formulation of personal care products.[12] Potassium N-cocoyl glycinate is mild to skin and applied in faces cleansers to remove soil and

make-up. N-acyl-L-glutamic acid has two carboxylic groups, which lead to better water solubility. The products based upon C12 fatty acid are used as face cleansers to remove soil and make up. Those based upon C18 are used as emulsifiers in skin care cosmetics. N-dodecanoyl alaninate is able to create creamy foam and it is nonirritating to skin, therefore it is often employed to formulate baby care products. N-lauroyl sarcosinate, which is used in toothpaste, shows good detergency like soap and strong enzyme-inhibiting effect.

### 2.3 Surfactant synthesized during this study

Two types of surfactants have been synthesized in this project. The first type includes  $\gamma$ -alkyl amino acids and di-alkyl amino acids, which are illustrated in the right column of Table 2. The second type is N-alkyl amino acids in the left column of Table 2. Both of them vary in the chain lengths and spacer lengths between two ester bonds. For  $\gamma$ -alkyl amino acids, the chain lengths include 8 carbons and 12 carbons and the spacer lengths include two methylene group and three methylene groups. For di-alkyl amino acids, the chain lengths include 12 carbons and 14 carbons and spacer lengths include two methylene groups and three methylene groups. The second type is N-alkyl amino acids based surfactants. The chain lengths include 8 carbons, 10 carbons and 12 carbons. Except for N-alkyl glycinate which has a single carboxylic group, the others surfactants have two with a spacer lengths including one methylene group, two methylene groups and three methylene groups. The effects of chain length and spacer length on physicochemical studies were studied and the role of linkage between the hydrophilic and hydrophobic moieties will be discussed in the latter chapters.

Name	Chemical structure	Name	Chemical structure
N-alkylglycinate		$\gamma$ -alkyl aspartate	
N-alkyl malonate		$\gamma$ -alkyl glutamate	
N-alkyl aspartate		Di-alkyl aspartate	
N-alkylglutamate		Di-alkyl glutamate	

## 2.4 Physicochemical study of the surfactants in solution

During this study the primary characteristics of the synthesized surfactants were determined. These properties are very important to discover the proper usage for such surfactants. They are the Krafft temperature, the critical micelle concentration, the solubility properties and the adsorption degree at the air water interface. Furthermore the surfactant prepared here are ionic surfactants and therefore their behavior will be modified by the presence of ions in solutions, especially calcium. The presence of calcium and magnesium in hard water is often an issue because it causes their precipitation and the ability of the surfactant to remain soluble in presence of calcium is referred as their lime resistance. In the present case, the dicarboxylic character of the polar head groups is susceptible to delay the precipitation and will be assessed.

Finally, because of their peculiar chemical structure, the surfactants are likely to be pH depending. This feature will be assessed from a solubility point of view.

### 2.4.1 Krafft temperature

Temperature plays very a important role in surfactant self-assembly phenomenon. The solubility of many ionic surfactants may be strongly temperature dependent. They may be insoluble at low temperature and then their solubility increases dramatically in a relatively narrow temperature range. This inflexion point of temperature is called Krafft point. And the value of this temperature depends on the specific structure of ionic surfactants, such as alkyl chain lengths or counter ions. Since all the surfactants synthesized in this project are ionic surfactants, it is significant to determine this temperature value before considering their applications.

#### 2.4.1.1 Definition

Krafft temperature is also known as Krafft point. Below this temperature, surfactants cannot reach their theoretical maximum activity. And they are unable to adsorb at the interface to the maximum extent.[9] Surfactants are most effective to reduce the surface tension of solution, only if the environmental temperature is above Krafft temperature. This theory can be applied to explain certain phenomena in our daily lives. For example, washing powder is most effective to remove soils when using hot water and sufficient amount of detergent.

### 2.4.1.2 Determination

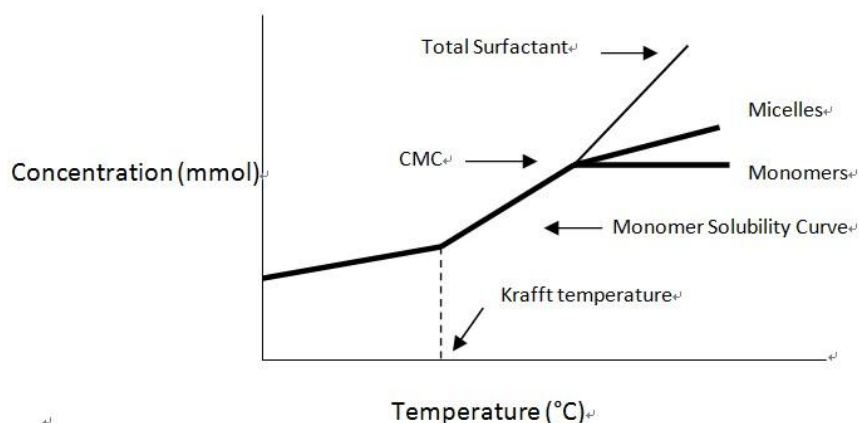


Figure 6 Temperature-solubility relationships for typical ionic surfactants

A scheme of temperature-solubility relationship for ionic surfactants is shown in Figure 6.[13] Ionic surfactants molecules exist as monomeric form in solution below Krafft temperature. Surfactant solubility is determined by the monomer solubility. Monomer concentration increases as the temperature rises. Surfactants start to aggregate above Krafft temperature and form micelles. Because micelles formation increases the solubility, the slope goes up at Krafft temperature. Concentration continues increasing until it reaches CMC. Although there are still certain amounts of monomers in solution, monomer concentration will keep constant and micelles become the predominate form. Micelle concentration increases as temperature rises. As a result, surfactant solubility turns to be determined by micelles solubility and there is another slope change at CMC.

The slope increase at Krafft temperature offers the method to measure it. Ionic surfactants act as electrolytes in solution. Therefore change of solution conductivity can representative change of concentration. It is convenient to get the value of Krafft temperature by measuring temperature-conductivity relationship. Figure 7 shows Krafft temperature of N-dodecyl glycine sodium salt at pH 13.



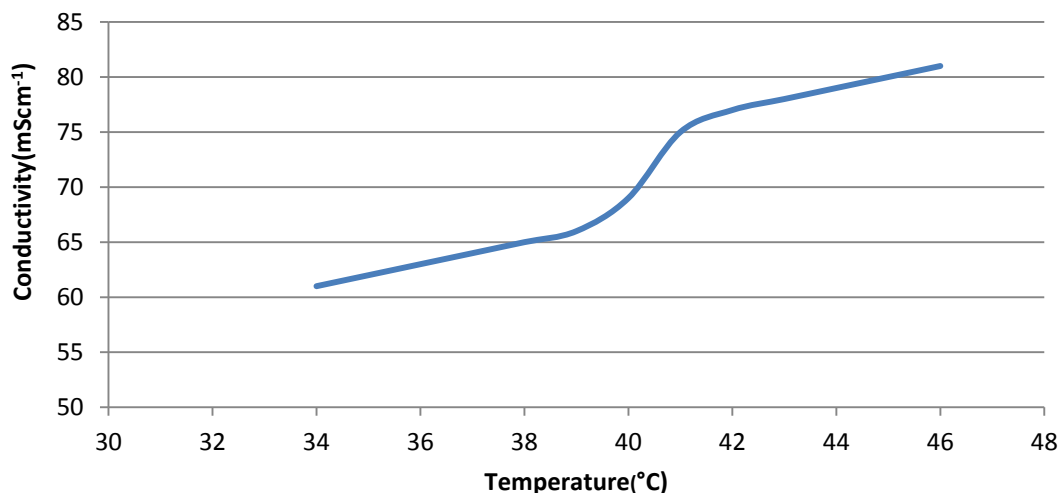


Figure 7 Temperature-conductivity plot of N-dodecyl glycine at pH 13

N-dodecyl-glycine sodium salt is not completely soluble in aqueous solution below 40°C and the solution is not transparent either. It becomes clear above Krafft temperature.

Nonionic surfactants do not exhibit a Krafft temperature because their solubilization mechanism is different. They become less soluble as the temperature increases, as consequence of the cloud point.[13]

## 2.4.2 Critical micelle concentration

Surfactant molecules possess amphiphilic structure so they pack at the surface after addition into the aqueous solution. By doing this, the surface tension of solution is significantly reduced. The value of surface tension decreases as increasing the surfactant concentration and it reaches the minimum when the surfactant concentration equals to its critical micelle concentration. If the concentration continues increasing after that, the new coming surfactant molecules will form an aggregation state called micelles. Micelle formation is a very important property of surfactants. Furthermore surfactant is the most effective on lowering the surface tension after it reaches the critical micelle concentration. Therefore determining critical micelle concentration is one of the most important steps in the study of surfactants.

### 2.4.2.1 Definition

Above a certain concentration, surfactants start to aggregate and form micelles. This concentration is referred as the critical concentration, i.e. CMC. For ionic surfactants, the molecules in solution act as simple electrolytes when the concentration is lower than CMC. In this situation, surfactants are unimers in solution. When the concentration is above the

CMC, every surfactants added cannot be unimer anymore. They prefer to switch to a self-assembled state called micelles. This transform from unimer to micelle brings great effects on the solution properties, such as osmotic pressure, light scattering and surface tension etc, which are dramatically changed as shown in Figure 8.[2]

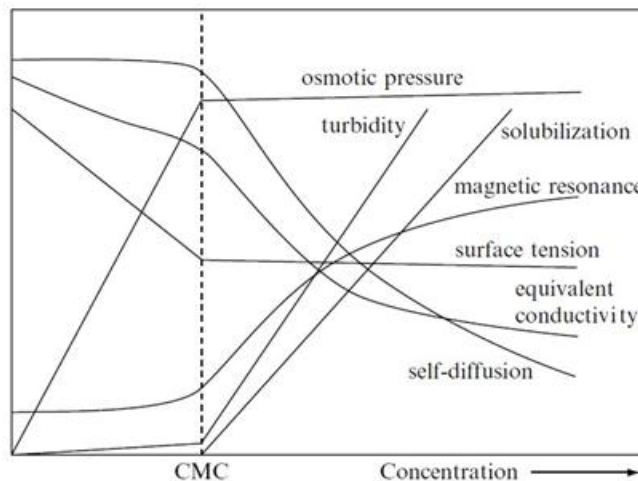


Figure 8 Surfactant property changes at CMC (from ref [2])

In the present study, we used surface tension and conductivity measurements.

#### 2.4.2.2 Determination of CMC

CMC is one of the most important parameter of surfactants. It is normally measured by tensiometry. The surface tension of a surfactant solution decreases as increasing the surfactant concentration. On the plot surface tension versus  $\log(\text{concentration})$  of a surfactant solution, a curve break occurs at CMC, as shown in Figure 8. Above CMC, the surface tension of surfactant solution reaches a constant minimum value, which indicates the interface is saturated with surfactant.[14]

In the plot of surface tension vs concentration, two other important data can be determined. The minimum value of surface tension can be directly read in the plot. This is a useful value when comparing the effectiveness of different surfactants. Area per molecule is another important value. It is achieved by calculation.

According to Gibbs equation, the surface concentration can be calculated from the slope of the plot. (from ref [12])

$$\Gamma = -\frac{1}{2.303nRT} \left( \frac{\partial \gamma}{\partial \log c} \right)$$

Where  $\gamma$  is the surface tension in mN/m,  $R=8.31 \text{ J/mol}^{-1} \text{ K}^{-1}$ ,  $\Gamma$  is in  $\text{mol}/1000\text{m}^2$

Area per molecule at the interface in  $\text{\AA}^2$  is calculated from the relation:

$$A = \frac{10^{23}}{N_A \cdot \Gamma}$$

Where  $N_A$ = Avogadro's number,  $\Gamma$  is in  $\text{mol}/1000\text{m}^2$

Area per molecule provides the information about how densely the surfactant molecules pack at the interface.

## 2.4.3 Lime tolerance

### 2.4.3.1 Definition

Hard water has high mineral content and concentration of calcium and magnesium ions is especially high, leading to trouble for practical applications. Sodium ions are commonly used as counter ions in surfactant synthesis. Compared with sodium ions, calcium ions are more favorable to bind to surfactants in solution. This is because calcium ions carry two charges. Binding with calcium ions makes surfactants precipitate from solution and lose activity. Surfactants precipitate in the form of lime soap. The reason for precipitation is that calcium ions neutralize ionic groups of surfactant, which is illustrated in Figure 9.[15]

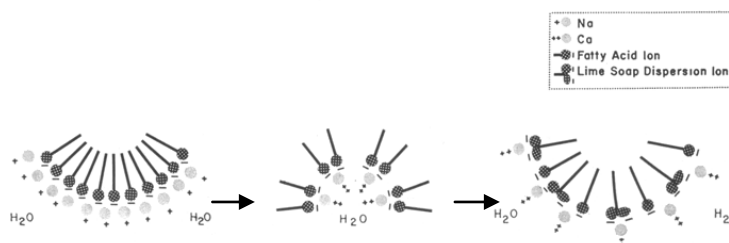


Figure 9 Scheme of lime soap dispersion mechanism (from ref [15])

Lime tolerance refers to the resistance of surfactant to precipitate as lime soap in the presence of hard ions like calcium ions. Surfactants which are very tolerant to high water hardness have found use in personal care products and detergent formulations. There is another reason for employing such surfactants in the formulation. They can form a mixed complex that shows high surface activity with lime soap. Such ability is also called lime soap dispersing power, which is defined as percentage or the number of grams of surfactant required to disperse the lime soap formed from 100g sodium oleate in water.[2] And those

surfactants are called lime soap dispersing agents. Such agents must possess an ester, ether, amido, or amino linkage between the terminal hydrophilic group and the hydrophobic group, as well as a straight-chain hydrophobic group.[12] It is found that anionic and particularly zwitterionic surfactants are the best for use as lime soap dispersing agent.[16] Anionic surfactants like sulfated alkanolamide[17] and zwitterionic surfactants like amidosulfobetaine[18, 19] were found to be very effective lime soap dispersing agents.

#### ***2.4.3.2 Determination of the lime tolerance***

Lime tolerance can be evaluated by measuring solubility and the surface activity change in the presence of calcium ions.[20] Another approach is to measure the percentage or the number of grams of surfactant required to disperse the lime soap formed from 100g sodium oleate in water.[2] In the present work, we have mainly focused on the solubility, which was determined by NMR, and on the modification of the surface activity upon the increase of concentration of calcium chloride in the subphase.

### **III Material and methods**

#### **3.1 Materials**

1-Bromooctane (Aldrich, 99%), 1-Bromodecane (Aldrich, 98%), 1-Bromododecane (Aldrich, 97%), 1-Dodecanol (Fluka,  $\geq 98.5\%$ ), 1-Tetradecanol (Aldrich, 97%), glycinylester hydrochloride (Aldrich, 99%), diethyl aminomalonate hydrochloride (Fluka,  $\geq 99\%$ ), dimethyl glutamate hydrochloride (Bachem, 98%), dimethyl aspartate hydrochloride (Bachem, 98%), DL- Pyroglutamic acid (Fluka,  $\geq 99\%$ ), p-Toluenesulfonic acid monohydrate (Aldrich, 99%), chloroform-d (Aldrich, 99.8%), L-Aspartic acid ( $\geq 97\%$ ), L-Glutamic acid ( $\geq 97\%$ ), calcium chloride anhydrous (Aldrich,  $>96\%$ ), sodium hydroxide (Fluka,  $\geq 98\%$ ), hydrochloric acid (Aldrich, 37%), sulfuric acid (Aldrich, 99%), triethylamine (Aldrich,  $\geq 99\%$ ), sodium bicarbonate (Fluka,  $\geq 99\%$ ) were used as received.

Synthesis and purification is conducted by classical methods, such as extraction and recrystallization etc. Column chromatography is avoided. In this way the synthesis of these surfactants can be repeated in a large scale according to the following protocols.

#### **3.2 Nuclear magnetic resonance (NMR)**

Proton Nuclear Magnetic Resonance Spectroscopy ( $^1\text{H-NMR}$ )

NMR is a spectroscopic method which is typically used to determine the structure of an organic molecule. All nuclei that have odd mass number or odd proton number can be

studied by NMR technique. The samples have to be liquid or dissolved. They are measured by applying a strong magnetic field and emit microwave radiation with different frequency. The position of the signal peaks are affected by chemical shift and spin-spin coupling.[4]

In surfactant analysis,  $^1\text{H}$  is most commonly used. This is because of hydrogen is the most abundant atom in organics and measurement time is very short.  $^1\text{H}$ -NMR is used to see the chemical structure and purity of the products. The nucleus of protons shows different reaction under high magnetic field. The protons in different chemical environment will give different chemical shifts or peak shape. However sometimes micelles formation cause peak broadening.[21]

NMR is a powerful analysis method because it can do both qualitative and quantitative analysis. In quantitative analysis, the exact amount of sample can be measured by read the peak ratio between sample and a reference with known content. However, the disadvantage of NMR is pure liquids or concentrated solutions of low viscosity must be available. And sample consuming of NMR is relatively large. The amount of each analysis is in the order of 10mg to achieve a good resolution.

### 3.3 Pendant drop tensiometer

Pendant drop tensiometer is the instrument which is used to measure the surface tension of all the samples in this project. Its measurement principle is based on an optical method, which is completely different with traditional force tensiometer. The principle of force tensiometer is making a solid ring touch the surface of the liquid sample and measuring the force between them. The force is further used to determine the surface tension of the liquid sample. In comparison with it, the principle of optical tensiometer is hanging a drop of liquid sample from a syringe tip, taking its picture by a high speed camera, and analyzing the shape of the drop. Surface tension of the liquid sample is determined through this equation:

$$\gamma = \frac{\Delta\rho \times g \times R_0}{\beta}$$

Where  $\gamma = \text{surface tension}$

$\beta = \text{shape factor}$

$\Delta\rho = \text{difference in density between fluids at interface}$

$g = \text{gravitational constant}$

$R_0 = \text{radius of drop curvature at apex}$

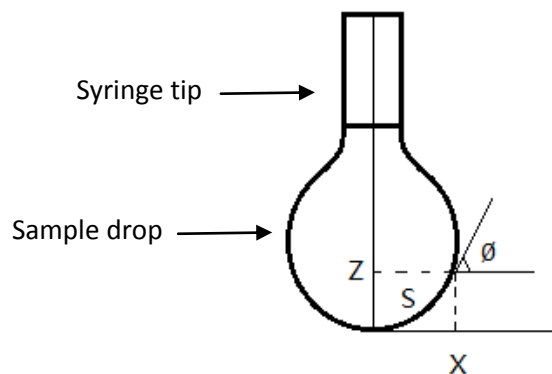


Figure 10 Scheme of shape factor calculation

The shape factor  $\beta$  is calculated with the following Young-Laplace equation by modern computational methods.

$$\frac{dx}{ds} = \cos \phi$$

$$\frac{dz}{ds} = \sin \phi$$

$$\frac{d\phi}{ds} = 2 + \beta z - \frac{\sin \phi}{x}$$

Both the optical and force tensiometer are available to conduct the common measurements such as interfacial tension and contact angle etc. Compared with each other, it is found that they have respective advantages and constraints. It is convenient to conduct the measurements with optical tensiometer because it consumes much less samples than force tensiometer and the time required to conduct each measurement is shorter. Moreover all the measurements have to be conducted at room temperature because the stability of the drop is reduced at higher temperature. The usability comparison of optical and force tensiometer is listed in Table 3.

	Optical tensiometer	Force tensiometer
Measurement type	Contact angel; Interfacial tension; Surface free energy	Contact angel; Interfacial tension; Surface free energy
Advantages	Less sample consuming	Automatic measuring CMC; Available at high temperature
Constraints	Only available at room temperature	More sample consuming

Table 3 Usability comparison of optical and force tensiometer

### 3.4 Conductometry measurements

The conductivity meter used was CDM 210 (Radiometer Copenhagen), and the surfactant solutions at a concentration above the CMC were heated with a cryostat Neslab RTE 200 ( $\pm 0.01^\circ\text{C}$ ).

### 3.5 Synthesis of the surfactants

Names and chemical structures of all the surfactants and yield of every reaction are listed in Table 4.

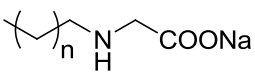
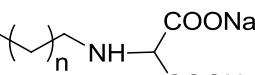
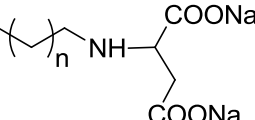
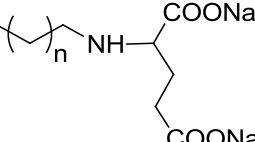
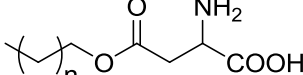
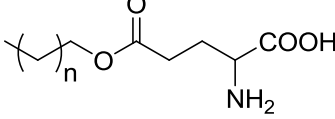
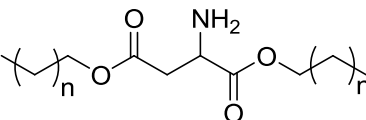
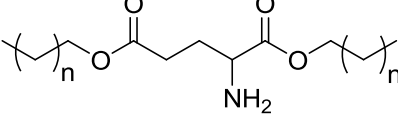
Name	Chemical structure	Yield
N-alkylglycine sodium salt		C8 45% C10 50% C12 86%
N-alkyl amino malonic acid di-sodium salt		C8 58% C10 60% C12 80%
N-alkyl aspartic acid di-sodium salt		C8 59% C10 60% C12 52%
N-alkylglutamic acid di-sodium salt		C8 37% C10 23% C12 26%
Y-alkyl aspartic acid		C8 37% C12 40%
Y-alkyl glutamic acid		C8 30% C12 35%
di-alkyl aspartic acid		C12 60% C14 82%
di-alkyl glutamic acid		C12 71% C14 69%

Table 4 Names, chemical structures and yields of all surfactants

### 3.5.1 N-alkyl amino acids synthesis

The chemical formulas of N-alkyl amino acids are shown in Figure 11:

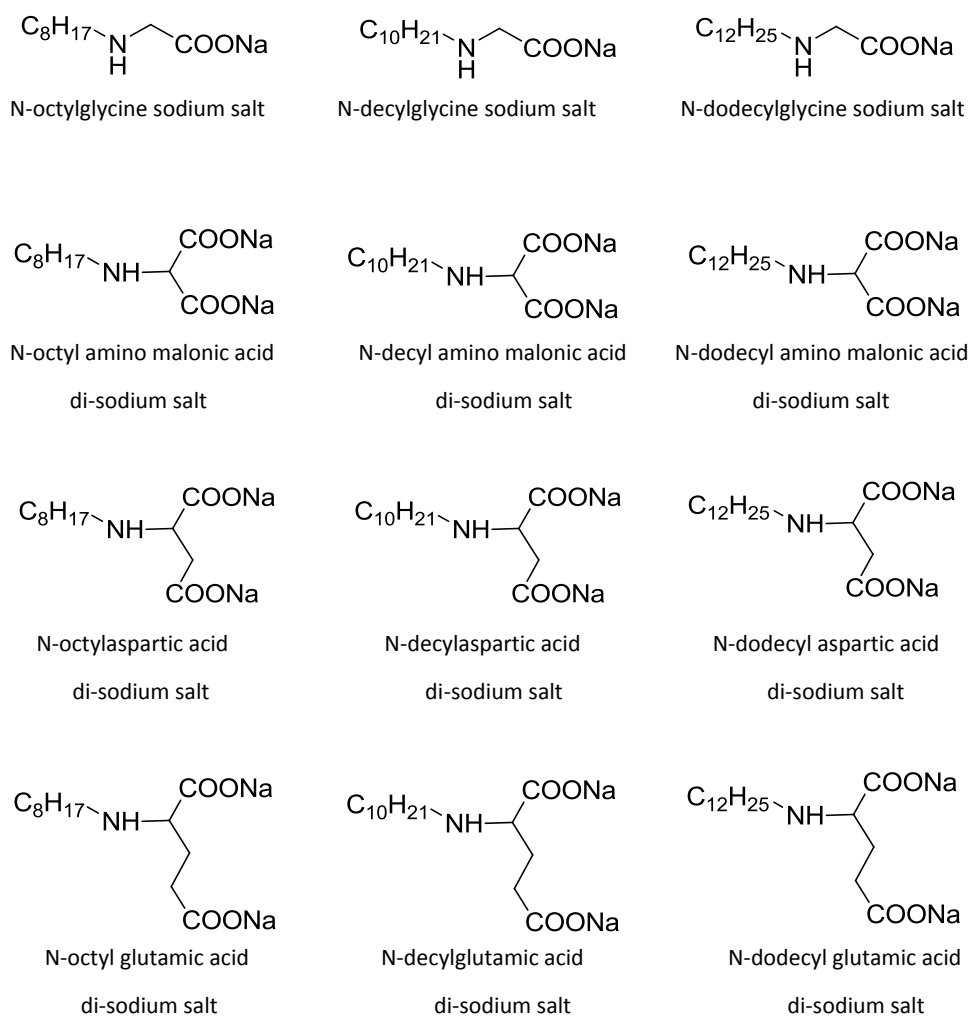


Figure 11 Names, chemical structures and yields of all surfactants

#### Synthesis of N-dodecyl glycine sodium salt:

##### Route 1

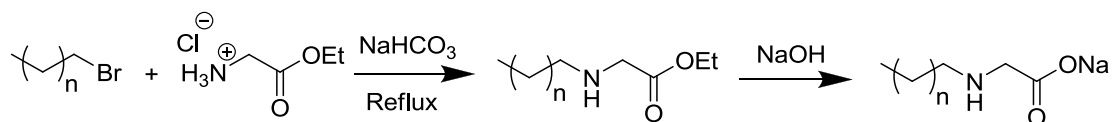


Figure 12 Synthesis Route 1 of N-alkyl glycine sodium salt



Glycine ethyl ester hydrochloride (1g, 7mmol) and sodium bicarbonate (1.2g, 14mmol) are suspended into acetonitrile (40mL). The suspension is stirred and dodecyl bromide (1.8, 7mmol) is added dropwise in it. The mixture is refluxed overnight. After that, acetonitrile is evaporated and the residue is dissolved in dichloromethane (25mL). It is washed with 0.1M hydrochloric acid (25mL×2) and distilled water (25mL×1). Then dichloromethane is evaporated and the residue is dissolved in ethanol (20mL). 4M sodium hydroxide (1.75mL) in ethanol (3mL) is added dropwise to the solution. The precipitate is filtered and washed with ethanol. It is further dried in vacuum.

Yield: 1.68g, 86%

### Route 2

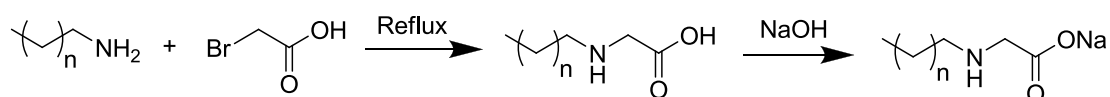


Figure 13 Synthesis Route 2 of N-alkyl glycine sodium salt

Bromoacetic acid (0.66g, 5mmol) and dodecyl amine (0.93g, 5mmol) are mixed into acetonitrile (25mL). The mixture is refluxed overnight. After that, acetone is added to precipitate the product. Such product is filtered and washed with acetone. Then it is dissolved in ethanol. 4M sodium hydroxide (1.25mL) in ethanol (3mL) is added dropwise to the solution. The precipitate is filtered and washed with ethanol. It is further dried in vacuum.

Yield: 0.23g, 18%

<sup>1</sup>H NMR (400MHz, D<sub>2</sub>O) δ 0.84 (3H, t), 1.27 (18H, s), 1.56 (2H, s), 2.68 (2H, s), 3.23 (2H, s)

**Synthesis of N-decyl glycine sodium salt:** Glycine ethyl ester hydrochloride (1g, 7mmol) and sodium bicarbonate (1.2g, 14mmol) are suspended into acetonitrile (40mL). The suspension is stirred and decyl bromide (1.58, 7mmol) is added dropwise in it. The mixture is refluxed overnight. After that, acetonitrile is evaporated and the residue is dissolved in dichloromethane (25mL). It is washed with 0.1M hydrochloric acid (25mL×2) and distilled water (25mL×1). Then dichloromethane is evaporated and the residue is dissolved in ethanol (20mL). 4M sodium hydroxide (1.75mL) in ethanol (3mL) is added dropwise to the solution which is stirred for 2 hours. The solvent is evaporated and the residue is recrystallized from DMSO water mixture (DMSO: water= 2:1).The solid is filtered and washed with ethanol to get white powder. It is further dried in vacuum.

Yield: 0.85g, 50%

$^1\text{H}$  NMR (400MHz,  $\text{D}_2\text{O}$ )  $\delta$  0.89 (3H, t), 1.29 (14H, s), 1.49 (2H, s), 2.56 (2H, s), 3.11 (2H, s)

**Synthesis of N-octyl glycine sodium salt:** Glycine ethyl ester hydrochloride (1g, 7mmol) and sodium bicarbonate (1.2g, 14mmol) are suspended into acetonitrile (40mL). The suspension is stirred and octyl bromide (1.38, 8mmol) is added dropwise in it. The mixture is refluxed overnight. After that, acetonitrile is evaporated and the residue is dissolved in dichloromethane (25mL). It is washed with 0.1M hydrochloric acid (25mL $\times$ 2) and distilled water (25mL $\times$ 1). Then dichloromethane is evaporated and the residue is dissolved in ethanol (20mL). Then 4M sodium hydroxide (1.75mL) in ethanol (3mL) is added dropwise to the solution which is stirred for 2 hours. After that, the solvent is evaporated and the residue is recrystallized from DMSO water mixture (DMSO: water= 2:1). The solid is filtered and washed with ethanol to get white powder. It is further dried in vacuum.

Yield: 0.66g, 45%

$^1\text{H}$  NMR (400MHz,  $\text{D}_2\text{O}$ )  $\delta$  0.83 (3H, t), 1.27 (10H, s), 1.45 (2H, s), 2.50 (2H, s), 3.11 (2H, s)

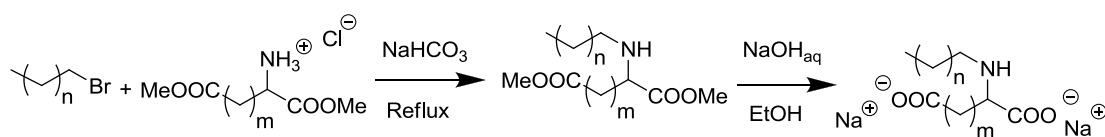


Figure 14 Synthesis of N-alkyl amino acids dicarboxylates ( $m=0, 1, 2$ ;  $n=6, 8, 10$ )

**Synthesis of N-dodecyl amino malonic acid di-sodium salt:** Amino malonic acid di-ethyl ester hydrochloride (1g, 5mmol) and sodium bicarbonate (0.8g, 10mmol) are suspended into acetonitrile (40mL). The suspension is stirred and dodecyl bromide (1.2g, 5mmol) is added dropwise in it. The mixture is refluxed overnight. After that, acetonitrile is evaporated and the residue is dissolved in dichloromethane (25mL). It is washed with 0.1M hydrochloric acid (25mL $\times$ 2) and distilled water (25mL $\times$ 1). Then dichloromethane is evaporated and the residue is dissolved in ethanol (20mL). Add 4M sodium hydroxide (2.5mL) in ethanol (3mL) dropwise into it. The precipitate is filtered and washed with ethanol. It is further dried in vacuum.

Yield: 1.26g, 80%

$^1\text{H}$  NMR (400MHz,  $\text{D}_2\text{O}$ )  $\delta$  0.81 (3H, t), 1.15 (18H, s), 1.43 (2H, t), 2.39 (2H, t), 3.61 (2H, t)

**Synthesis of N-decyl amino malonic acid di-sodium salt:** Amino malonic acid di-ethyl ester hydrochloride (1g, 5mmol) and sodium bicarbonate (0.8g, 10mmol) are suspended into acetonitrile (40mL). The suspension is stirred and decyl bromide (1.1g, 5mmol) is added dropwise in it. The mixture is refluxed overnight. After that, acetonitrile is evaporated and the residue is dissolved in dichloromethane (25mL). It is washed with 0.1M hydrochloric acid (25mL×2) and distilled water (25mL×1). Then dichloromethane is evaporated and the residue is dissolved in ethanol (20mL). Add 4M sodium hydroxide (2.5mL) in ethanol (3mL) dropwise into it. The precipitate is filtered and washed with ethanol. It is further dried in vacuum.

Yield: 0.86g, 60%

$^1\text{H NMR}$  (400MHz,  $\text{D}_2\text{O}$ )  $\delta$  0.83 (3H, t), 1.25 (14H, s), 1.45 (2H, t), 2.42 (2H, t), 3.61 (2H, t)

**Synthesis of N-octyl amino malonic acid di-sodium salt:** Amino malonic acid di-ethyl ester hydrochloride (1g, 5mmol) and sodium bicarbonate (0.8g, 10mmol) are suspended into acetonitrile (40mL). The suspension is stirred and octyl bromide (0.97g, 5mmol) is added dropwise in it. The mixture is refluxed overnight. After that, acetonitrile is evaporated and the residue is dissolved in dichloromethane (25mL). It is washed with 0.1M hydrochloric acid (25mL×2) and distilled water (25mL×1). Then dichloromethane is evaporated and the residue is dissolved in ethanol (20mL). Add 4M sodium hydroxide (2.5mL) in ethanol (3mL) dropwise into it. The precipitate is filtered and washed with ethanol. It is further dried in vacuum.

Yield: 0.76g, 58%

$^1\text{H NMR}$  (400MHz,  $\text{D}_2\text{O}$ )  $\delta$  0.82 (3H, t), 1.24 (10H, s), 1.45 (2H, t), 2.42 (2H, t), 3.61 (2H, s)

**Synthesis of N-dodecyl aspartic acid di-sodium salt:** Aspartic acid di-methyl ester hydrochloride (2g, 10mmol) and sodium bicarbonate (1.7g, 20mmol) are suspended into acetonitrile (40mL). The suspension is stirred and dodecyl bromide (2.5g, 10mmol) is added dropwise in it. The mixture is refluxed overnight. After that, acetonitrile is evaporated and the residue is dissolved in dichloromethane (25mL). It is washed with 0.1M hydrochloric acid (25mL×2) and distilled water (25mL×1). Then dichloromethane is evaporated and the residue is dissolved in ethanol (20mL). Add 10M sodium hydroxide (2mL) in ethanol (3mL) dropwise into it and stirred for 2 hours. 20mL of acetonitrile are added to precipitate the product. This product is filtered and washed with acetonitrile. It is further dried in vacuum.

Yield: 1.8g, 52%

$^1\text{H}$  NMR (400MHz,  $\text{D}_2\text{O}$ )  $\delta$  0.84 (3H, t), 1.25 (18H, s), 1.44 (2H, s), 2.24-2.44 (2H, m), 2.46-2.59 (2H, m), 3.33-3.37 (1H, m)

**Synthesis of N-decyl aspartic acid di-sodium salt:** Aspartic acid di-methyl ester hydrochloride (2g, 10mmol) and sodium bicarbonate (1.7g, 20mmol) are suspended into acetonitrile (40mL). The suspension is stirred and decyl bromide (2.2g, 10mmol) is added dropwise in it. The mixture is refluxed overnight. After that, acetonitrile is evaporated and the residue is dissolved in dichloromethane (25mL). It is washed with 0.1M hydrochloric acid (25mL $\times$ 2) and distilled water (25mL $\times$ 1). Then dichloromethane is evaporated and the residue is dissolved in ethanol (20mL). Add 10M sodium hydroxide (2mL) in ethanol (3mL) dropwise into it and stirred for 2 hours. . 20mL of acetonitrile are added to precipitate the product. This product is filtered and washed with acetonitrile. It is further dried in vacuum.

Yield: 1.35g, 60%

$^1\text{H}$  NMR (400MHz,  $\text{D}_2\text{O}$ )  $\delta$  0.82 (3H, t), 1.22 (14H, s), 1.46 (2H, s), 2.21-2.28 (2H, m), 2.51-2.59 (2H, m), 3.38-3.39 (1H, m)

**Synthesis of N-octyl aspartic acid di-sodium salt:** Aspartic acid di-methyl ester hydrochloride (2g, 10mmol) and sodium bicarbonate (1.7g, 20mmol) are suspended into acetonitrile (40mL). The suspension is stirred and octyl bromide (2.2g, 10mmol) is added dropwise in it. The mixture is refluxed overnight. After that, acetonitrile is evaporated and the residue is dissolved in dichloromethane (25mL). It is washed with 0.1M hydrochloric acid (25mL $\times$ 2) and distilled water (25mL $\times$ 1). Then dichloromethane is evaporated and the residue is dissolved in ethanol (20mL). Add 10M sodium hydroxide (2mL) in ethanol (3mL) dropwise into it which is stirred for 2 hours. . 20mL of acetonitrile are added to precipitate the product. This product is filtered and washed with acetonitrile. It is further dried in vacuum.

Yield: 1.7g, 59%

$^1\text{H}$  NMR (400MHz,  $\text{D}_2\text{O}$ )  $\delta$  0.83 (3H, t), 1.25 (10H, s), 1.47 (2H, s), 2.23-2.30 (2H, m), 2.49-2.61 (2H, m), 3.39-3.41 (1H, m)

**Synthesis of N-dodecyl glutamic acid di-sodium salt:** Glutamic acid di-methyl ester hydrochloride (2g, 9mmol) and sodium bicarbonate (1.35g, 18mmol) are suspended into acetonitrile (40mL). The suspension is stirred and dodecyl bromide (2.2g, 9mmol) is added dropwise in it. The mixture is refluxed overnight. After that, acetonitrile is evaporated and the residue is dissolved in dichloromethane (25mL). It is washed with 0.1M hydrochloric acid (25mL $\times$ 2) and distilled water (25mL $\times$ 1). Then dichloromethane is evaporated and the residue

is dissolved in ethanol (20mL). Add 10M sodium hydroxide (1.8mL) in ethanol (3mL) dropwise into it and stirred for 2 hours. 20mL of acetonitrile are added to precipitate the product. Such product is filtered and washed with acetonitrile. It is further dried in vacuum.

Yield: 0.8g, 26%

$^1\text{H}$  NMR (400MHz,  $\text{D}_2\text{O}$ )  $\delta$  0.84 (3H, t), 1.25 (18H, s), 1.44 (2H, s), 1.62-1.82 (2H, m), 2.12-2.17 (2H, m), 2.24-2.46 (2H, m), 3.30 (1H, t)

**Synthesis of N-decyl glutamic acid di-sodium salt:** Glutamic acid di-methyl ester hydrochloride (2g, 9mmol) and sodium bicarbonate (1.35g, 18mmol) are put into acetonitrile (40mL). The suspension is stirred and decyl bromide (2g, 9mmol) is added dropwise in it. The mixture is refluxed overnight. After that, acetonitrile is evaporated and the residue is dissolved in dichloromethane (25mL). It is washed with 0.1M hydrochloric acid (25mL $\times$ 2) and distilled water (25mL $\times$ 1). Then dichloromethane is evaporated and the residue is dissolved in ethanol (20mL). Add 10M sodium hydroxide (1.8mL) in ethanol (3mL) dropwise into it and stirred for 2 hours. 20mL of acetonitrile are added to precipitate the product. Such product is filtered and washed with acetonitrile. It is further dried in vacuum.

Yield: 0.68g, 23%

$^1\text{H}$  NMR (400MHz,  $\text{D}_2\text{O}$ )  $\delta$  0.82 (3H, t), 1.23 (18H, s), 1.45 (2H, s), 1.70-1.82 (2H, m), 2.14-2.18 (2H, m), 2.33-2.47 (2H, m), 3.18 (1H, t)

**Synthesis of N-octyl glutamic acid di-sodium salt:** Glutamic acid di-methyl ester hydrochloride (2g, 9mmol) and sodium bicarbonate (1.35g, 18mmol) are put into acetonitrile (40mL). The suspension is stirred and octyl bromide (1.7g, 9mmol) is added dropwise in it. The mixture is refluxed overnight. After that, acetonitrile is evaporated and the residue is dissolved in dichloromethane (25mL). It is washed with 0.1M hydrochloric acid (25mL $\times$ 2) and distilled water (25mL $\times$ 1). Then dichloromethane is evaporated and the residue is dissolved in ethanol (20mL). Add 10M sodium hydroxide (1.8mL) in ethanol (3mL) dropwise into it and stirred for 2 hours. 20mL of acetonitrile are added to precipitate the product. Such product is filtered and washed with acetonitrile. It is further dried in vacuum.

Yield: 1g, 37%

$^1\text{H}$  NMR (400MHz,  $\text{D}_2\text{O}$ )  $\delta$  0.82 (3H, t), 1.26 (18H, s), 1.46 (2H, s), 1.73-1.83 (2H, m), 2.13-2.17 (2H, m), 2.46-2.50 (2H, m), 3.04 (1H, t)

### 3.5.2 $\gamma$ -alkyl surfactant synthesis

The structures of  $\gamma$ -alkyl surfactants are shown in Figure 15:

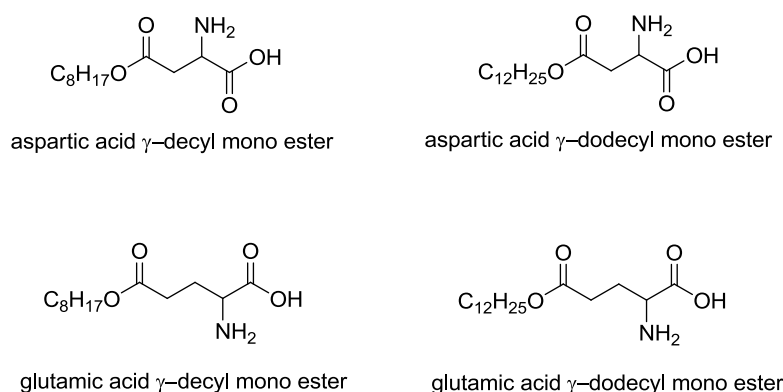


Figure 15 Chemical structures of  $\gamma$ -alkyl amino acids

The synthesis of these compounds has been described previously.[22]

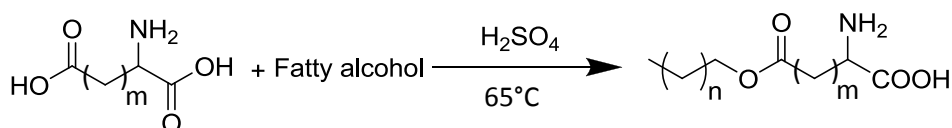


Figure 16 Synthesis of  $\gamma$ -alkyl aspartic acids ( $m=1, 2$ ;  $n=6, 10$ )

**Synthesis of  $\gamma$ -octyl L-aspartate:** The mixture of L-aspartic acid (4g, 30mmol) and 1-octanol (4.7g, 36.5mmol) was dissolved in tert-butanol (60ml). 95%-98% sulfuric acid 2.4 ml was added to the mixture dropwise by a syringe. The suspension was heated up to  $65^\circ\text{C}$  for 1 hour. After that ethanol (50ml) and triethylamine (6ml) were added to precipitate the product. The solid was filtrated and washed with methanol and water.

Yield: 2.7g, 37%

$^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3$ )  $\delta$  0.87 (3H, t), 1.28 (12H, s), 1.61 (2H, s), 2.19-2.30 (2H, m), 4.06-4.17 (1H, m)

**Synthesis of  $\gamma$ -dodecyl L-aspartate:** The mixture of L-aspartic acid (4g, 30mmol) and 1-dodecanol (6.8g, 36.5mmol) was dissolved in tert-butanol (60ml). 95%-98% sulfuric acid 2.4 ml was added to the mixture dropwise by a syringe. The suspension was heated up to  $65^\circ\text{C}$  for 1 hour. After that ethanol (50ml) and triethylamine (6ml) were added to precipitate the product. The solid was filtrated and washed with methanol and water.

Yield: 3.6g, 40%

**Synthesis of  $\gamma$ -dodecyl L-glutamate:** The mixture of L-glutamic acid (4g, 27mmol) and 1-dodecanol (10g, 53.8mmol) was dissolved in tert-butanol (60ml). 95%-98% sulfuric acid 2ml was added to the mixture dropwise by a syringe. The suspension was heated up to 65°C for 1 hour. After that ethanol (50ml) and triethylamine (8ml) were added to precipitate the product. The solid was filtrated and washed with methanol and water. The product was further purified by recrystallized in a 2:1 mixture of hot isopropanol/water.

Yield: 2.1g, 30%

**Synthesis of  $\gamma$ -dodecyl L-glutamate:** The mixture of L-glutamic acid (4g, 27mmol) and 1-dodecanol (10g, 53.8mmol) was dissolved in tert-butanol (60ml). 95%-98% sulfuric acid 2ml was added to the mixture dropwise by a syringe. The suspension was heated up to 65°C for 1 hour. After that ethanol (50ml) and triethylamine (8ml) were added to precipitate the product. The solid was filtrated and washed with methanol and water. The product was further purified by recrystallized in a 2:1 mixture of hot isopropanol/water.

Yield: 3g, 35%

### 3.5.3 Di-alkyl amino acids synthesis

The structures of di-alkyl amino acids are shown in Figure 17:

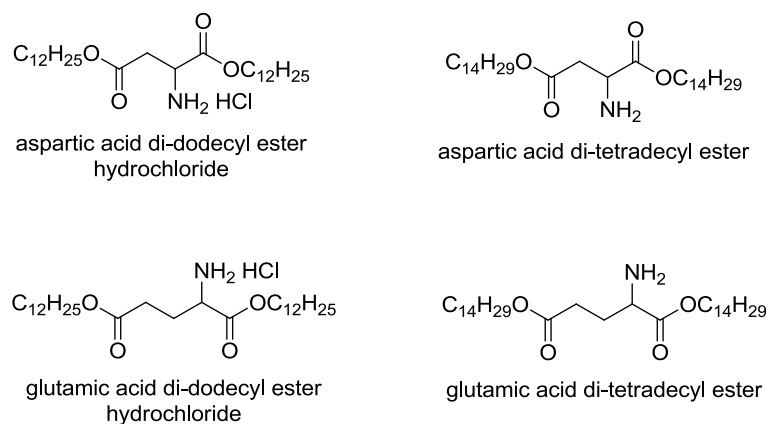


Figure 17 Chemical structures of di-alkyl amino acids

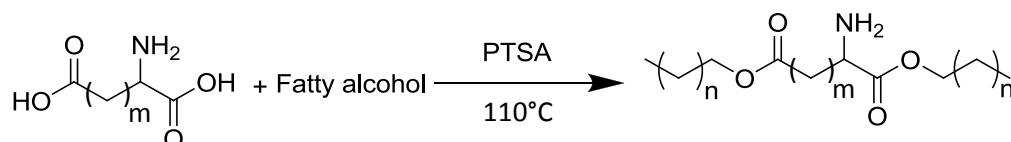


Figure 18 Synthesis of di-alkyl amino acids ( $m=1, 2$ ;  $n=10, 12$ )

**Synthesis of aspartic acid di-tetradecylester:** L-aspartic acid (4g, 30mmol) and p-Toluenesulfonic acid (6.5g, 32mmol) were dissolved in toluene (100ml) and refluxed for 1 hour at 110°C. 1-Tetradecanol (13.7g, 64mmol) was added to the solution, followed by stirring for 12 hours under reflux. The reaction mixture was then evaporated and dissolved in ethyl acetate (100ml). The ethyl acetate solution was washed with 10% sodium carbonate solution (100ml×2) and distilled water (100ml×1). After that ethyl acetate was evaporated and the residue was recrystallized from methanol to obtain a white powder. Such powder was further dried in vacuum.

Yield: 13g, 82%.

$^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3$ )  $\delta$  0.87 (6H, t), 1.29 (44H, s), 1.56-1.62 (4H, m), 2.70-2.78 (2H, m), 3.78-3.80 (1H, m)

**Synthesis of aspartic di-dodecyl ester hydrochloride acid:** L-aspartic acid (4g, 30mmol) and p-Toluenesulfonic acid (6.5g, 32mmol) in Toluene (100ml) and refluxed for 1 hour at 110°C. Dodecanol (12g, 64mmol) was added to the solution, followed by stirring for 12 hours under reflux. The reaction mixture was then evaporated and dissolved in ethyl acetate (100ml). The ethyl acetate solution was washed with 10% sodium carbonate solution (100ml×2) and distilled water (100ml×1). After that 12M hydrochloric acid solution (3ml) was added to precipitate the product. The product was filtered and washed with ethyl acetate. It was further dried in vacuum to obtain a white powder.

Yield: 9g, 60%.

$^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3$ )  $\delta$  0.86 (6H, t), 1.29 (36H, s), 1.53-1.59 (4H, m), 2.70-2.78 (2H, m), 3.73-3.74 (1H, m)

**Synthesis of glutamic acid di-tetradecyl ester:** L-glutamic acid (4g, 27mmol) and p-Toluenesulfonic acid (6.5g, 32mmol) were dissolved in toluene (100ml) and refluxed for 1 hour at 110°C. 1-Tetradecanol (12.8g, 60mmol) was added to the solution, followed by stirring for 12 hours under reflux. The reaction mixture was then evaporated and dissolved in ethyl acetate (100ml). The ethyl acetate solution was washed with 10% sodium carbonate solution (100ml×2) and distilled water (100ml×1). After that ethyl acetate was evaporated and the residue was recrystallized from methanol to obtain a white powder. Such powder was further dried in vacuum.

Yield: 10g, 69%.



$^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3$ )  $\delta$  0.87 (6H, t), 1.25 (44H, s), 1.59-1.62 (4H, m), 1.84-2.08 (2H, m), 2.43-2.47 (2H, m), 3.66 (1H, s)

**Synthesis of glutamic di-dodecyl ester hydrochloride acid:** L-glutamic acid (4g, 27mmol) and p-Toluenesulfonic acid (6.5g, 32mmol) in Toluene (100ml) and refluxed for 1 hour at  $110^\circ\text{C}$ . Dodecanol (11g, 60mmol) was added to the solution, followed by stirring for 12 hours under reflux. The reaction mixture was then evaporated and dissolved in ethyl acetate (100ml). The ethyl acetate solution was washed with 10% sodium carbonate solution (100ml $\times$ 2) and distilled water (100ml $\times$ 1). After that 12M hydrochloric acid solution (3ml) was added to precipitate the product. The product was filtered and washed with ethyl acetate. It was further dried in vacuum to obtain a white powder.

Yield: 10g, 71%

$^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3$ )  $\delta$  0.86 (6H, t), 1.25 (36H, s), 1.59-1.65 (4H, m), 1.84-2.11 (2H, m), 2.44-2.48 (2H, m), 3.47-3.50 (1H, m)

### 3.5.4 Other synthesis paths

The following synthesis paths were tried but the reactions did not happen.

Trial 1:

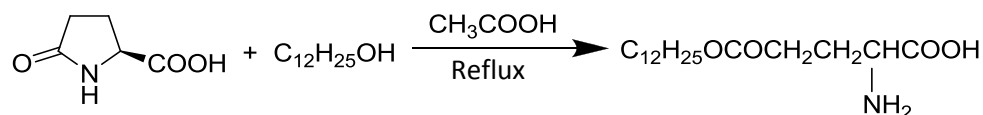


Figure 19 Planned synthesis paths of  $\gamma$ -dodecyl L-glutamate

**Synthesis of  $\gamma$ -dodecyl L-glutamate:** Pyroglutamic acid (0.33g, 2.5mmol), 1-dodecanol (0.48g, 2.6mmol) and acetic acid (14 $\mu$ l, 0.25mmol) were dissolved in acetonitrile (25ml). The suspension was refluxed for 6 hours. After that, the solvent was evaporated and the product was analyzed by NMR.

**Problem:** The product was expected to precipitate after the reaction was completed. However it has not happened. Instead the residue was evaporated and analyzed by NMR. The spectrum only showed the peaks of pyroglutamic acid and 1-dodecanol. Thus we have concluded that the reaction did not occur.

**Limitation:** Because the lactam ring that consists of three or four atoms is unstable, the selection of the starting material is limited to pyroglutamic acid, which contains five atoms in the lactam ring. Therefore this route can only be applied in the synthesis of dodecyl

glutamate. However even dodecyl glutamate is not able to precipitate after the reaction. So the following synthesis routes were tried thereafter.

Tria 2:

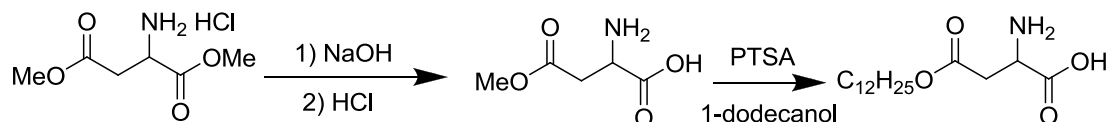


Figure 20 Planned synthesis paths of  $\gamma$ -dodecyl L-aspartate

**Step 1 - Synthesis of  $\gamma$ -methyl L-aspartate:** A two-neck flask was charged with aspartic acid dimethyl ester hydrochloride (0.5g, 2.37mmol) and acetonitrile (23ml). The flask was immersed in ice bath and 1M potassium hydroxide (4.74mmol) was added dropwise to the suspension. The mixture was kept in ice bath for 2 hours.

**Problem:** Niwayama and Cho (2009)[23] described that the experiment of two symmetric ester bonds selectively hydrolysis could be achieved at 0-4°C. However, in this experiment neither of the ester bonds was broken at such low temperature. When the temperature was raised to 25°C, one of the ester bond was broken. In this situation the reaction is incomplete and consequently the mono ester was contaminated with the di-ester. They are difficult to separate due to similar polarity.

**Limitation:** Even if  $\gamma$ -methyl L-aspartate was synthesized, in step 2 transesterification of methyl group would compete with esterification of carboxyl group in the presence of fatty alcohol. The similarity of their reaction conditions will bring more variables. As a result, the purification process would be more complicated.

Trial 3:

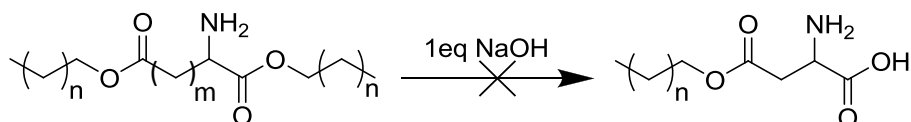


Figure 21 Planned synthesis paths of  $\gamma$ -dodecyl L-glutamate

**Limitation:** Originally such di-alkyl esters are planned as the starting material to synthesize  $\gamma$ -alkyl mono ester, which is illustrated in Figure 21. And the synthesis of di-alkyl esters works very well. However, the hydrolysis selectivity of these two ester bonds is difficult to control. As a result, the hydrolysis product is a mixture including both  $\alpha$ -alkyl mono ester

and  $\gamma$ -alkyl mono ester. And such mixture is difficult to separate because they show very similar polarity. Consequently this plan is abandoned.

## **IV Results and discussion**

### **4.1 Synthesis**

#### **4.1.1 Synthesis of $\gamma$ -alkyl amino acids**

$\gamma$ -alkyl amino acids were synthesized according to the strategy of fundamental path 1 as described in Figure 5 with slight modifications. The only difference is that the reacted carboxylic group of amino acids is on  $\gamma$  position of the side chain, not on  $\alpha$  position as illustrated in fundamental path 1. Following this strategy three possible preparation procedures were tried in this study. The first one is the ring-opening reaction of pyroglutamic acid, which is shown in Figure 19 that did not lead to the right products. The second one is to selectively hydrolyze one of the ester bonds of the di-alkyl esters according to the method of Niwayama and Cho (2009)[23], which are described in Figure 20 and 21. However the selectivity was poor and it resulted in difficulties of purification. The third one is to selectively create an ester bond according to the patent of Wassermann et al. (1966)[22] which works fine. Therefore  $\gamma$ -alkyl derivatives were obtained by reactions of primary alkyl alcohol of at least 8 carbon atoms with glutamic acid or aspartic acid in the presence of a strong acid catalyst, which is illustrated in Figure 16. A tertiary alcohol was used as a solvent and the temperature was kept under 65° in order to avoid any di-alkyl amino acids contaminates. However, the low solubility of  $\gamma$ -alkyl derivatives in various deuterium solvents made the characterization difficult. In further investigations it was found that this type of surfactants could be dissolved only when the amine group was completely protonated. Therefore the NMR characterization of this type of surfactants was conducted in deuterium chloroform with a drop of trifluoacetic acid. The yields of  $\gamma$ -alkyl derivatives range from 30% to 40% and they are half of the values of di-alkyl derivatives. It can be seen that low temperature and short reaction time effectively avoid forming corresponding di-alkyl derivatives but the yields are reduced due to the incomplete reaction of feedstock.

#### **4.1.2 Synthesis of di-alkyl amino acids**

A synthetic procedure modified from the literatures was used for the synthesis of the desired di-alkyl amino acids.[24, 25, 26] Primary alcohol of at least 12 carbons was reacted with aspartic acid or glutamic acid in the presence of PTSA. The reactants were dissolved in toluene and refluxed overnight. The extraction of products in the literatures was conducted in chloroform/water system. However it was found in the study that this combination may result in the formation of stable emulsions in the presence of di-alkyl amino acids

surfactants. Therefore chloroform/water was replaced by ethyl acetate/water in this study. The latter system shows evident phase separation compared with the other one. After ethyl acetate was evaporated, di-tetradecyl aspartate and glutamate were recrystallized from methanol at 4 degree. While di-dodecyl amino acids as their HCl salts were crystallized when a little amount of concentrated hydrochloric acid was dropped into the ethyl acetate solution. The di-alkyl derivatives were obtained in high yields (60%-82%).

#### **4.1.3 Synthesis of N-alkyl amino acids**

N-alkyl amino acids were prepared according to the strategy of path 4, which is illustrated in Figure 5. Methyl or ethyl esters of amino acids were used as the starting materials in order to protect the carboxylic groups. Esters of glycine, amino malonic acid, aspartic acid and glutamic acid hydrochloric salts were reacted with primary alkyl halogens with 8-12 carbons under refluxing overnight. The reactions were run in acetonitrile using 2 equivalents sodium bicarbonate as base. 1 equivalent is to activate the amine group of amino acids and the other equivalent is to neutralize the produced acid. The ester bonds were subsequently cleaved by alkali. The hydrolysis was performed in ethanol and sodium hydroxide solution. The sodium salts of N-dodecyl glycine and malonate were directly precipitated out under this condition while the other products not. N-octyl glycine and N-decyl glycine were crystallized in DMSO/water mixture. Aspartate and glutamate were crystallized by an addition of acetonitrile in the ethanol and sodium hydroxide solution. Glycinates, malonates and aspartates were obtained in overall good yields (ca. 60%). Glutamates have relatively lower yields due to their tendency of precipitation in ethanol and sodium hydroxide solution is not apparent as expected.

## **4.2 Physicochemical study of surfactants**

There are three types of surfactants synthesized in this study, which are  $\gamma$ -alkyl amino acids, di-alkyl amino acids and N-alkyl amino acids. Their physicochemical properties will be shown and discussed respectively in this chapter.

### **4.2.1 $\gamma$ -alkyl amino acids**

$\gamma$ -alkyl amino acids are insoluble in both water and most common organic solvent at room temperature. This is due to the intermolecular electrostatic attraction between ammonium and carboxylate group, which is illustrated in Figure 22. However, when the pH of the solution is adjusted to acid or alkaline, dispersing them becomes easier. This is because in acidic condition, the amine group is protonated, and in alkaline condition, the carboxylic acid is deprotonated thus negatively charged.

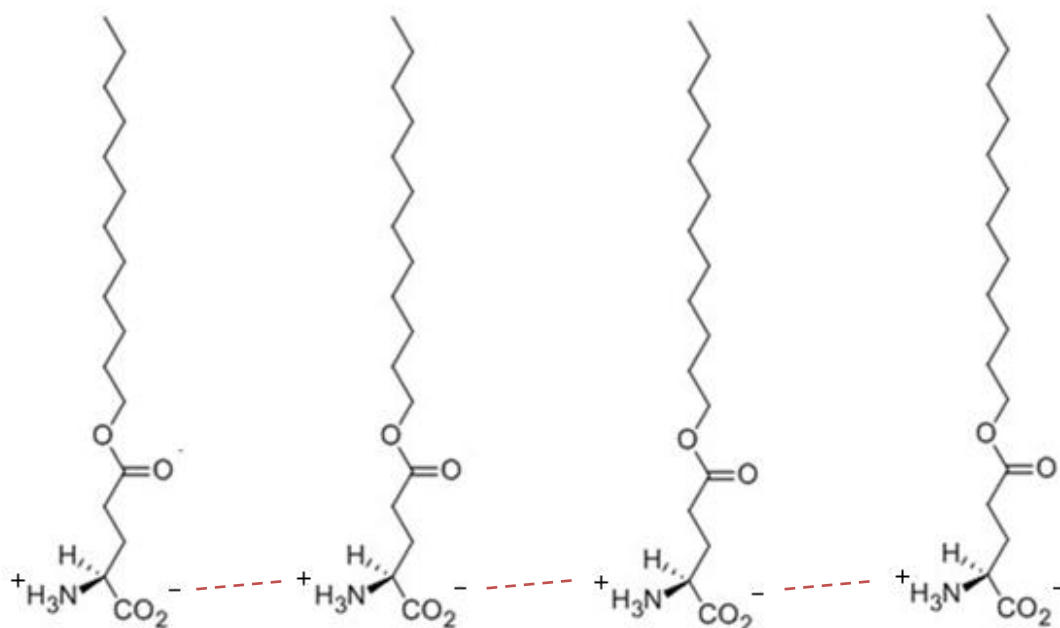


Figure 22 Inhibition of hydrophilicity due to electrostatic attraction between ammonium and carboxyl group

This situation can also be observed for N-lauroyl lysine, which is also called AMINHOPE LL as its commercial name. It is the functional powder developed by Ajinomoto Company in the formulation of foundations, lotions and creams. The chemical structure is compared in Table5.

Name	Structure
γ-dodecyl glutamic acid	
N-lauroyl lysine	

Table 5 Chemical structure comparison between γ-dodecyl glutamic acid and N-lauroyl lysine

Four properties are mentioned in the product description of AMIHOPE LL.[27]

1. Lubricity  
It has smooth and silky skin feel. When adding in foundations, it dramatically decreases the frictional coefficient.
2. Water and oil repelling property (except in strong acid and alkaline condition)

3. High adhesion property
4. Layer by layer structure observed by SEM.

The basic structure between this two chemical is very similar. Water and oil repelling property is also the same. Therefore it is possible that  $\gamma$ -alkyl amino acids also have the potential as functional powders and used in the formulation of cosmetics like AMIHOPE LL. Their property comparison has not been reported in any publication yet. Due to time restriction, it has not been further studied. It can be imagined that the presence of an amide bond increases the solubility of AMIHOPE LL, compared with the ester bond of  $\gamma$ -alkyl derivatives.

#### **4.2.2 Di-alkyl amino acids**

Di-alkyl amino acids have two hydrophobic tails, which make them easily soluble in organic solvents, such as chloroform and dodecane. However, their water solubility at room temperature is greatly decreased. According to Bancroft's rule, oil-soluble emulsifier tends to give water in oil emulsion[28] which is true for di-alkyl surfactants. For aspartic acid di-dodecyl ester the HLB number is calculated according to Davies method.[2] Tertiary amine gives 9.4, free ester gives 2.4, and methylene or methyl group gives -0.475. As a result, HLB number of aspartic acid di-dodecyl ester is 2.8. ( $HLB=9.4+2.4\times 2-0.475\times 24=2.8$ ) So it is supposed to be water in oil emulsifier. Theoretically two hydrophobic chains can be very helpful to stabilize emulsions. Proved by experiment, the emulsion of aspartic acid di-dodecyl ester in water/chloroform system does not separate for weeks. Moreover this surfactant is liquid at room temperature, which means it is very easy to disperse in other liquid system. Therefore this type of surfactants is believed to be very effective in formulating emulsions.

#### **4.2.3 N-alkyl amino acids**

N-alkyl amino acids are based on four types of structures, which are glycinate, malonate, aspartate and glutamate. Every type of surfactant has 8-carbon, 10-carbon and 12-carbon as the hydrophobic chain. Selecting glycine, amino malonic acid, aspartic acid and glutamic acid as study objects is due to their similar chemical structures. Glycine has only one carboxylic group whereas amino malonic acid, aspartic and glutamic acid have two carboxylic groups. The distance between two carboxylic groups is also different. Amino malonic acid has  $-\text{CH}-$  as the spacer group, whereas aspartic acid has  $-\text{CHCH}_2-$  and glutamic has  $-\text{CHCH}_2\text{CH}_2-$ . In the following part the effects of similar structures on physicochemical properties of surfactants will be discussed and eventually the results will be compared with the findings of previous work.[20]

#### 4.2.3.1 Krafft temperature

Prior to other measurements, the solubility of the surfactants at various pH was assessed in order to determine if the Krafft temperature was above or below room temperature. Krafft temperature was determined with conductivity measurements.

It is found that except C12Glycinate, C8, C10 and C12Malonate, the Krafft temperature of the rest surfactants are below room temperature, i.e. around 23°C. The Krafft temperatures of C8, C10 and C12Malonate are 40°C, 53°C and 67°C, respectively. It indicates that the solubility of these surfactants decrease as increasing the length of hydrophobic chain, as it could be expected.

In contrast with them, the Krafft temperature of C12Glycinate is pH dependent in a narrow range. It is below room temperature at pH12 but significantly increases at pH 11 and pH 13, which are shown in Figure 23 and 24.

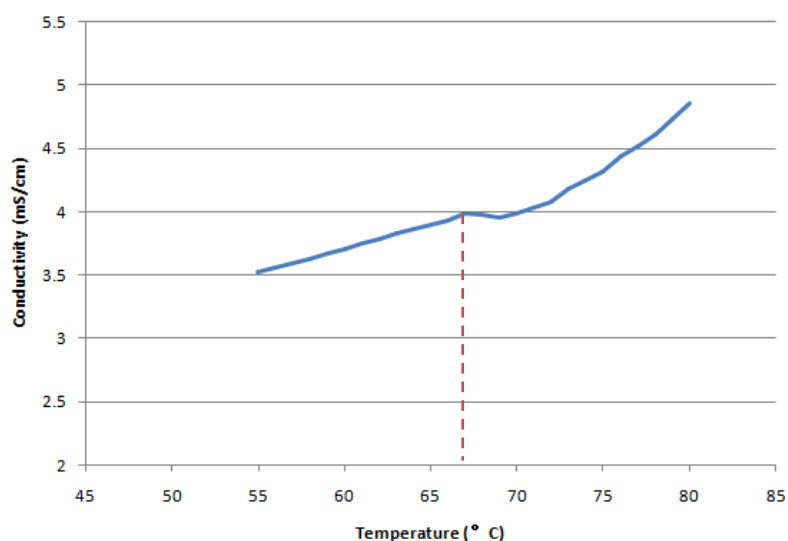


Figure 23 Krafft temperature of C12Glycinate at pH11

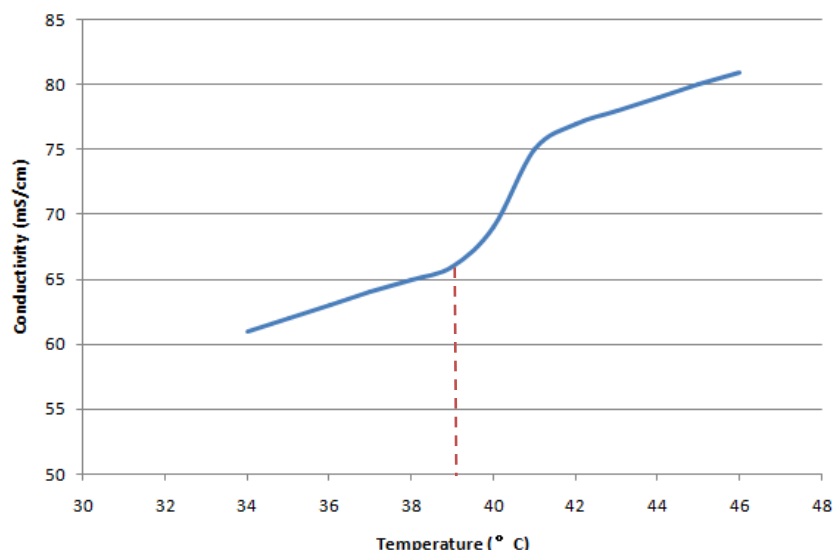


Figure 24 Krafft temperature of C12Glycinate at pH13

From the two figures above, the Krafft temperatures of C12Glycinate are 67°C at pH11 and 39°C at pH13. In addition its phase diagram was plotted at 20°C, which is illustrated in Figure 25. Different concentrations of C12Glycinate solutions were prepared and titrated with 0.1M HCl and 0.1M NaOH solution. The values of pH were determined with a pH electrode and the solubility was directly observed by the experimenter.

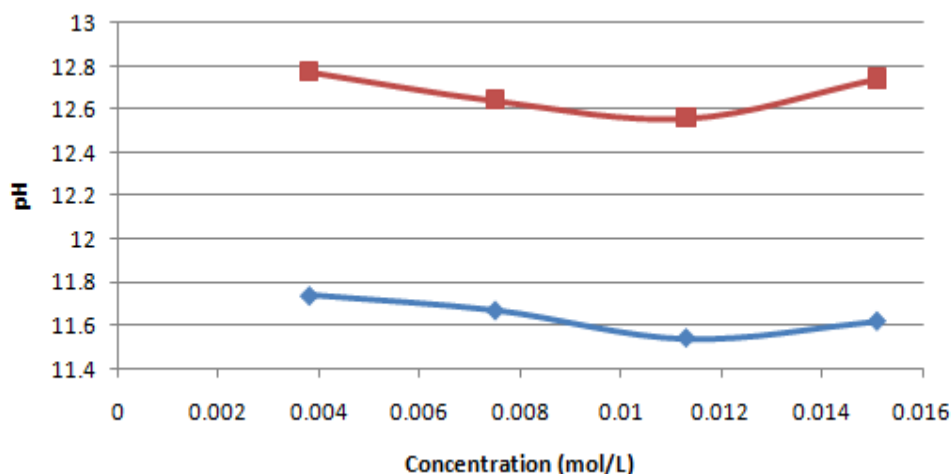


Figure 25 Phase diagram of C12Glycinate at 20°C

It was found that C12Glycinate was only soluble at 20°C in the range of pH11.8 and pH 12.5, below and above the Krafft temperature increases rapidly. N-alkyl type of surfactants has both amino groups and carboxyl groups in the molecule. For C12Glycinate, it only has one carboxyl group. The solubility dramatically decreases after the amino group or such carboxyl group is protonated. Although it has the amphoteric surfactant structure as well, it can only



behave as an anionic surfactant at room temperature. The protonation of amino group leads to significant increase of Krafft temperature. However C8Glycinate and C10Glycinate are soluble in a wider pH range compared with C12Glycinate due to a decrease of hydrophobicity. More pH effects on the solution properties of N-alkyl amino acids will be detailed in the later chapters. The Krafft temperature measurements above are used to settle the experimental conditions for determining the critical micelle concentrations in the following part.

#### **4.2.3.2 Surface tension comparison concerning the effect of alkyl chain length**

The critical micelle concentrations were determined by surface tension measurements. Different concentrations of surfactant solutions were prepared and their surface tensions were measured with a pendant drop tensiometer.

The critical micelle concentrations were also determined by conductivity measurements. The conductivity meter was a CDM 210 (Radiometer Copenhagen). The experiments were performed both automatically and manually. In automatic mode, the surfactant solution at a concentration above the CMC was diluted by addition of 0.2mL water every 20 second with an automatic burette 765 Dosimat (Metrohm). The conductivity meter and the automatic burette were controlled by computer. In manual mode, different concentrations of surfactant solutions were prepared and their conductivities were measured respectively. However, the break in the conductance-surfactant concentration curves, which was used to determine the value of CMC, was absent for all the measurements. One of the results is shown in Figure 26.

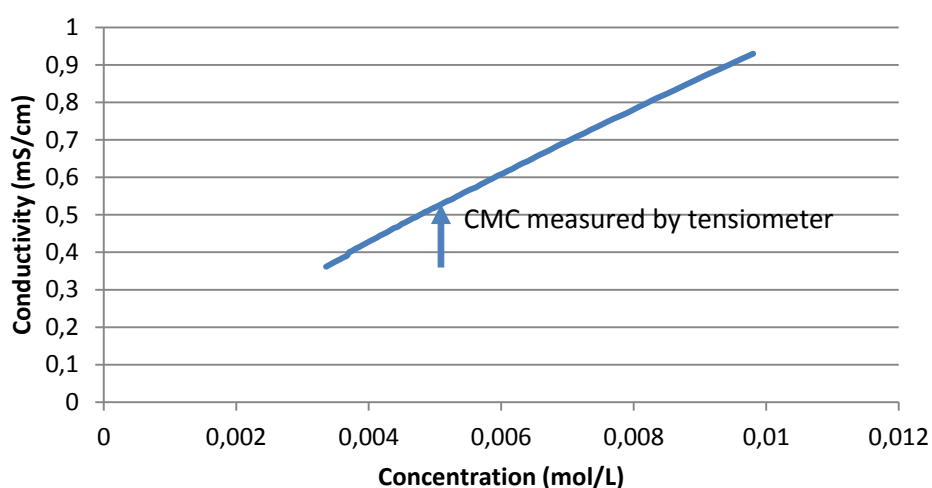


Figure 26 Plot of conductivity versus C8Glycinate concentration in aqueous solution at 20°C

It has been found that this curve break may also be smaller than expected or absent for anionic surfactants of structure  $RC(O)NR^1CH_2CH_2COONa$  or similar.[29, 30, 31] It was concluded in the literatures that this may be due to the release of the sodium ions upon micellization.

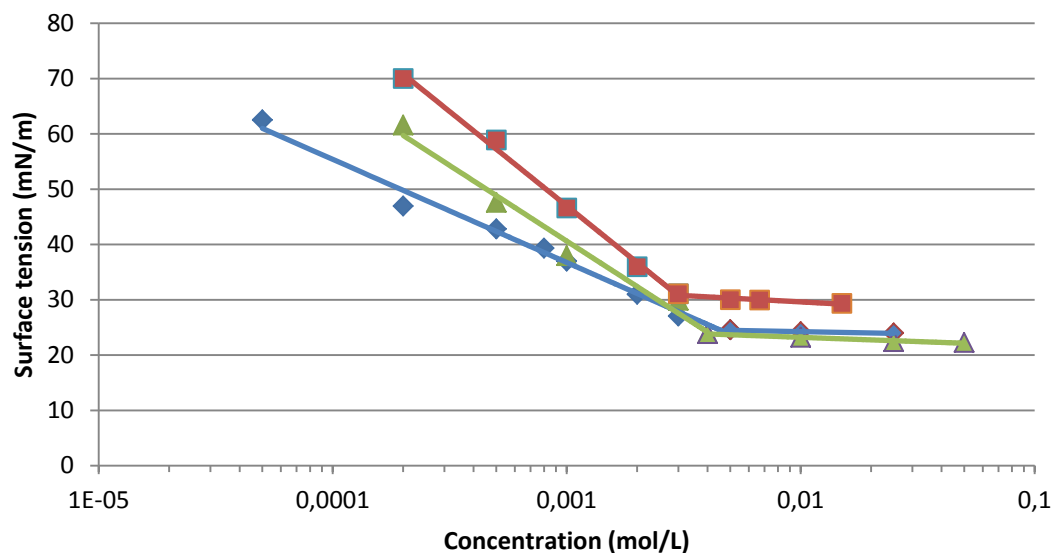


Figure 27 Surface Tension vs Concentration plot of N-alkyl glycine sodium salt at 20°C

C8Glycinate ( $\diamond$ ); C10Glycinate ( $\triangle$ ); C12Glycinate ( $\square$ )

Figure 27 shows concentration-surface tension plot of N-alkyl glycine sodium salt at 20°C. The surface tensions of C12Glycinate solutions were measured at pH12 due to the high Krafft temperature at other pH whereas for C8Glycinate and C10Glycinate the pH was not adjusted and was around. Data concerning CMC, surface tension at CMC ( $\gamma_{min}$ ) and area per molecule at interface are summarized in Table 6. As expected, the curves show first a decrease of the surface tension followed by a clear break at the CMC. The CMC value becomes smaller with an increase in hydrophobicity of alkyl groups (C12Glycinate < C10Glycinate < C8Glycinate), because stronger hydrophobic effect contributes to Gibbs energy of micellization. However, although the reason is not clear, the surface tension of C12Glycinate at CMC ( $\gamma_{min}$ ) is slightly higher than C8Glycinate and C10Glycinate.  $\gamma_{min}$  indicates the effectiveness of surface tension reduction. Therefore C8 and C10Glycinate are slightly more effective on surface tension reduction compared with C12Glycinate. With respect to the occupied area per molecule at the interface, the value of C12Glycinate is smaller than the other two. Such value generally decreases by the addition of an inorganic electrolyte because of the decrease in the electrostatic repulsion between the ionic head groups.[32] In the present case NaOH was added for controlling the pH.

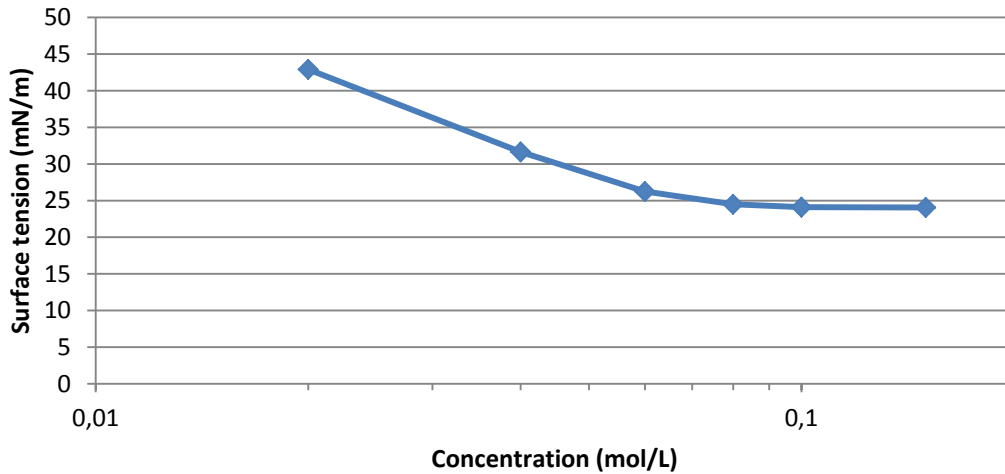


Figure 28 Surface Tension vs Concentration of N-alkyl amino malonic acid di-sodium salt at 40°C

C8Malonate (◇)

Figure 28 shows concentration-surface tension plot of N-octyl amino malonic acid di-sodium salt at 40°C. Krafft temperatures of N-decyl and N-dodecyl malonate are too high for surfactants that can be applied in daily life and render the CMC measurements very difficult. Therefore they are not plotted. No further studies are conducted concerning C10Malonate and C12Malonate due to their high Krafft temperature.

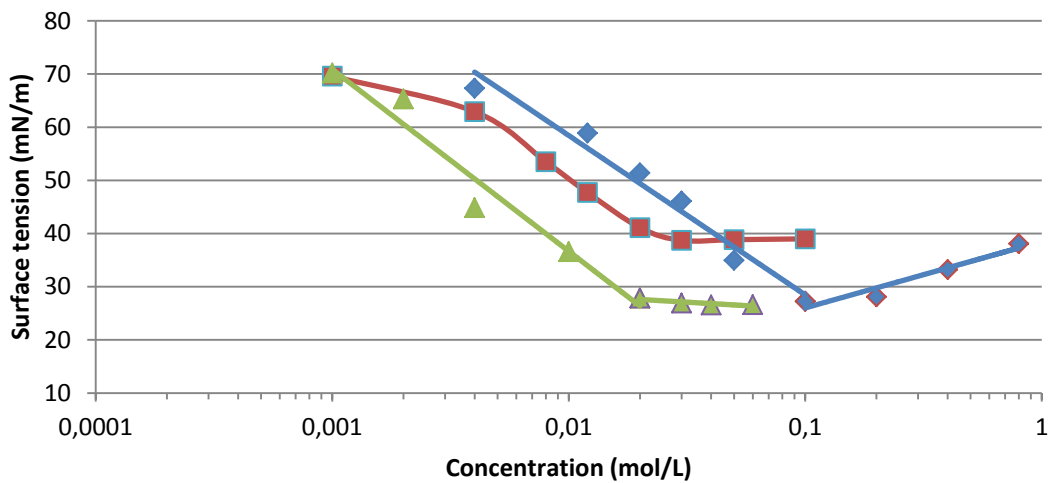


Figure 29 Surface Tension vs Concentration of N-alkyl aspartic acid di-sodium salt at 20°C

C8Aspartate (◇); C10Aspartate (△); C12Aspartate (□)

Figure 29 shows concentration-surface tension plot of N-alkyl aspartic acid di-sodium salt at room temperature. Three surfactants with different chain lengths are tested, which are

C8Aspartate, C10Aspartate and C12Aspartate. Data concerning CMC, surface tension at CMC ( $\gamma_{\min}$ ) and area per molecule at interface are summarized in Table 6. It is unexpected that the order of CMC is C12Aspartate $\approx$ C10Aspartate<C8Aspartate. There is very little difference between the minimum value of surface tension of C8Aspartate and C10Aspartate. However this value of C12Aspartate is higher compared with the two others. It is unexpected that C12Aspartate are less effective on reduction of surface tension. Concerning the value of CMC and  $\gamma_{\min}$  C10Aspartate shows a higher surface active than C12Aspartate. It may be because the surface tensions were measured by pendant drop tensiometer. Compared with the other surfactants, C10Aspartate are not able to keep the drop shape at the tip of syringe during the measurements. The drops of C10Aspartate easily fell down without respect to the concentration of solution. It rendered difficult to take the pictures and consequently brought errors on calculation of the surface tension. This may be solved by applying a different method for determination of surface tension of C10Aspartate. Nevertheless, we find here a similar trend as for the glycinate based surfactant. Concerning area per molecule at water/air interface, the values vary from 83Å<sup>2</sup> (C10Aspartate) to 95Å<sup>2</sup> (C8Aspartate), but they are at the same level.

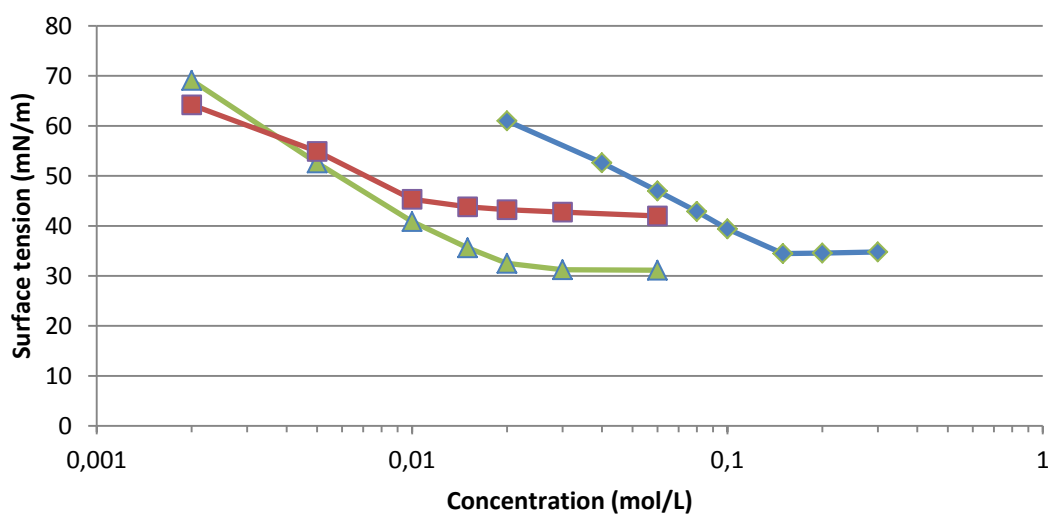


Figure 30 Surface Tension vs Concentration of N-alkyl glutamic acid di-sodium salt at 20°C

C8Glutamate (◇); C10Glutamate (△); C12Glutamate (□)

Figure 30 shows concentration-surface tension relationships of N-alkyl glutamic acid di-sodium salt at 20°C. Three surfactants with different chain lengths are tested, which are C8Glutamate, C10Glutamate and C12Glutamate. Data concerning CMC, surface tension at CMC ( $\gamma_{\min}$ ) and area per molecule at interface are summarized in Table 4. CMC follows a trend as C12Glutamate< C10Glutamate <C8Glutamate as expected due to the increase of

hydrophobicity. However, the order of  $\gamma_{\min}$  is C10Glutamate < C8Glutamate < C12Glutamate. C12Glutamate is unexpected less effective on reduction of surface tension than the other two and the reason is unclear. Concerning to area per molecule at interface the order is C10Glutamate < C8Glutamate < C12Glutamate. It is surprising that C12Glutamate are less surface active than C8Glutamate and C10Glutamate. But this shows the same picture as for the glycinate and the aspartate based surfactants.

Data concerning CMC, area per molecule and minimum value of surface tension of N-alkyl amino acids are listed in Table 6.

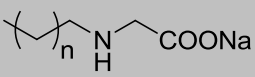
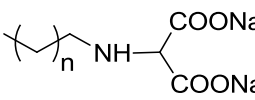
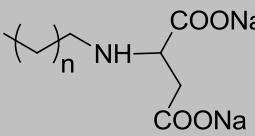
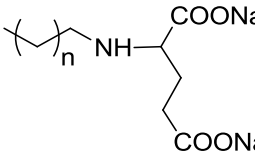
		CMC	Area per molecule	$\gamma_{\min}$
	C8	0.005mol/L	93 Å <sup>2</sup>	24.56 mN/m
	C10	0.004mol/L	85 Å <sup>2</sup>	25.61 mN/m
	C12	0.003mol/L	56 Å <sup>2</sup>	31.06 mN/m
	C8	0.07mol/L (40°C)	81 Å <sup>2</sup>	24.49 mN/m
	C10	KT 53°C		
	C12	KT 67°C		
	C8	0.1mol/L	95 Å <sup>2</sup>	27.25 mN/m
	C10	0.02mol/L	83 Å <sup>2</sup>	27.85 mN/m
	C12	0.02mol/L	91 Å <sup>2</sup>	41.10 mN/m
	C8	0.15mol/L	92 Å <sup>2</sup>	34.46 mN/m
	C10	0.025mol/L	74 Å <sup>2</sup>	32.52 mN/m
	C12	0.01mol/L	106 Å <sup>2</sup>	43.25 mN/m

Table 6 Data summarized from plots of surface tension vs. concentration of N-alkyl amino acids

In addition, an empirical equation was proposed by Klevens concerning the relation between CMC and the number of carbon atoms in the hydrophobic chain.[33]

$$\log \text{CMC} = A - BN$$

where A is a constant for a particular ionic head at a given temperature and B is constant  $\approx -0.3$  at 35°C for an ionic type of surfactant. According this equation, the relationship between log CMC and carbon number of glycinate, aspartate and glutamate are plotted in Figure 30.

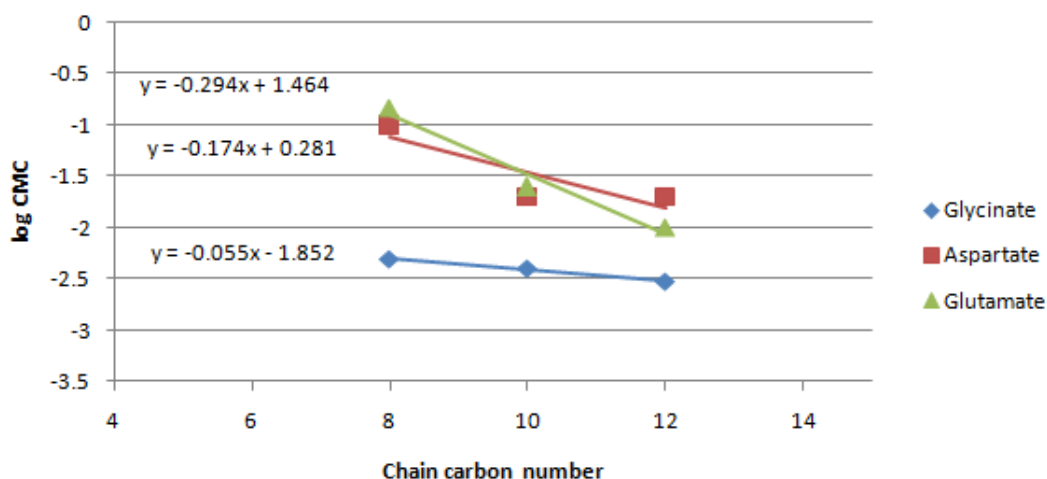


Figure 31 Plot of log CMC vs Chain length

Glycinate and glutamate have a better linear relationship than aspartate due to the latter one has similar CMC value in 10-carbon and 12-carbon. The obtained intercept of y-axis of the linear fits for glycinate, aspartate and glutamate is -1.85, 0.28 and 1.46, respectively. The order of it is glutamate > aspartate > glycinate, which indicates that the hydrophilicity of the surfactants decreases in the same order. As expected, additional carboxylic group gives better hydrophilicity. The values of conventional ionic surfactants like Na carboxylate soaps[34] and alkyl trimethylammonium bromides[35] are 1.8 at 20°C and 2.0 at 25°C respectively. They are higher than those values of N-alkyl amino acids, which indicate that they have better solubility than the latter ones. The obtained slope of the linear fits for glycinate, aspartate and glutamate is -0.06, -0.17 and -0.29, respectively. The value of glutamate is in line with those of conventional ionic surfactants (-0.3), meaning roughly 3-fold decrease in CMC per CH<sub>2</sub> group added. Those values of glycinate and aspartate are higher than those of conventional ionic surfactants, which indicate they still have high surface activity with relatively short hydrophobic chain.

#### 4.2.3.3 Surface tension comparison concerning spacer length between two carboxyl groups

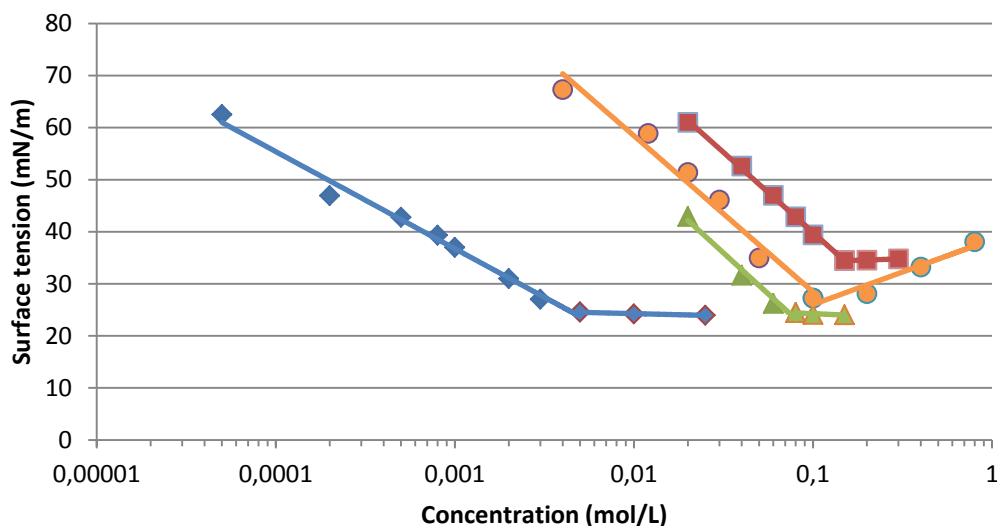


Figure 32 Comparison of N-alkyl amino acids with the same chain length

C8Glycinate ( $\diamond$ ); C8Malonate ( $\triangle$ ); C8Aspartate (O); C8Glutamate ( $\square$ )

Figure 32 shows the surface tension comparison of N-octyl derivatives concerning the spacer length between two carboxyl groups. Glycinate is used as a reference here. It is found that the CMC values of the surfactants with the same hydrophobic chain follow a trend as Glycinate << Malonate < Aspartate < Glutamate (even if the malonate has a Krafft temperature at 40°C). Surfactants with one carboxylic group have much lower CMC value than surfactants with two carboxyl groups. The difference is more than one order of magnitude. Among surfactants with two carboxylic groups, CMC values increase as the length of spacer group increases. The order of  $\gamma_{\min}$  shows the same result with CMC. The values of area per molecule at the interface are fairly similar. Therefore the order of surface activity is Glycinate > Malonate > Aspartate > Glutamate. Surface activity increases as decreasing the spacer length between two carboxyl groups when the surfactants have the same carbon number in the hydrophobic chain.

The possible reason is described as follows. Two carboxylic groups carry the same negative charges and have electrostatic repulsion when surfactants pack at the interface or form micelles. The increase of the spacer length enhances the repulsion. Therefore the packing of surfactants with larger spacer length is less dense.

#### 4.2.3.4 Surface tension comparison concerning amino and amido linkage surfactants

Amino linkage	CMC	Amido linkage	CMC (Reference) <sup>16</sup>
	0.005mol/L		0.01mol/L
	0.04mol/L		0.05mol/L
	0.01mol/L		0.05mol/L

Table 7 CMC comparison between amino and amido linkage surfactants with 12 carbon-atom chain

N-acyl derivatives of glycine, aspartic acid and glutamic acid have an amide group as the linkage between hydrophobic and hydrophilic moiety and therefore are called amido linkage surfactants. The CMC values of such surfactants with 12 carbon-atom chain are 0.01mol/L, 0.05mol/L and 0.05mol/L respectively. They were synthesized and measured in our laboratories during previous studies.[20] N-alkyl amino acids synthesized in this study have the same structures except the replacement of amido linkage by amino linkage. Amino linkage surfactants have lower CMC values and higher surface activities compared with amido linkage surfactants, which is listed in Table 7. They are both derived from amino acids, however the cationic property of amino linkage surfactants are lost because the amino group of the surfactants are acylated. The amino linkage surfactants are amphoteric and their amino group can carry a positive charge in micelles. The electrostatic attraction between the amino group and the carboxylic group makes the surfactants pack more densely at the interface and micellization is more favorable.

#### 4.2.3.5 Influence of pH on solubility and surface tension

N-alkyl type of surfactants has amino groups and carboxyl groups in the molecule. Their solution properties probably depend on the pH of the solution. Therefore C12Glycinate, C12Aspartate and C12Glutamate are selected to study the pH effects on both solubility and surface tension.



Solubility was measured by NMR according to Bordes et al.[20] The surfactant solutions were prepared at twice the CMC in D<sub>2</sub>O and the same amount of acetonitrile (5μL for 1mL of surfactant solution) was added as an internal standard. Required amount of hydrochloric acid was added and thoroughly mixed with the solutions. After that they were kept overnight before measurement. Relative amount of surfactants in solution was calculated by the ratio of the area of the peak of the terminating -CH<sub>3</sub> of the hydrophobic chain and -CH<sub>3</sub> of acetonitrile. Relative amount of dissolved surfactant as a function of molar ratio of added HCl to surfactant is plotted in Figure 33. The results of amido linkage surfactants with the same hydrophobic chains, which were measured at the same condition, are shown in Figure 34.[20] They were compared with each other to understand the role of amino and amido linkage at various pH.

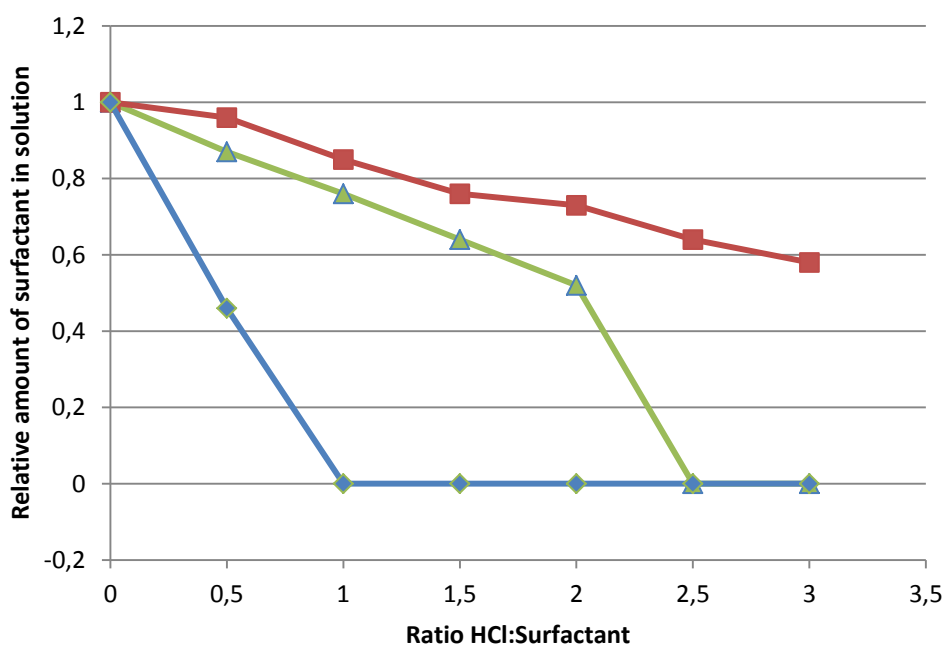


Figure 33 Relative amount of amino linkage surfactants in solution vs HCl/surfactant ratio

C12Glycinate (◇); C12Aspartate (△); C12Glutamate (□)

The plot shows that C12Glycinate is very sensitive to pH compared with the two others. It is easier to precipitate when pH is in acidic regime, which is due to the dramatic increase of Krafft temperature. C12Aspartate and C12Glutamate have higher acid tolerance than C12Glycinate as expected due to the additional carboxyl group which is able to be protonated. However it is still interesting to see that after the two carboxyl groups are protonated by 2 equivalents acid, C12Aspartate is still at 50% in solution and C12Glutamate is at 70% in solution (express in mole). If the titration continues, C12Aspartate completely precipitate by adding 2.5 times of hydrochloric acid. And C12Glutamate has even higher acid

tolerance. When adding 3 times of hydrochloric acid, there is still 60% of C12Glutamate in solution. This result is very different with amido linkage aspartate and glutamate, see Figure34. It is believed that the difference of acid tolerance is because of the presence of amino linkage.

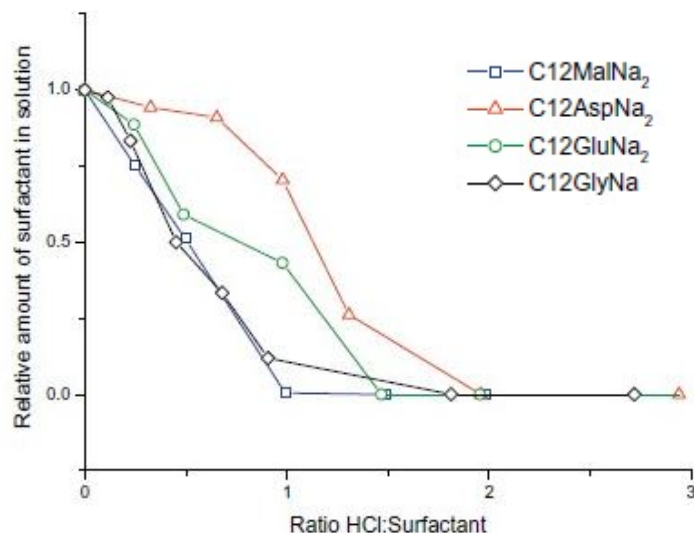


Figure 34 Relative amount of amido linkage surfactants in solution vs HCl/surfactant ratio [20]

By comparison, it is found that C12Glycinate with amino linkage has slightly lower acid tolerance than corresponding amido linkage surfactant while C12Aspartate and C12Glutamate have higher acid tolerance than their corresponding homologues. The difference of amino linkage and amido linkage is that the former one can be protonated in presence of acid while the latter one cannot. This protonation has negative effect on acid tolerance of C12Glycinate due to the tail biting effect, which is illustrated in Figure 35. The tail biting effect reduces the hydrophilicity of the polar headgroup. This effect can also be found with other alkyl  $\alpha$ -amino acid based surfactants.[11]

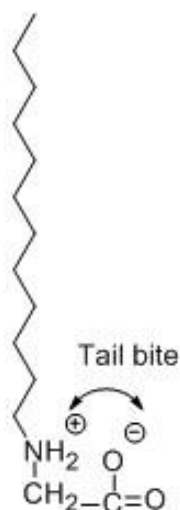


Figure 35 Tail biting effect of C12Glycinate

However, the protonation of amino linkage have positive effect on acid tolerance of C12Aspartate and C12Glutamate. The corresponding surfactants with amido linkage completely precipitated by adding 1.5 equivalent and 2 equivalent hydrochloric acid respectively, of which the curves are steeper and the resistance to precipitation on addition of acid are weaker. The positive effect of protonation of amino linkage on acid tolerance can be explained by three reasons. Firstly the tail biting effect is absent in two-carboxyl group surfactants. Secondly the protonation is a slow equilibrium process, which is illustrated in Figure 36. The surfactant solutions achieve the resistance to pH change due to the presence of an equilibrium between protonated surfactant (weak acid on the left) and unprotonated surfactant (its conjugate base on the right). When acid was added to the equilibrated mixture of protonated and unprotonated surfactant solution, the equilibrium is shifted to the left according to Le Chatelier's principle.

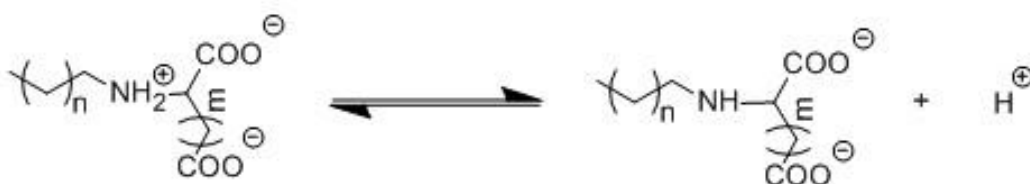


Figure 36 Equilibrium equation of N-alkyl aspartate and N-alkyl glutamate in the presence of acid (m=1, 2; n=6, 8, 10)

Thirdly, aspartate and glutamate have three dissociation states at different concentration of acid. When no acid is added, the surfactants produce two anions and both of the

counterions are  $\text{Na}^+$ . The solubility is provided by two carboxyl groups. After 1 equivalent acid is added, the carboxyl group on  $\alpha$  position is protonated. Positive charge is transferred to the amine group and these two groups keep neutrality. The other carboxyl group is still ionized. Meanwhile the solubility decreases due to the lost of sodium ion but it is compensated by the protonation of amine group. After 2 equivalent acid is added, two carboxyl groups are protonated and the surfactant produce a cation on the amine group. At this time the solubility only relies on the protonated amine group and still a certain amount of surfactants are soluble. However, amido linkage surfactants completely precipitate in this situation due to the lack of ammonium.

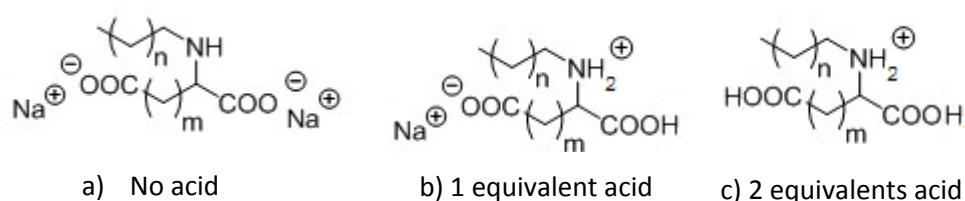


Figure 37 Dissociation state of N-alkyl aspartate and N-alkyl glutamate in the presence of acid ( $m=1, 2$ ;  $n=6, 8, 10$ )

Beside the bulk study, the effect of pH on surface tension was also studied. The surfactant solutions were prepared at twice the CMC and required amount of hydrochloric acid was added. They were thoroughly mixed and the surface tensions were measured by a pendant drop tensiometer respectively. The advantage of using pendant drop method instead of the du Noüy ring method here is because the viscosity is not an influent factor for the former method.

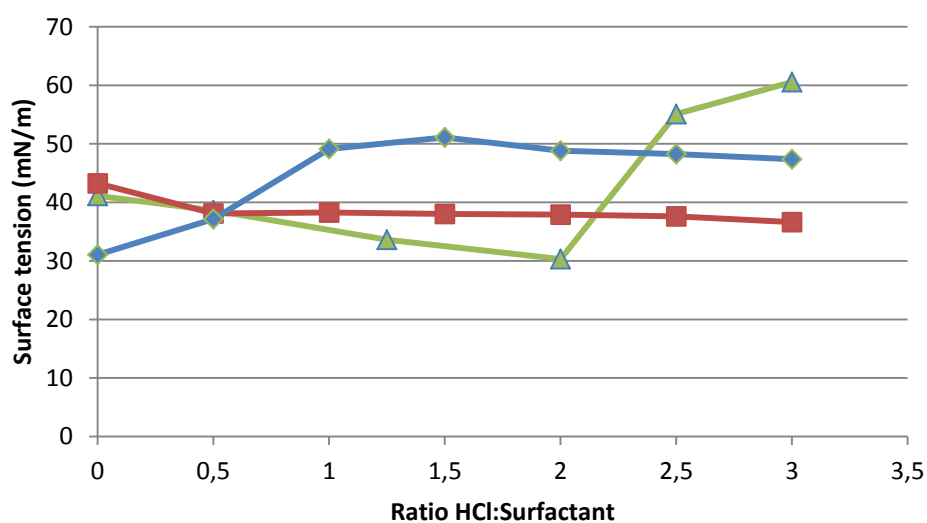


Figure 38 Surface tension vs HCl/surfactant ratio at 20°C

C12Glycinate ( $\diamond$ ); C12Aspartate ( $\triangle$ ); C12Glutamate ( $\square$ )

Figure 38 shows the surface tension change by adding 1M hydrochloric acid at 20°C. It helps to understand the aggregation behavior of N-alkyl amino acids at various pH. Surface tension of C12Glycinate increase by adding hydrochloric acid due to the precipitation of surfactant, which is in agreement with Figure 33. It keeps constant when ratio reaches 1:1. This ratio indicates surfactant is completely protonated at that moment the surface tension is at  $50 \text{ mN}\cdot\text{m}^{-1}$ . Surface tension of C12Aspartate and C12Glutamate decrease by adding acid. It indicates that the surface activity of form b) in dissociation state increases compared with form a). It is because the zwitterionic structure of form b) (see Figure 37) makes the molecules pack more densely due to electrostatic attraction and micellization is therefore more favorable. Meanwhile the value of CMC and area per molecule at the interface can probably be reduced in form b). After the addition of 2 equivalents of acid, the surface tension of C12Aspartate starts to significantly increase due to the precipitation. This is corresponding with the dramatic decrease of solubility, which is shown in Figure 33. Surface tension of C12Glutamate keeps constant at this condition due to the combined influence of the surfactant precipitation and decrease of CMC. Most of C12Glutamate are still dissolved and the value of CMC is reduced. Therefore surface tension equals to  $\gamma_{\text{min}}$  and keeps constant.

#### **4.2.3.5 Influence of calcium ion on solubility and surface tension**

Lime tolerance is evaluated by measuring the surfactant solubility during the addition of calcium chloride to the surfactant solution. Solubility was measured by NMR according to Bordes et al.[20] Surfactant solutions were prepared at twice the CMC in  $\text{D}_2\text{O}$  and acetonitrile ( $5\mu\text{L}$  for  $1\text{mL}$  of surfactant solution) was added as an internal standard. Required amount of calcium chloride was added and thoroughly mixed with the solutions. After that they were kept overnight before measurement by NMR. Relative amount of surfactants in solution was calculated by the ratio of the area of the peak of the terminating  $-\text{CH}_3$  of the hydrophobic chain and  $-\text{CH}_3$  of acetonitrile. Relative amount of dissolved surfactant as a function of molar ratio of added  $\text{CaCl}_2$  to surfactant is plotted in Figure 39. The results of amido linkage surfactants with the same hydrophobic chains, which were measured at the same condition, are shown in Figure 40.[20]

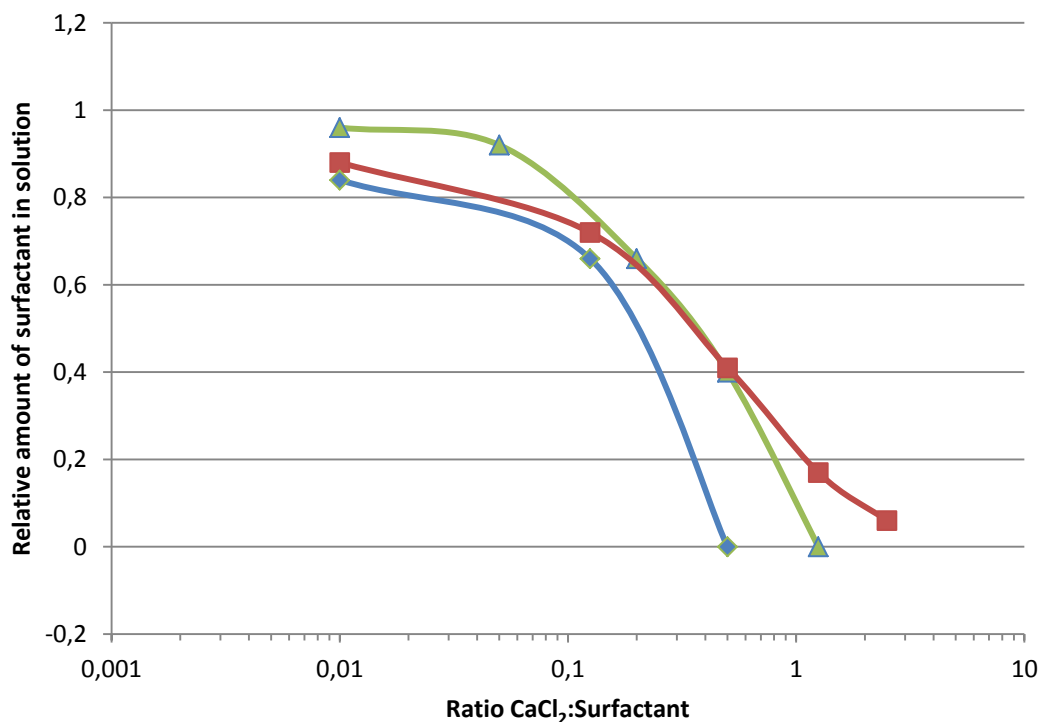


Figure 39 Relative amount of surfactant in solution vs CaCl<sub>2</sub>/surfactant ratio at 20°C

C12Glycinate (◇); C12Aspartate (△); C12Glutamate (□)

The curve of C12Glycinate is steeper than the two others, which indicates that it has the lowest lime tolerance. This is due to C12Glycinate which has only one carboxylic group and one equivalent calcium chloride will make two equivalents C12Glycinate precipitate. And this is true from the observation that the precipitation of C12Glycinate is immediate while C12Glutamate precipitate slowly. C12Glutamate has the highest lime tolerance in the presence of high concentration of CaCl<sub>2</sub>. There is still 6% C12Glutamate in solution when adding 2.5 equivalents CaCl<sub>2</sub>. However the decrease tendency of C12Glutamate is more apparent than C12Aspartate at moderate addition of CaCl<sub>2</sub>. Also, it is observed that C12Aspartate forms white gels at high concentration of CaCl<sub>2</sub>. Gelification can induce a broadening of the peak in NMR spectrum which can also be interpreted as a loss of solubility.

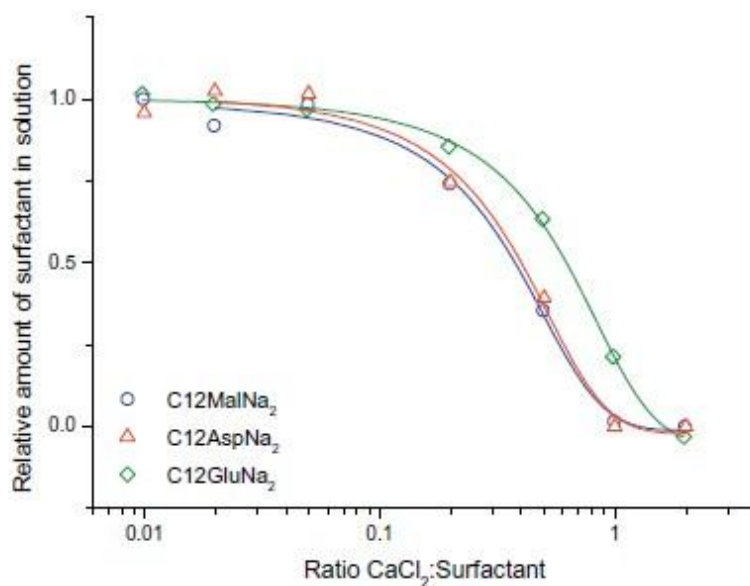


Figure 40 Relative amount of amido linkage surfactants in solution vs CaCl<sub>2</sub>/surfactant ratio at 20°C [20]

The most significant difference with amido linkage appears to be the gelation of C12Aspartate with amino linkage. Furthermore the decrease tendency of amino linkage surfactants are steeper at moderate addition of CaCl<sub>2</sub> and secondly amino linkage C12Glutamate do not completely precipitate at high concentration of CaCl<sub>2</sub>, while amido linkage surfactants are completely precipitated.

In addition, the effect of CaCl<sub>2</sub> on surface tension was also studied. The surfactant solutions were prepared at twice the CMC and required amount of CaCl<sub>2</sub> was added. They were thoroughly mixed and the surface tensions were measured by a pendant drop tensiometer. The concentration of CaCl<sub>2</sub> in seawater is marked as a line in the plot to consider the usability of N-alkyl amino acids in seawater. The results are compared with the plot of amido linkage surfactants, which is illustrated in Figure 42.

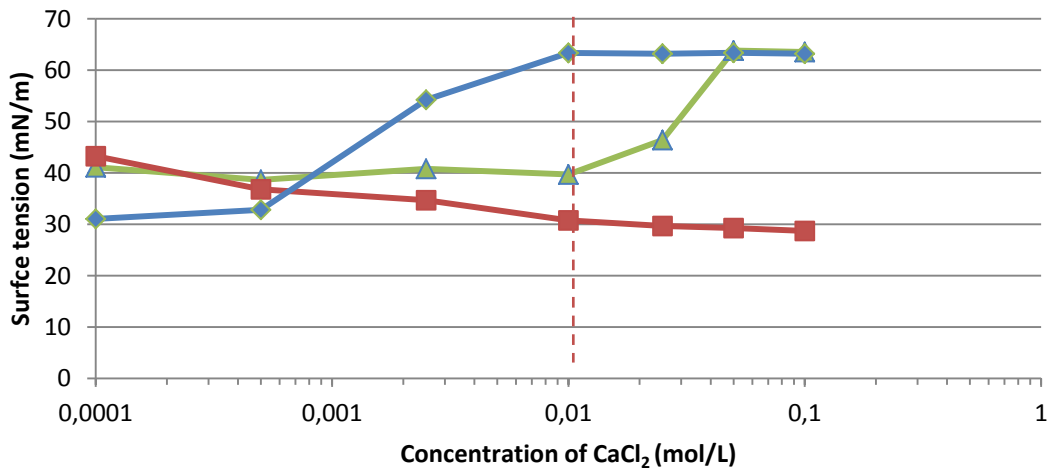


Figure 41 Surface tension vs CaCl<sub>2</sub> concentration at 20°C

C12Glycinate (◇); C12Aspartate (△); C12Glutamate (□)

Figure 41 shows the plot of surface tension as a function of CaCl<sub>2</sub>. Surface tension of C12Glycinate increases as increasing CaCl<sub>2</sub> concentration due to their precipitation. It reaches a maximum value and keeps constant in the presence of 10mM CaCl<sub>2</sub>. Therefore it will not show a good surface activity in seawater. Surface tension of C12Aspartate keeps constant at moderate addition of CaCl<sub>2</sub> and it starts to increase above the concentration of CaCl<sub>2</sub> in seawater due to gelation. The situation is different for C12Glutamate. For this surfactant the surface tension gradually decreases by adding CaCl<sub>2</sub> Even if it was observed that there was a considerable precipitation of C12Glutamate.

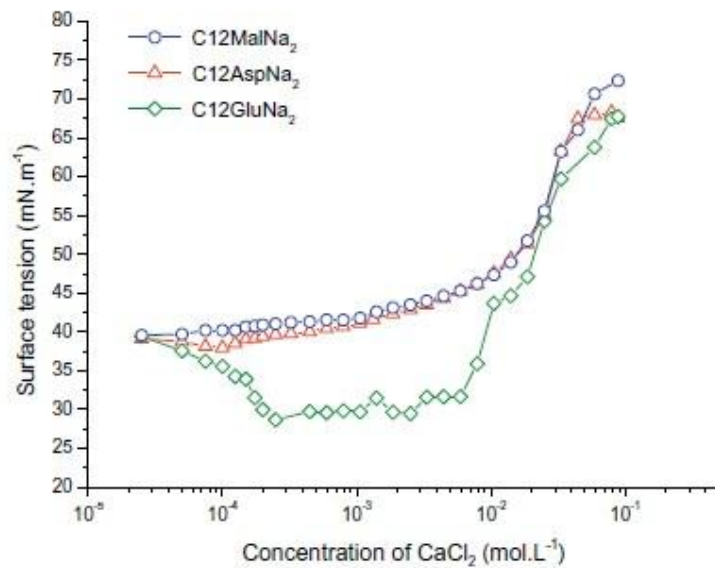


Figure 42 Surface tension of amido linkage surfactants vs concentration of CaCl<sub>2</sub> at 20°C [20]



The curve of C12Aspartate with amino linkage is similar to the amido aspartate. Both of them lead to an increase of surface tension due to precipitation in the presence of 0.01-0.02 mol/L calcium ions. C12Glutamate differs from its amido linkage equivalent. Although there is a considerable precipitation of C12Glutamate with amino linkage in high concentration of calcium chloride solution as well, the surface tension slightly decreases instead of the rapid increase of amido glutamate. It can be explained that CMC of C12Glutamate with amino linkage is reduced to a very low value and 6% of C12Glutamate is enough for micellization, because the calcium ions bind with two carboxyl groups of adjacent surfactant molecules (Figure 43 right) instead of two carboxyl groups within the same molecule (Figure 43 left). The former situation reduces the Gibbs energy for micellization.  $\gamma_{\min}$  is slightly reduced as well and the surface tension equals to  $\gamma_{\min}$  at high concentration of  $\text{CaCl}_2$ . The latter situation leads to the immediate precipitation.

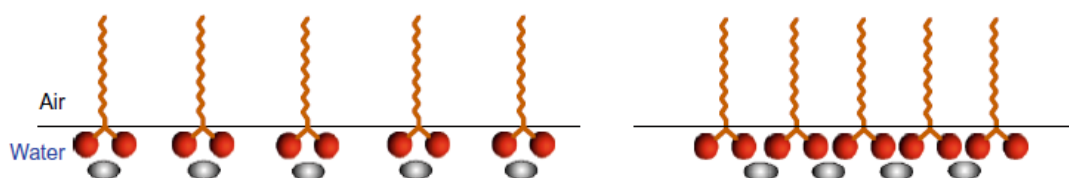


Figure 43 Intramolecular (left) and intermolecular (right) binding of calcium by dicarboxylic surfactant at air-water interface. [20]

## VI Conclusion

Three types of amino acid based surfactants are synthesized and studied in this work, which are  $\gamma$ -alkyl amino acids, di-alkyl amino acids and N-alkyl amino acids.

$\gamma$ -alkyl amino acids include  $\gamma$ -octyl aspartic acid,  $\gamma$ -octyl glutamic acid,  $\gamma$ -dodecyl aspartic acid and  $\gamma$ -dodecyl glutamic acid. All of them are insoluble in water and in most common organic solvents due to intermolecular electrostatic attraction between ammonium and carboxyl group. It may be possible to employ them as the functional additives in the formulation of powder cosmetics.

Di-alkyl amino acids include di-dodecyl aspartic acid, di-dodecyl glutamic acid, di-tetradecyl aspartic acid and di-tetradecyl glutamic acid. All of them are very soluble in organic solvents but insoluble in water at room temperature. They are able to form stable water in oil emulsions due to good dispersing ability and double hydrophobic chains.

N-alkyl amino acids consist of N-alkyl glycinate, N-alkyl amino malonate, N-alkyl aspartate and N-alkyl glutamate. Their hydrophobic chains are 8-carbon, 10-carbon and 12-carbon, respectively. Krafft temperature of malonate is above room temperature and it increases as the chain length increases. C12Glycinate are soluble in aqueous solution at room temperature only when the pH is between 11.8 and 12.5 due to tail biting effect. Except C12Glycinate and Malonate, the other surfactants are very soluble in aqueous solution at room temperature.

CMC of N-alkyl amino acids cannot be measured by conductivity because the curve break is absent. It was determined by tensiometry a linear relationship of log CMC with chain length for all surfactants. The values of glutamate are similar with conventional ionic surfactants while glycinate and aspartate show high surface activity with relatively shorter hydrophobic chain.

Concerning spacer length between two carboxyl groups, the values of CMC follow a trend as Glycinate << Malonate < Aspartate < Glutamate. CMC of surfactant with one carboxylic group is much lower than for dicarboxylate surfactants. CMC increases as distance between carboxylic groups increases. Surface activity increases as decreasing the spacer length between two carboxyl groups when the surfactants have the same hydrophobe.

As amphoteric surfactants, surface activity of amino linkage N-lauryl amino acids is higher than anionic amido linkage N-lauroyl amino acids.

Acid and lime tolerance are dramatically increased by additional carboxyl group and they further increase as the length of spacer group between two carboxyl groups increase. The order of acid and lime tolerance is C12Glycinate < C12Aspartate < C12Glutamate.

Compared with dicarboxyl amido linkage surfactants, dicarboxyl amino linkage surfactants have wider pH range and the surface activity increases with moderate addition of acid.

Dicarboxyl N-alkyl amino acids have a chelating effect in the presence of calcium ions and C12Aspartate forms white gel. C12Glutamate shows high surface activity at high concentration of calcium ions and has the potential to be used in seawater.

## **VII Further studies**

Biodegradability of N-alkyl amino acids needs further study. Usability of  $\gamma$ -alkyl amino acids as functional powders in cosmetic can be investigated and compared with N-lauroyl lysine. The performance of di-alkyl amino acids as emulsifier and emulsion stabilizer can be further studied.

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