Oil spill remediation by mixtures of amino acid-based surfactants and dodecyl glucoside: Effect of pH and salinity

Master of Science Thesis in the Master Degree Programme, Materials and Nanotechnology

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Master Thesis

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Abstract

Marine oil spills are a major threat to our marine environment and it is of interest to find environmentally friendly and efficient ways of removing oil spill from the ocean. A conventional method today is to use oil dispersants, which will reduce the surface tension at the oil/water interface and thereby create small oil drops, which can be degraded by oceanic microbes. Today, environmentally harmful surfactants are commonly used for oil spill remediation after a big oil leak. The demand is to find a more environmentally friendly surfactant for oil spill remediation applications. In this work, three amino- acid based biodegradable surfactants, dodecyl glutamate, dodecyl glycinate, dodecyl sarcosinate and one sugar based biosurfactant, dodecyl glucoside, are evaluated for their potential to perform as environmentally friendly oil dispersants.

The goal was to find mechanisms governing interfacial behavior of biosurfactants mixed systems and the role of structural variations. In this project, it was determined how well ionic biosurfactants align at interfaces and surfaces by evaluating structure property-performance related to molecular structure of the biosurfactants. Mixtures of biosurfactants were also evaluated for antagonistic or synergistic effects that may have occurred between a nonionic and an ionic biosurfactants.

Surface tension and CMCs, emulsion stability and interfacial tension were measured for both pure and mixed systems of biosurfactants. The biosurfactant systems were evaluated under conditions similar to oceanic and the effect of sodium chloride, calcium chloride and pH was studied. The analysis showed very promising results for the use of mixtures of biosurfactants. Systems containing dodecyl glutamate were of particular interest because these showed emulsification capacity even in the presence of a concentration of $CaCl_2$ that was the same as in sea water. Dodecyl glycinate on the other hand gave good results when used in a NaCl solution of the same ionic strength as in sea water.

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Content

1. Introduction

1.1 Oil spills are a major threat to the environment

Oil spills are by far the most serious threat to the marine environment. The number of major oil spills have decreased in the past decade [1], but in April 2010, BP experienced a huge oil leak in the gulf of Mexico and the oil discharge had to be stopped before it would have contaminated the entire gulf [2] . Assessments of the impact on the environment show that more than 8,000 sea turtles, birds and marine mammals were injured or dead, six months after the spill and the long term damages from the oil and use of surfactants are hard to evaluate [3]. If most of the oil spill would have stayed at sea, it would have affected fisheries but less spawning and shoreline habitats than previous giant spills [2].

1.2 Ways of removing the oil from vast oil spills

Common ways of removing oil spill include mechanical containment booms, skimmers and sorbents [1]. Containment booms are used to prevent the oil from spreading. It acts like a fence, trapping the oil, which makes it easier for removal and prevents spreading to the shoreline. Skimmers are used similarly to a household vacuum cleaner or a pump to suck up oil from the surface. The drawback with this technique is that the surface needs to be free of debris to prevent clogging of the skimmer. A debris free surface is a condition that is difficult to achieve in the ocean. In areas that cannot be reached by skimmers, sorbents can be used to either adsorb or absorb small traces of oil by using different materials, natural organic, natural inorganic or synthetic[4] .

One of the major ways of treating marine oil spills is by using oil dispersants. The surface tension is reduced at the oil/water interface, which makes the oil´s surface forces lessened and the oil can be broken up, and the spill is less likely to reach shore. Natural forces like currents and waves increases the dispersion of the droplets and eventually drops are broken down by the ocean´s microbes and bacteria [1][5]. An unusual group of marine hydrocarbon-degrading bacteria has proven to have an important role in the biological removal of hydrocarbons from polluted oceans. When an oil spill occurs, a successive bloom of limited amount of marine micro bacteria takes place. The blooms of bacteria result in a rapid degradation of many oily constituents and this process can be accelerated by limiting nutrients for the bacteria [6].

In the research investigations on oil spill remediation it is of interest to evaluate greener oil dispersants. Therefore, biosurfactants based solutions to the problems of spreading of oil and cleaning of the spill from the shore are evaluated. The demand and interest for biosurfactants has been steadily increasing and they are likely to eventually replace their environmentally unfriendly counterparts in many applications [7].

Historically synthetic dispersants have been used for oil spill remediation but are debated to be toxic [8]. In this regard, biosurfactants are a less harmful alternative since they are biodegradable. One of the major problems with biosurfactants is to identify a suitable one, in a mixture or pure, that will create a stable emulsion with regard to oil dispersion under sea conditions. Another problem is to find the amount of biosurfactants needed to achieve stable oil in water emulsion, also under sea conditions. Fundamentally, the role of structure and property relationship of biosurfactants in oil dispersions and particularly under conditions similar to sea is not known, nor is the synergistic and antagonistic behavior of biosurfactant mixtures.

1.3 Aim of the project

In this master thesis project four different kinds of biosurfactants are evaluated, three amino-acid based and one sugar-based, in an oil/water system. The goal is to find mechanisms governing interfacial behavior of biosurfactants mixed systems and the role of structural variations. In this project, it is determined how well ionic biosurfactants align at interfaces and surfaces by evaluating structure-property-performance related to molecular structure of the biosurfactants. Mixtures of biosurfactants are also evaluated for antagonistic or synergistic effects that may occur between a nonionic and ionic biosurfactants. The understanding of those behaviors is important in order to obtain insight in how biosurfactants perform as oil dispersants.

Interfacial properties and solution behavior of the sugar-based biosurfactant *n* -dodecyl-*β*-D-glucoside has previously been investigated at Columbia University in a solid/liquid system. Romain Bordes at Chalmers University of Technology has synthesized amino-acid based surfactants, dodecyl glutamate and dodecyl glycinate. Those amino-acid based surfactants have potential to be used for oil spill remediation, especially when mixed with *n* -dodecyl-*β*-D-glucoside. Dodecyl sarcosinate is a commercially available amino- acid based surfactant with similar structure to dodecyl glycinate and is also studied in this master thesis.

1.4 Method

The work consists of a literature study including an evaluation of structural/performance relationship and stability of oil in water emulsions for selected biosurfactants. This information was used to construct the set-up for the experiments where CMC, surface tension and emulsion stability were determined.

The laboratory work includes determining surface tension as a function of surfactant concentration by using Du Noüy Ring method. Surface tension measurements were performed on the pure surfactants and the surfactant mixtures, at various pH, salinity and with 0.42 ‰ calcium chloride added. Interfacial tension for oil in surfactant solution was determined by the same tensiometry method.

Emulsion stability was another part of the laboratory work and evaluated for all surfactants by observing the change of the generated emulsion as a function of time. Pictures were taken with different time interval to follow the emulsion stability.

1.5 Limitations

A limited range of pH and salinity has been considered with respect to the variation of pH and salinity range that may occur in the ocean. This is however not exactly in line with natural oceanic conditions since there is a significant variation of pH and salinity depending on what ocean is considered. In this master thesis the focus was to first evaluate a simple system and eventually make it more advanced.

Systems at different temperatures will not be studied due to the lack of equipment to do this. The relevance of this would also probably be mostly for nonionic ethoxylated surfactants. It would have been interesting to study systems at temperatures close to the freezing temperature of water since an oil spill might as well occur in the polar regions of the world, and at temperatures close to zero effects of viscosity and solubility also plays a role for the emulsification properties.

2. Theory and analysis

2.1 Conditions in the sea, pH and salinity

There are numerous factors that could influence the behavior of surfactants in the ocean since it contains many different chemicals and biological matter. Two major factors are pH and salinity. The pH in oceans is slightly basic and varies between 7.5 and 8.4 [9]. Scientists believe that the ocean is getting more acidic due to the greenhouse effect [10]. The salinity varies from about 0.7-0.8 % in the Baltic Sea [11] to 4% in the Red Sea and the average salinity in the oceans is approximately 3.5% [12]. The main salt is sodium chloride but there are also other salts present, see Table 1.

Table 1 Principal constituents of seawater [12]

Chemical constituent	Contents (parts per thousand)	Contents (mol/liter)
Calcium (Ca)	0.419	0.010
Magnesium (Mg)	1.304	0.536
Sodium (Na)	10.710	0.466
Potassium (K)	0.309	0.008
Bicarbonate (HCO3)	0.146	0.002
Sulfate (SO4)	2.690	0.028
Chloride (Cl)	19.350	0.546
Bromide (Br)	0.07	8.7609*10^-4
Total salinity	35.079	

2.2 Ionic and nonionic surfactants

Since the majority of surfaces, including minerals, fibers and cell membranes are negatively charged, cationic surfactants adsorb very well to must surfaces, which might not always be desired. Hydrolytically stable cationics also show higher aquatic toxicity than most other classes of surfactants [13].

Some facts can be noted about ionic surfactants. One example is that ionic surfactants physiochemical properties are markedly affected by electrolytes. Another example is that anionic surfactants are in general more skin irritating than nonionics. The nonionic surfactants are normally compatible with all other types of surfactants and are not sensitive to hard water. Anionic surfactants are generally sensitive to hard water. The sensitivity decreases in the order carboxylate > phosphate > sulfate/sulfonate. A short polyoxyethylene chain between hydrocarbon chain and the anionic group can improve the salt tolerance. If the polyoxyethylene chain is replaced by a polyoxypropylene chain the anionic surfactants solubility in organic solvents will be improved, but this will reduce the rate of biodegradation though.

Sugar based nonionic surfactants solubility increases as the temperature goes up. This behavior is contrary to other nonionic surfactant with ethoxylated head groups. These become less water soluble with higher temperature, due to the dehydration of the polar head group at higher temperatures = more hydrophobic.

2.3 CMC concept

2.3.1 General facts regarding the CMC

Low CMC values are desired since a low CMC usually mean that good packing at interfaces is obtained at lower concentration. A lower CMC also means more surfactants in micelles and fewer free surfactants in solution. These two things may give better solubilization, less skin and eye irritation and less foaming [13].

The CMC of a surfactant solution depends on the chemical structure of the surfactant and also on temperature, added electrolytes etc. Some general remarks regarding variations in CMC due to the chemical structure of the surfactants and additional changes to the surfactant solution are:

- Increasing alkyl chain length of the surfactant will strongly decrease CMC.
- CMCs of nonionic surfactants are much lower than those for ionic surfactants.
- Cationic surfactants typically have slightly higher CMCs than anionic surfactants.
- For ionic surfactants, the valency of the counterion is significant. Increasing the valency will give a decrease in CMC by a specific factor, depending on if the counterion is organic or inorganic.
- Alkyl chain branching, double bonds, aromatic rings, partial fluorination or any other gruop that give polar character to the hydrophobic part of the surfactant can give changes to the CMC. Introduction of for example a benzene ring to the chain will reduce CMC while the introduction of a polar group will increase the surfactants hydrophilicity and thereby increase the CMC. The presence of a double bond will increase the CMC by two to three times.
- At increasing temperature the CMC of ionic surfactants is increasing and the CMC of nonionic surfactants of the polyoxyethylene type is decreasing.
- Salt addition to an ionic surfactant gives a lowering of the CMC. The added electrolytes will give a pronounced effect on the micelle formation of the ionic surfactants since they can screen the repulsions between the charged headgroups, this will lead to the lowering of CMC. For nonionic surfactants, simple salts produce only small variations in the CMC where both decreases and increases are possible.
- Addition of low molecular cosolutes (for example alkanes, alcohols and aromatic compounds) can give increases or decreases in the CMC to a very different extent depending on the polarity and amphiphilic character of the cosolute. Decreases in the CMC is observed for most uncharged cosolutes, as alcohols for example.
- Pressure has little influence on the CMC [13][14].

2.3.2 CMC and mixed micelles

When surfactants are mixed in a solution, other factors will also apply. Surfactant mixtures will usually form micelles that will consist of a mixture of the individual components. These surfactant mixtures might have lower CMCs than the individual components alone; in that case there is a synergistic effect and a net attraction between the surfactants. When mixing an anionic surfactant with a nonionic surfactant the nonionic surfactant will shield the repulsion between the head groups of the anionic surfactants in the micelle. A very large synergistic effect can arise when there is a large net attraction between the surfactants, for example a cationic and an anionic surfactant, this will give a lowering of the CMC. A requirement is that the hydrocarbon chains of the surfactants are short (around 10 carbon atoms). In such a system the micellar composition will be kept constant which means that the electrostatic attraction between the two surfactant species should be the dominating driving force.

The CMC of a mixture of two surfactants will depend on the concentration/mole fraction of the two surfactant species and also on the interaction between them. How much the CMC will be lowered can be expected by using the formula:

$$\frac{1}{CMC} = \frac{x_1}{f_1^m CMC_1} + \frac{x_2}{f_2^m CMC_2} \qquad (1)$$

where CMC = the CMC of the mixed surfactants system, CMC_1 and CMC_2 are the CMCs of the individual included surfactants and x_1 and x_2 are the molar fractions in solution of the included surfactants. f_1^m and f_2^m are the activity coefficients of the surfactants in the micelle according to:

$$ln f_1^m = (x_2^m)^2 \beta \qquad ln f_2^m = (x_1^m)^2 \beta \qquad (2)$$

where x_2^m and x_1^m are molar fractions of the individual surfactants in the micelles. These values can be iterated by using the Newton-Raphson method:

$$F = (x_1^m)^2 ln(x_1 CMC) - (x_1^m)^2 ln(x_1^m CMC_1) - (x_2^m)^2 ln(x_2 CMC) + (x_2^m)^2 ln(x_2^m CMC_2) = 0 \qquad (3)$$

$$F' = \frac{dF}{dx_1^m} = 1 + 2x_2^m[ln(x_2 CMC) - ln(x_2^m CMC_2)] + 2x_1[ln(x_1 CMC) - ln(x_1^m CMC_1)] \qquad (4)$$

The β-parameter is an interaction parameter quantifying the net interaction between the surfactant species in the micelle. The value of the β-parameter indicates if there will be a net repulsion or a net attraction between the surfactants in the mixture, where positive values means repulsion and negative values mean attraction. When the β-parameter is zero there is no interaction between the two different surfactants in the mixture and the activity will be unity. A positive β value can exist when there for example is a mixture of normal hydrocarbon-based surfactants with fluorinated ones. When the attraction between the surfactants increases the β-parameter becomes increasingly more negative and the CMC of the mixture decreases. A value of -2 is typical for mixtures of anionic and nonionic surfactants. For a mixture between an anionic and a cationic surfactant values between -10 to -20 are typical. When salt is added to mixtures like these the value of the β-parameter decreases in magnitude, the decrease is larger with a higher salt concentration this is due to that the salt shields the electrostatic repulsions of the ionic surfactants, which makes the interaction between the nonionic and the ionic surfactant weaker [13].

$$x_1^m(2) = x_1^m(1) - \frac{F(x_1^m(1))}{F\prime(x_1^m(1))} \qquad (5)$$

$$\beta = \frac{1}{(x_1^m)^2} ln\left(\frac{x_2 CMC}{x_2^m CMC_2}\right) = \frac{1}{(x_2^m)^2} ln\left(\frac{x_1 CMC}{x_1^m CMC_1}\right) (6)$$

2.4 Emulsions

An emulsion is a dispersion of one liquid in another liquid, in the case of this study; oil is dispersed into the continuous phase water in form of oil droplets, i.e. an oil-in-water emulsion [13]. The emulsion is obtained by shearing the two fluids and thereby incorporating one phase into the other [15]. If oil is dispersed in water without any surfactants or other kind of stabilizer added the stability will be very poor. In that case the droplets will soon collide and this will lead to droplet fusion/coalescence, which means that the emulsion droplets merge into each other, and the eventual phase separation of the emulsion [13].The rate of the collisions of the oil droplets is dependent on several factors such as the density difference between the dispersed and the continuous phase, the droplet radius, the polydispersity, temperature and the viscosity of the continuous phase [13][14][15][10]. The larger the droplets the faster they will move in the solution, it is therefore important to have a small droplets size to increase the stability of the emulsion. The breakdown of an emulsion can occur in different ways. Creaming, Sedimentation, Ostwald ripening (larger droplets grow at the expense of small ones since the liquid from the small droplets is transferred to the larger ones) and flocculation are all processes that might lead to coalescence of the emulsion droplets. Creaming and sedimentation are mechanisms that occur as a result of the density differences between the phases. The Ostwald ripening depends on the solubility of the oil in water. If the oil is a small hydrocarbon, like hexane, the process is much more pronounced [13][14]. The lifetime of emulsions may vary from a few seconds to many years, depending on the surfactant and other conditions[16][10].

Stabilization of an emulsion can be induced by using surfactants. If an ionic surfactant is used this will give an electrostatic stabilization of the emulsion. This means that when the oil droplets, stabilized by ionic surfactants, starts to emerge a repulsive interaction will arise due to that the diffuse double layers around the particles will start to overlap. This overlap will give an increase in ion concentration, which will result in a loss of entropy and therefore lead to repulsion (Born repulsion). If electrolytes are added to the system this will shield the charges on the emulsion droplets and will therefore give less stabilization to the emulsion. According to DLVO theory, at a salt concentration of 0.1 M there is virtually no electrostatic repulsion left. Other forms of stabilization can be steric stabilization or particle stabilization, often the different modes of stabilization are combined to give for example "electrosteric stabilization" [13].

For a surfactant to be a good emulsifier (to create the emulsion in the first place) two requirements must be fulfilled. The first criteria is that the surfactant must reduce the oil-water interfacial tension to low values and the second criteria is that the surfactant must rapidly diffuse to a newly created interface (the oil droplet that is created when shearing is applied to the system). Low molecular weight surfactants are therefore superior when it comes to creating emulsions since these will rapidly diffuse and cover the new interface. If a low molecular weight surfactant is used to create the emulsion other emulsion stabilizers might be needed to create a stable emulsion [13].

2.5 General facts about biosurfactants

Biosurfactants can be of different kinds, e.g. fatty acids, phospholipids, glycolipids, lipopeptides/lipoproteins and polymeric/particulate biosurfactants. Biosurfactants can be synthesized by microorganisms such as bacteria and yeast or via other paths using fatty acids, amino acids and hydrocarbons as starting materials [1][5][4] . It is important that biosurfactants fulfill criteria regarding aquatic toxicity, rate of biodegradation and bioaccumulation, issued by Organization from Economic Cooperation and Development (OECD). Amino-acid based surfactants have, according to literature, low aquatic toxicity and high rate of biodegradation [18]. Some advantages with using biosurfactants are biodegradability, low toxicity, environmental compatibility and digestibility; this also makes biosurfactants useful in applications such as cosmetics and pharmaceuticals. Except cosmetics and pharmaceuticals, there are also other areas where the use of biosurfactants would be suitable. Some other examples are in control of oil spills, handling of industrial emulsions, biodegradation and detoxification of industrial effluents and in bioremediation of contaminated soil [19]. Biosurfactants made from renewable sources are in principle carbon dioxide neutral. This is an advantage compared to surfactants synthesized from petrochemical origin. Another aspect is that oil prices keep rising, which will make chemicals from renewable feedstock more and more competitive [18].

For solving major environmental problems large quantities of surfactants are needed. This makes the toxicity factor and the cost of some chemically synthesized surfactants major obstacles [19]. Biosurfactants are therefore chemicals of the future because of the low toxicity and that they can be produced from several cheap raw materials which are available in large quantities. The carbon source, for the hydrophobic tale, may come from hydrocarbons, carbohydrates and/or lipids that can be used separately or in combination with each other. Also, depending on the application, biosurfactants can be made from several inexpensive waste substrates such as industrial waste and byproducts [1][4]. However, more efficient bioprocesses are needed to make the biosurfactants competitive towards the chemically synthesized surfactants in the market. The production costs are high for the biosurfactants due to the inefficient bioprocessing methodology available and the need to use expensive substrates [8].

Current research and literature has not yet shown any single biosurfactant suitable for all the potential applications. Therefore, the development and research of more multifunctional biosurfactants is important. Also biosurfactant mixtures need to be evaluated in order to find potential synergistic effects [8].

2.5.1 Dodecyl glutamate

Dodecyl glutamate is a dicarboxylic acid based on the non-essential amino acid, glutamic acid [18]. It is synthesized by the Schotten–Baumann reaction, a derivatization of the regular amino acid and it can easily be made into its chiral form [16].

Dodecyl glutamate is considered to be biodegradable. It is somewhat structure-related to the glutamatediacetates, which are not surface active. Glutamatediacetates have been synthesized in L- and D- form and the aerobic biodegradation of those two has been studied. It was found that both were biodegradable but only the L-form was willingly biodegradable. It is expected that the same will take place for surface active glutamates [18]. Dodecyl glutamate contains an amide link between the hydrophobic tail and the polar head, which could be subjected to enzymatic hydrolysis.

The two carboxylic groups in the structure of dodecyl glutamate, Figure 1, are separated from the chiral center by methylene groups. The carboxylic groups give the surfactant chelating properties and good tolerance towards calcium containing inorganic materials [18] and is therefore interesting for hard water applications. The tolerance towards calcium is explained by the ability of dodecyl glutamate to form an intermolecular complex with calcium at the air-water interface. It also becomes surface active in the presence of calcium and could therefore be very interesting to use for oil-spill remediation. A very small amount of calcium salt is used (molar ratio of ion to surfactant 1:100), to lower the surface tension from 40nM to 30mM [16].

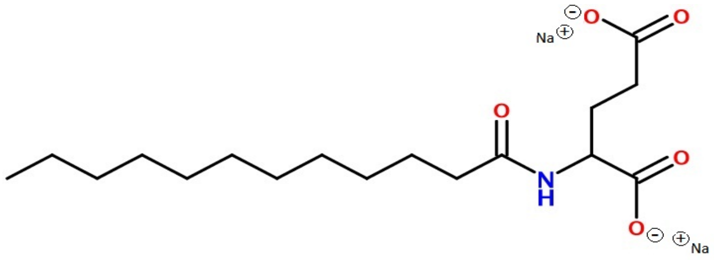


Figure 1. Structure of dodecyl glutamate

The close-packing of dodecyl glutamate is enhanced by the presence of calcium ions because of intermolecular binding [16]. The close packing of the surfactants aligned at the surface may also be favored by hydrogen bonding between the amide linkages [16].

In protonated form, the pka value of dodecyl glutamate cannot be determined since the protonated form will make the surfactant insoluble. However the pka values for dodecyl glutamate can be obtained by evaluating the protonation of glutamic acid by addition of acid. The first carboxyl group has a pka of 2.10, the second carboxyl group 4.07 and the ammonium group has a pka of 9.47 [16].

Dodecyl glutamate has CMC values of 50mM by tensiometry and 74 mM by conductivity [16].The chain of dodecyl glutamate has twelve carbons but the effect of the chain length has shown that an increase of the length would give a decrease of the CMC. The CMC is also very dependent on pH [18].

2.5.2 Dodecyl glycinate

Dodecyl glycinate has a twelve-carbon hydrophobic tail, Figure 2, and in comparison to dodecyl glutamate, which has a dicarboxylic group, dodecyl glycinate is monocarboxylic. It can be produced from a non-essential amino acid available in significant quantities in soybeans, meats, seafood and nuts [20]. Obtaining glycine from its natural source is made by hydrolysis of proteins and can be expensive. Another way to get dodecyl glycinate is to produce it synthetically by the Schotten Baumann reaction [18].

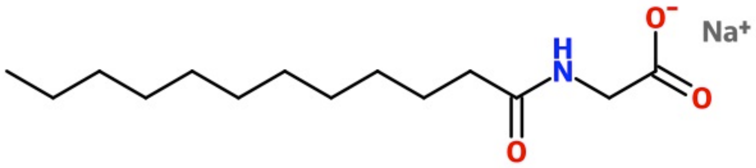


Figure 2. Structure of dodecyl glycinate

Dodecyl glycinate has a CMC of 10mM with tensiometry and 14mM with conductivity. The area/molecule is 63Å^2 and the plateau value of surface tension above the CMC is 39 mN/m according to literature [16].

Addition of acid will precipitate the surfactant. Below the CMC it is possible to see that a only a small amount of acid precipitates dodecyl glycinate compared to dodecyl glutamate, which is more acid resistant [16].

2.5.3 Dodecyl sarcosinate

Dodecyl sarcosinate is a twelve-carbon amino acid based surfactant with one carboxylic group and differs from glycinate since dodecyl sarcosinate has a methyl group on the nitrogen atom in the amide group. Dodecyl sarcosinate is an anionic surfactant, Figure 3, and has good solubility, detergency and resistance to calcium [21].

Dodecyl sarcosinate is well studied and is commercially available in large volumes [18]. It can be produced from an amino acid derivative, sarcosine, but can also be synthetically produced in high yields by a two-step synthesis, which is explained in Figure 4. [18].

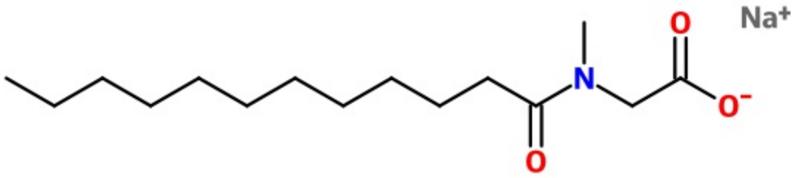


Figure 3. Structure of dodecyl sarcosinate

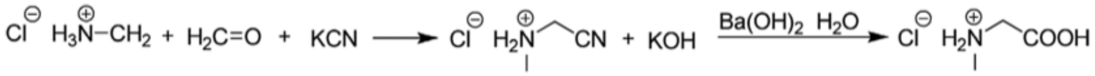


Figure 4. Synthesis of sarcosinate

Different chain lengths are available for dodecyl sarcosinate. A study that examined sarcosinate with 12, 14, 16 and 18 carbons determines that the greatest lowering in surface tension at CMC could be obtained with Na-N-lauroyl sarcosinate. The effectiveness can also be improved by increasing temperature [22].

2.5.4 Dodecyl glucoside

n-dodecyl-β-D-glucoside, also known as dodecyl glucoside, Figure 5 is a sugar- based surfactant belonging to the group alkylpolyglucoside. Sugar-based surfactants like dodecyl glucoside have strong interactions between the head groups because of the intermolecular hydrogen bonds between the hydroxyl groups in the sugar moieties. They are relevant to use as biosurfactants since they are stable against hydrolysis, but willingly biodegradable [23] and the aquatic toxicity is low [13] .

Alkylpolyglucosides (APGs) are prepared by a process based on Fischer glycosylation, a reaction between an alcohol and a glucoside with glucose. Another way to prepare it is by transacetalization of a short chain alkyl glucoside with a long chain alcohol. Alkyl glucoside can also be made from enzymatic synthesis [13]. An increase in chain length increases the surface activity but decreases the water solubility [24]. Conventional nonionics show an inverse solubility vs. temperature relationship, which APGs do not and this makes it different in solution behavior compared to normal nonionics [13].

The CMC for dodecyl glucoside used in this study is 0.19mM and the purity is higher than or equal to 99%, determined by HPLC. It is insoluble in water at 20°C [25].

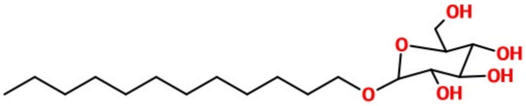


Figure 5. Structure of n-dodecyl-β-D-glucoside

2.6 Distribution of species

Depending on predominate pH, carboxylic and amine groups on the surfactant are either protonated or deprotonated. A protonated or deprotonated form will give the surfactant different characteristics and are of importance in evaluation of surface tension results at different pH. To determine what species of the surfactant are present at a specific pH, a species distribution diagram can be constructed. This graphical presentation surveys the interrelationships of equilibrium concentrations of the species in solution, as a function of pH [26]. To construct the graph it is of interest to find all concentrations of species present at various pH. Equilibrium expressions and one massbalance can be used to calculate all present species of dicarboxylic and carboxylic surfactants dissolved in water. First of all, it is important to know the pka values for the groups on the surfactant that can be protonated and deprotonated. From the pka values the equilibrium constant for proton transfer can be calculated by (7).

$$K_a = 10^{-pK_a} \qquad (7)$$

The simplest example of a species distribution diagram is that for acid-base equilibria involving a monoprotic acid. For a monoprotic acid, the equilibrium constant is obtained by (8).

$$K_{a=} \frac{[H^+][A^-]}{[HA]} \qquad (8)$$

The total surfactant concentration, C_{tot} is equal to a mass balance of all of the species added together divided by the volume of the vessel they are kept in. Combining the equilibrium expressions (7) with (8) and C_{tot}, will give the equations that define the concentration vs. pH dependence in a species distribution diagram.

4. Experimental procedures

4.1 Preparation of surfactant solutions

The samples that were used for fluorescence, surface tension and conductivity measurements were prepared in vials according to a dilution series. 10 ml of the solutions were prepared in each vial. The dilution series of the surfactants had a highest concentration of approximately 3 times the CMC from literature data of the investigated surfactant and a lowest concentration of 1/3 times CMC literature data. The dilutions of the surfactants were done by using a stock solution containing the 3 times CMC concentration of the surfactant and were then diluted according to $C_1V_1 = C_2V_2$ using a pipette and scale.

Since the amount of surfactants was rather small an approach of how to use the material as effective as possible was developed. This resulted in that the vials that first were prepared with water + surfactant also later on was salted with 50 mg NaCl to get solutions of 0.5% salt concentration and also an additional amount of 300 mg NaCl to get 3.5% salt concentration. The preparation of the dilution series with 0.42 parts per thousand of $CaCl_2$ was done with a stock solution of $CaCl_2$ which held this concentration, the following dilutions were then also made with this stock solution so that every vial held exactly the same $CaCl_2$-concentration.

4.2 Fluoresence

Fluoresence measurements were performed to the pH 7 solution of the dodecyl glutamate to determine CMC. 20 ml vials were first prepared and covered with alumina foil. Pyrene dissolved in chloroform was added to the vial in an amount that would give 10-6 Molar, i.e. 100 µl was added to a vial that contained 10 ml of surfactant solution. After the addition of pyrene the vial was set in a fume hood for 30 min to let the chloroform evaporate. The surfactant solution, at different concentrations and pH, was kept stirred for ~24 hours. After this ~3 ml of solution were transferred into a quartz cuvette and run in the Fluorimeter. The CMC was detected to be in the surfactant concentration interval where the I3/I1 ratio shifted from 0.5-0.6 to 0.7-1.0.

4.3 pH measurements and titrations

In order to evaluate the surfactants and surfactants mixtures at the desired pH range for this study, pH 5-pH 10, HCl and NaOH was added to the vials. The acid and bases were titrated dropwise with a pipette into the vials of different concentration of dodecyl glutamate. The pH was detected with an accumet basic pH-meter, AB15 from Fisher Sceintific.

4.4 Du Noüy Ring measurements

4.4.1 Surface tension

The surface tension was determined by tensiometer, using the Du Noüy Ring method. The machine used was a Krüss digital tensiometer K-10. Dodecyl glutamate was measured at concentration series of 0.3-180 mM and the surfactant was dissolved in deionized water, approximately 10 ml. The analyzed series had pH7, pH 9.7 and 0.5% NaCl as well as 3.5% NaCl respectively and $CaCl_2$. The laboratory was not automatically temperature controlled but the temperature was determined before measuring each series.

The surface tension was obtained in order to order to determine the CMC and plateau value surface tension above CMC .The procedure when using the machine was the same for every measurement. First, the ring was washed with deionized water and then burned with a flame torch. The same beaker was used for each series and it was washed with detergent, ethanol and deionized water before surfactant solution was added. The Krüss digital tensiometer was set to a zero level before the start of every series.

4.2.2 Interfacial tension

Interfacial tension was measured by the Du Noüy Ring method, Krüss digital tensiometer K-10, using a ring. The interfacial tension was determined at a surfactant concentration above CMC, to ensure that all surfaces would be covered by surfactant to achieve best possible lowering of interfacial tension between surfactant solution and hexane. For dodecyl glutamate, a solution of 10 ml with a concentration of 120mM was added in a beaker that had been washed with detergent, ethanol and deionized water. 10 ml of hexane was added to this solution and an interface between the aqueous phase and the oil phase appeared. Before submerging the ring, it was washed with deionized water and burned with a flame torch. After submerging the ring, it was pulled up from the aqueous phase and the interfacial tension was determined when the ring passed the interface of the aqueous phase and the oil phase. Temperature was not kept constant during those measurements but always measured before the start of each measurement.

4.4 β-parameter calculations

Using the CMC estimated from the graphs for the mixtures of the anionic surfactants and the nonionic dodecyl glucoside the β-parameter and the molar ratios of each surfactant in the micelles, x_{1m} and x_{2m} could be calculated, see equations 1-4 under the section 2.3.2. The equations were incorporated in a script in the programme MatLab which did the Newton-Raphson iteration to get the right molar ratios in the micelles. See appendix for the MatLab script.

4.5 Conductivity measurements

Conductivity measurements were performed at two locations with two different conductometers. The reason for this was a breakdown of a tensiometer at Columbia University. To get reliable and comparable results it was important to measure the conductivity the same day as the surface tension measurements were performed. The second conductivity measurements were performed as control measurements to the first ones. The conductometer used at Cytec in Stamford was a 712 Conductometer, Metrohm, BRINKMANN and the conductometer used at Columbia was an OAKTON CON 510 Series conductometer.

4.6 Emulsion stability/capacity

The efficiency of emulsions can be determined in many different ways. In this study the emulsification capacity was evaluated according to the following:

- 10 ml of surfactant solution and 10 ml of hexane was added to a 20 ml vial.
- The vial was shaken vigorously for two minutes.
- The emulsification capacity (EC) was calculated by dividing the measured height of the emulsion layer by the total height of the mixture and multiplying it by 100 [27].
- This was done at three occasions: after 5 minutes, after 30 minutes and after 24 hours.
- The foam height of the emulsions was also noted.
- The measurements were done at room temperature.

5. Results

5.1 Results from Fluorescence

From the Fluorescence measurements, Figure 6, it is possible to see the interval for where the CMC of dodecyl glutamate is located; this is between 60mM – 120mM. It would be possible to make dilution series between 60mM and 120mM and repeat fluorescence to get a more specific CMC value. However other techniques can also give this information. This interval agrees with the results from surface tension measurements that showed a CMC of approximately 57 mM (see section 5.2.2).

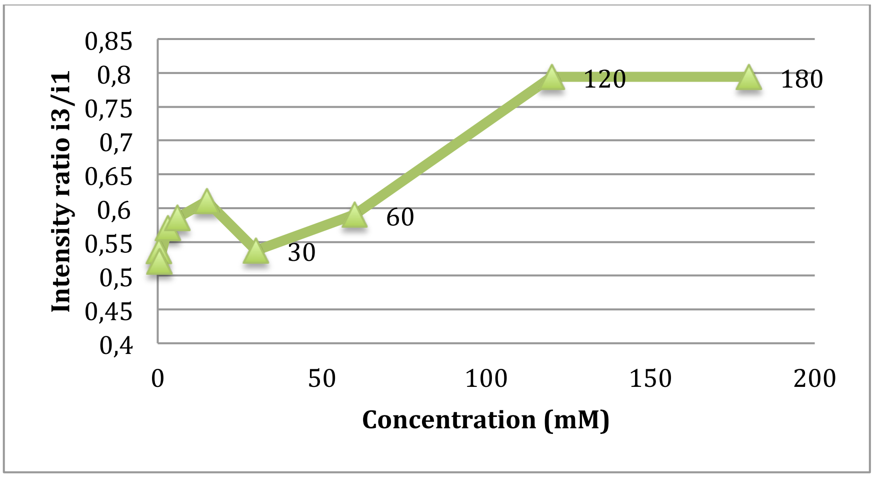


Figure 6. Fluorescence results for dodecyl glutamate at pH 7

5.2 Results from Du Noüy Ring measurements

The results from the tensiometer tests by the Du Noüy Ring are presented here, both interfacial tension and surface tension results, at varying pH, salinity and with different mixing ratios with dodecyl glucoside.

5.2.1 Dodecyl glucoside

The analyses were performed one day after solutions were prepared in order to determine the CMCs and plateau value of surface tension above the CMC. The CMCs cannot be obtained from each graph in Figure 7 which displays results at a temperature from 21-22°C. Higher concentrations would be required in order to get the CMCs, which were unobtainable because of limited solubility of glucoside. The CMC for glucoside presented in Table 2 is a value from literature.

Since glucoside is a nonionic surfactant, the hypothesis was that salt addition would not influence the surface tension of glucoside. This was however not the case, which can be seen from Figure 7. The effect of salt is not very major but significant enough to be discussed. The effect of increasing pH was not evaluated for a series of glucoside because one sample of glucoside at pH 9 had the same surface tension as a pH 7 sample. Hence it was concluded that glucoside is not affected by pH.

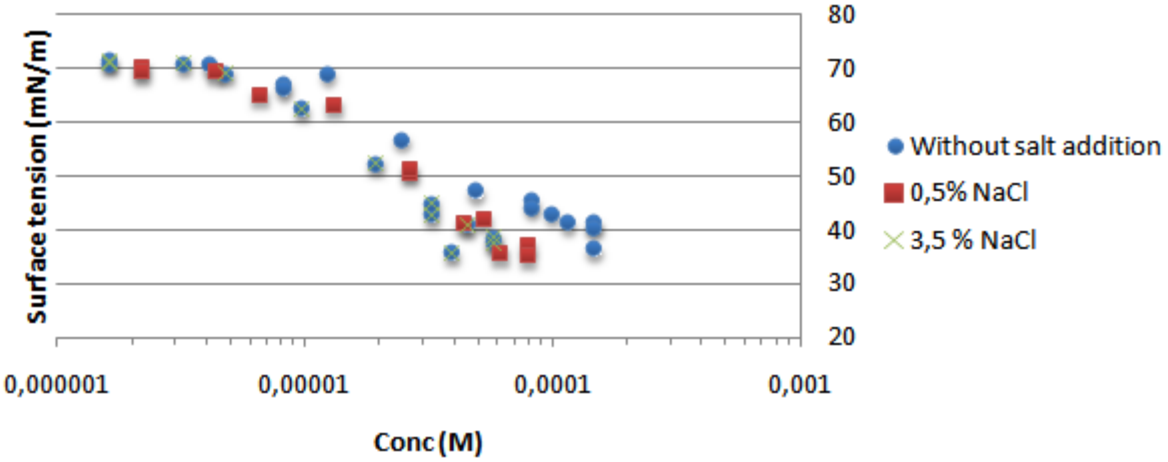


Figure 7. Comparison of surface tensions with and without salt at various concentrations of dodecyl glucoside.

Table 2. Results from surface tension measurements fordodecyl glucoside under varying conditions.

Condition	CMC (mM) By tensiometer	Plateau value of surface tension above CMC (mN/m)	Area per molecule (Å^2)	Interfacial tension with hexanes (mN/m)	Emulsification capacity (EC) after 24 hours	Comments
pH 7	To low concentrations dissolved to be able to tell! Therefore need to make new concentrations or use literature data! CMC from literature is 0.15-0.3 mM.		27.2	-		
pH 9				10.5		
0.5% NaCl			22.2	-		
3.5% NaCl			36.6	N.A.	0 (0.148 mM)	Was not able to measure!
0.42 ‰ CaCl2			28.4	-		

The interfacial tension between aqueous solution of glucoside and hexane was evaluated at 21.8°C and when 3.5% NaCl was added to a sample with glucoside concentration 0.079 mM the interfacial tension was not measurable. However, at pH 9 the interfacial tension was 10.5 between hexane and an aqueous solution with glucoside concentration of 0.148mM, Table 2.

5.2.2 Dodecyl glutamate

Solutions of dodecyl glutamate with pH 7 were analyzed at a temperature of 21.1°C and the solutions with pH 9.7°C were analyzed at a temperature of 21.9°C. The results in Figure. 8 were obtained three weeks after the solutions were prepared.

As can be interpreted from Figure. 8 there is an increase in CMC at pH 9.7 compared to the CMC at pH 7 and the results from surface tension measurements can be seen in Table 4. The plateau value of surface tension above CMC seems to be unaffected by an increase in pH, Figure. 8.

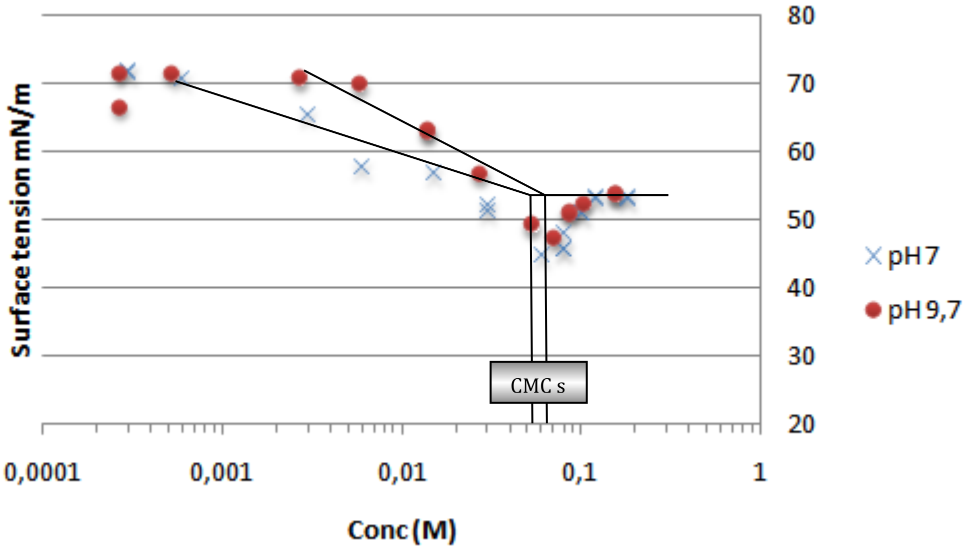


Figure 8. Comparison of surface tensions at neutral pH and pH 9.7 as a function of dodecyl glutamate concentration. The lines indicate how the CMCs are calculated and is 67mM at pH 9.7 and 57 mM at pH 7.

The results from adding sodium chloride to the glutamate solutions at pH 7, implies expected lowering in CMC Figure. 9. One can also determine from Figure.9 that the plateau value of surface tension above CMC is unaffected by salt addition.

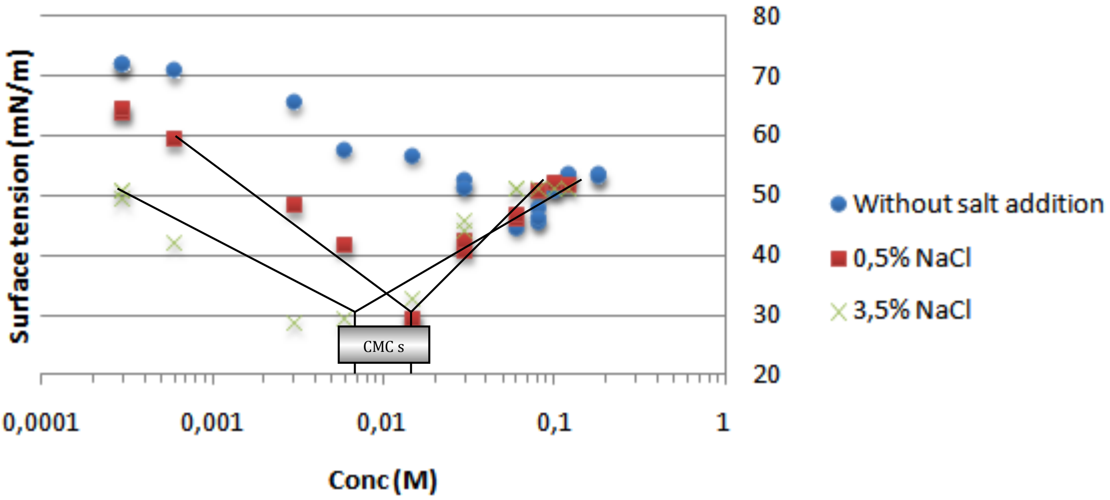


Figure 9. Comparison of surface tensions with and without salt addition as a function of dodecyl glutamate concentration. The lines indicates how the CMCs for salt addidtion were determined and the CMC for adding 3.5% NaCl was 6mM and 16mM when 0.5% NaCl was added.

Glutamate is prone to become very surface active in the presence of calcium ions [16]. Figure. 10 shows that calcium addition certainly lowers the surface tension above CMC, however the CMC is unaffected. Turbidity was detected at dodecyl glutamate concentrations between 0.6-120mM when calcium was added, Figure. 11.

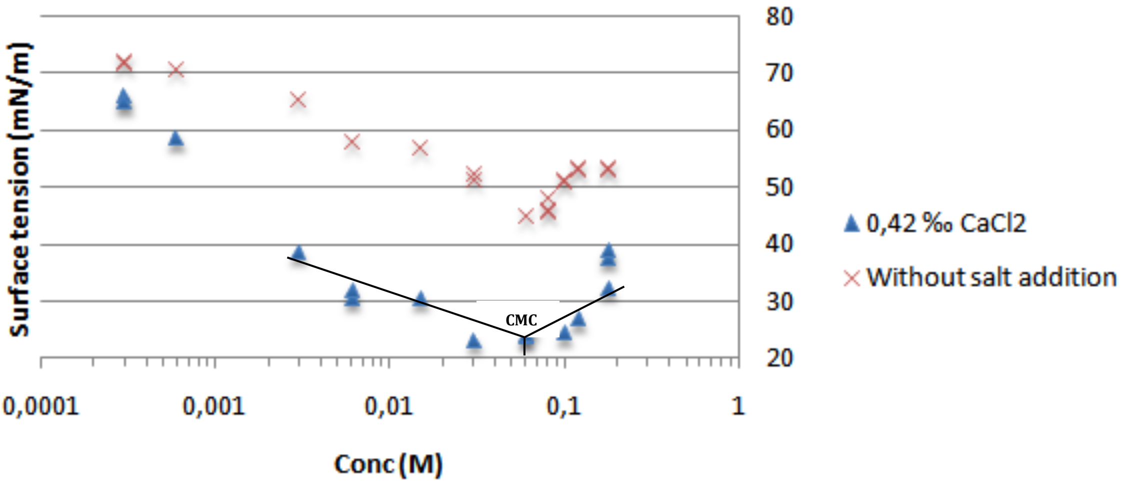


Figure 10. Comparison of surface tensions with 0.42 ‰ calcium chloride addition and without salt addition vs. dodecyl glutamate concentration. The lines show how the CMC was determined when and it is 60mM when calcium chloride was added.

Figure 11. Concentrations of dodecyl glutamate, from left to right: 0.3 mM, 0.6 mM, 3 mM, 6 mM, 15 mM, 30 mM, 60 mM, 64.7 mM, 100 mM, 120 mM and 180 mM

The glutamate concentration can be determined by TOC, other methods can be used to determine the calcium ion content. Due to a lack of time, all concentrations of the precipitated samples were not determined; hence it is very difficult to make a surface tension curve to determine the CMC.

5.2.3 Dodecyl glutamate and dodecyl glucoside mixtures

The results from surface tension measurements on the dodecyl glucoside mixtures with dodecyl glutamate can be seen in Table 4.

5.2.3.1 Molar ratio 1:1

A mixture of dodecyl glutamate and dodecyl glucoside at a molar ratio of 1:1 precipitated at concentrations between 0.819-98mM. Data from the surfactant mixture samples in this interval, see shaded area in Figure 12, are therefore not that reliable. At least an approximate CMC can be calculated from Figure 12, this value is about 0.34 mM. The line in Figure 12 indicates how the CMC is determined, however it is very difficult to draw the correct lines from this graph to determine the CMC since more data points would be required to give a more reliable CMC.

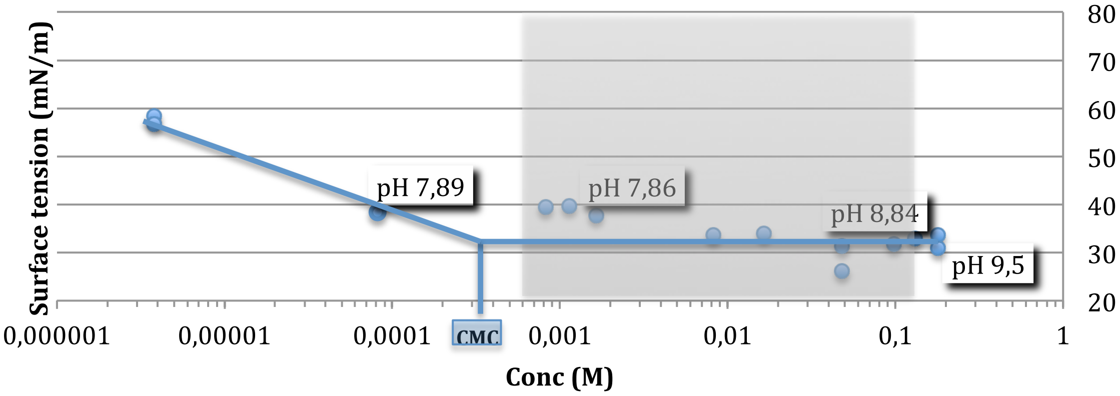


Figure 12. Surface tension of dodecyl glutamate/dodecyl glucoside mixture with molar ratio 1:1 vs. Surfactant mixture concentration. The shaded area indicates surfactant mixture concentrations where precipitation occurs. pH varies from 7.89-9.7. The line shows how the CMC is calculated.

Precipitation in the solutions induces different degrees of turbidity to the samples, Figure 13. Since precipitation was observed, surface tension behavior when adding salt was not evaluated for a dodecyl glutamate/dodecyl glucoside mixture at molar ratio 1:1.

Figure 13. Samples of dodecyl glutamate and dodecyl glucoside solutions at mixing ratio 1:1, from left to right: 0.0038 mM, 0.0819 mM, 1.14 mM, 1.64 mM, 8.19 mM, 16.4 mM, 48 mM, 131 mM and 180 mM

Calcium has a demonstrated effect on the surface behavior of dodecyl glutamate Figure 10, hence calcium addition was interesting to evaluate for a dodecyl glutamate/dodecyl glucoside mixture and the surface tension behavior as a function of surfactant mixture concentration can be observed in Figure 14. The CMC is calculated from the lines in Figure 14 and is also difficult to determine from this graph since more measuring points are required for a good value of the CMC. From Figure 14 one can say that the CMC of the mixture seems unaffected by calcium addition and is roughly 0.3 mM.

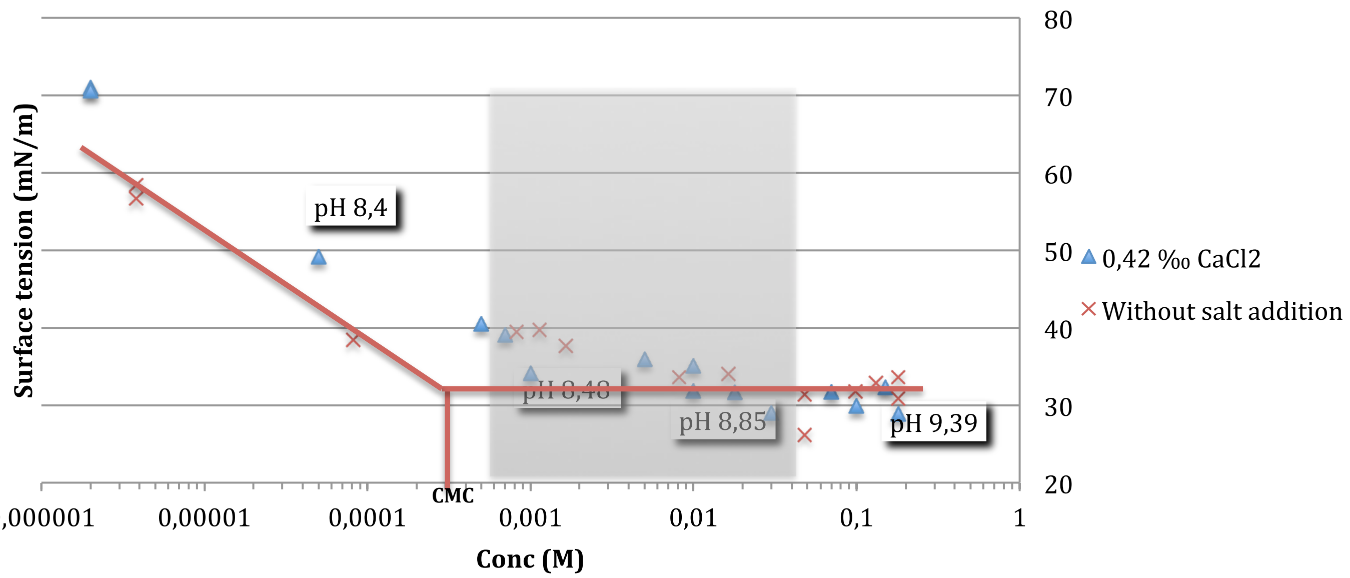


Figure 14. Calcium effect on surface tension as a function of surfactant mixture concentration a mixture consisting of dodecyl glucoside and dodecyl glutamate in a 1:1 molar ratio. The grey rectangle indicates the surfactant mixture concentration range where precipitation occurred for a dodecyl glucoside/dodecyl glutamate mixture with 0.42 ‰ CaCl2. pH values are presented for surfactant mixtures which contains addition of $CaCl_2$. The red line shows how the CMC is calculated with and without $CaCl_2$ addition.

In conformity with mixtures at a molar ratio of 1:1 without calcium, addition of 0.42 ‰ $CaCl_2$ shows precipitation at surfactant mixture concentrations from 1mM to 70mM, Figure 15.

Figure 15. Samples of dodecyl glutamate and dodecyl glucoside at mixing ratio 1:1 with 0.42 ‰ CaCl2 added. From left to right: 0.05mM, 0.5 mM, 0.7 mM, 1 mM, 5 mM, 10 mM, 18 mM, 30 mM, 70 mM, 100 mM, 150 mM and 180 mM

5.2.3.2 Molar ratio 19:1

Solutions with 95% dodecyl glutamate and 5% dodecyl glucoside (19:1 ratio), were prepared and analyzed two days after preparation. All measurements occurred at a temperature from 21.7°C to 22°C and pH varied from 6.95-9.65. All samples were clear except the 5 mM and 60mM sample, when salt was added it was no longer turbid.

From Figure 16 it is possible to evaluate the CMCs and the surface tension above the CMC for the surfactant mixture. With no salt the CMC is 2.9 mM and adding salt seems to have no/very little effect on the CMCs, determined from the line in, Figure 16. From the graph it looks as the effect for 3.5% might be a slightly lower CMC, but this is very hard to determine. Again, the

CMC is unreliable since it is based on only a few data points. The surface tension above the CMC is about 40mN/m from Figure 16.

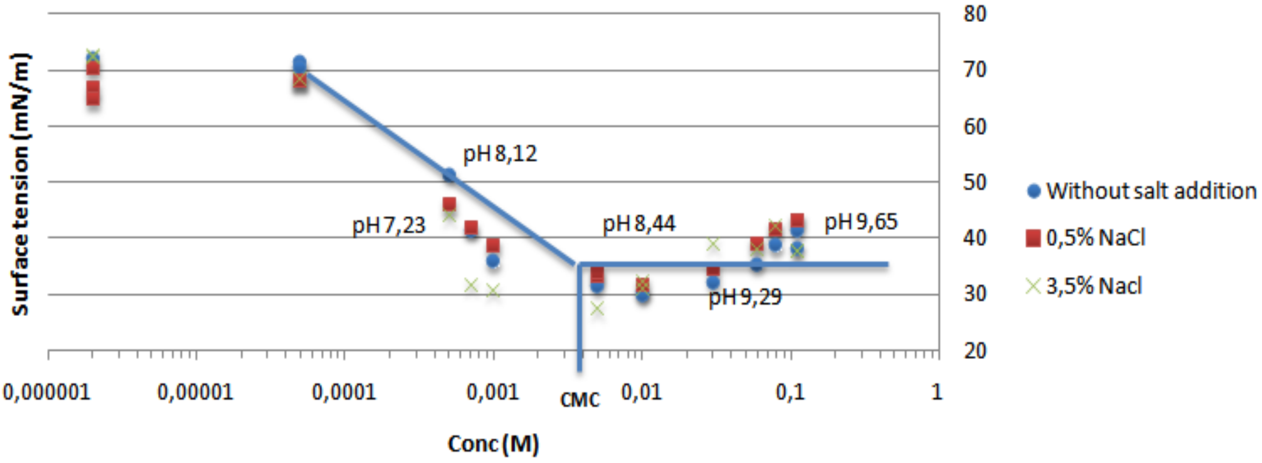


Figure 16. NaCl addition to the mixture of dodecyl glutamate and dodecyl glucoside at 19:1 ratio. The line shows how the CMC is calculated. The pH is increasing with increasing surfactant concentration.

The intention was to also evaluate the effect of calcium for the 19:1 mixture, however precipitation occurred when a stock solution of 110mM of the dodecyl glutamate/glucoside mixture with 0.42 ‰ $CaCl_2$ was prepared, see Figure. 17, therefore no further studies was done.

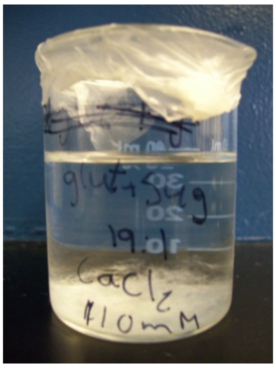


Figure 17. The stock solution of 110 mM dodecyl glutamate/dodecyl glycinate, ratio 19:1 with 0.42‰ CaCl2 added. Precipitation can be seen on the bottom of the beaker. The precipitate was in a crystal-like state. The picture was taken two weeks after the sample had been stirred for 24 with magnetic stirring.

5.2.4 Table of results for dodecyl glutamate solutions

When using the calculated CMCs from the graphs and the Matlab program for the mixtures of glucoside and glutamate at the two different ratios and salinities the data in Table 3 is received.

Table 3. β-parameters and ratio of anionic surfactant in micelles for dodecyl glutamate + dodecyl glucoside mixtures

Mixture glutamate:glucoside	CMC of mixture (mM)	Molar ratio of glutamate in micelle	β-parameter
19:1 ratio, pH 7	2.9	0.184	-2.0
19:1 ratio, pH 7 05% NaCl	2.9	0.29	-1.77
19:1 ratio, pH 7, 3.5% NaCl	< 2.9	~0.35	-2.0
1:1 ratio, pH 7	0.34	0.082	-3.9
1:1 ratio, pH 7, 0.42‰ CaCl2	0.34	0.082	-3.9

In Table 4 the results from surface tension measurements presented in Figure 8,9,10,12,14,16 are summarized.

Table 4. Summary Table of the results from the experiments on glutamate and its mixtures

glutamate solutions	**CMC (mM)**	**Plateau value of surface tension above CMC (mN/m)**	**Area per molecule (Å^2)**	**Interfacial tension with Hexanes**	**Emulsification Capacity (EC) after 24 hours**	**Comments**
pH 7	57	53	130	18.6 (conc. 120 mM)	0 % (conc. 120 mM)	
pH 9.7	67	53	120	-	-	
pH7, 0.5% NaCl	16	52	102	18.5 (conc. 120 mM)	-	
pH7, 3.5% NaCl	6	51	120	17.2 (conc. 120 mM)	-	
pH 7, 0.42 ‰ **CaCl2**	60	52	141	16.3 (conc. 120 mM)	2% (conc. 180 mM)	Precipitation at 0.6-120mM
MIXTURES **glutamate:glucoside**	**CMC (mM)**	**Plateau value of surface tension above CMC (mN/m)**	**Area per molecule (Å^2)**	**Interfacial tension with hexanes**	**Emulsification Capacity (EC) after 24 hours**	**Comments**
19:1 ratio pH 7	2.9	40	52.3	7.5 (conc. 110 mM)	52% (conc. 110 mM)	Precipitation at 5mM and 60 mM
19:1 ratio, pH 7 0.5% NaCl	2.9	40	97	-	-	
19:1 ratio, pH 7, 3.5% NaCl	2.9	40	61.6	10.5 (conc. 110 mM)	0 % (conc. 110 mM)	
19:1 ratio, pH 7, 0.42‰ CaCl2	-	-	-	-	-	Precipitation of stock solution at 110mM
1:1 ratio, pH 7	0.3	32	148 (hard to tell)	2.95 (conc. 131 mM)	54% (conc. 131 mM)	Precipitation at 0.819-98 mM
1:1 ratio, pH 7, 0.42‰ CaCl2	0.3	31	284	2.75 (conc. 150 mM)	53% (conc.150mM)	Precipitation at 1-70mM

5.2.5 Dodecyl glycinate

Dodecyl glycinate was evaluated at temperatures between 20.1°C and 21.3°C. The surfactant solutions were prepared one day before analysis occurred. From Figure 17 it is clear that the effect of salt is substantial and the CMCs are lowered from 10mM (without salt) to 5 mM (0.5% NaCl) and 2.2 mM (3.5% NaCl). Figure 17 presents that the surface tension above the CMC is lowered when NaCl is added, from 40mM/m without NaCl to 33mN/m with 0.5% NaCl. A further reduction to 28mN/m occured when 3.5% NaCl is added. At a concentration of 3.5% NaCl, some precipitation could be detected in samples with surfactant concentration 7mM, 20mM and 30mM.

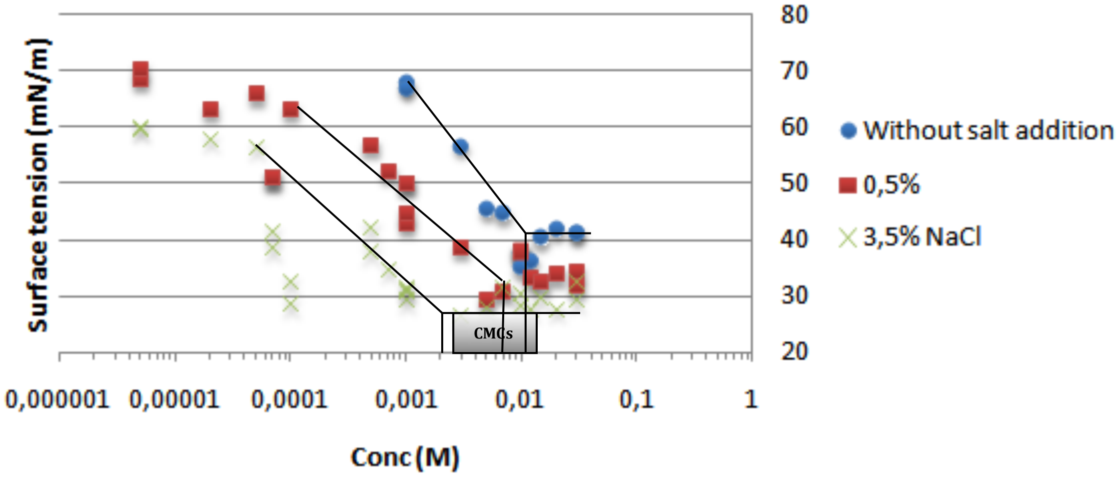


Figure 17. Comparision of surface tensions vs. dodecyl glycinate surfactant concentration with and without salt addition. The lines indicate how the CMCs are determined. At 3.5% NaCl the CMC is 2.2mM and at 0.5% NaCl the CMC is 5.5mM. When no salt is added the CMC is 10mM.

Figure 18 displays how an increase in pH would affect the CMCs and surface tension of dodecyl glycinate. The pH is not accurately 7 and 9 for all samples in Figure 18, but the graph gives an estimation of how the surface tension as a function of glycinate concentration is behaving in basic surroundings.

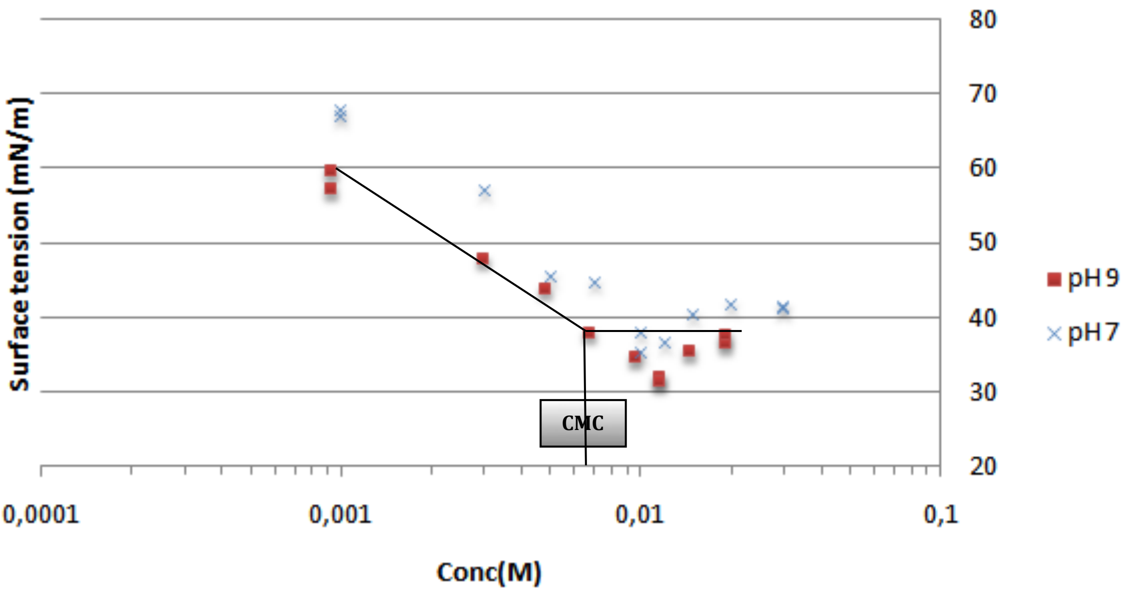


Figure 18. Comparision of surface tensions vs. dodecyl glycinate surfactant concentration at different pH. The line indicate how the CMC is determined. At pH 9 the CMC is about 6.7 mM.

The calcium effect on glycinate was also of interest to evaluate. When a concentration of 0.42 ‰ CaCl2 was added to the sample with the highest concentration of glycinate, 30 mM, precipitation was observed, Figure 19.

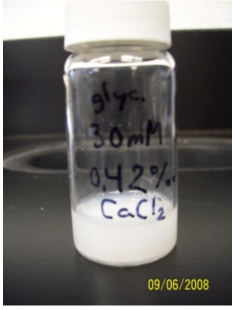


Figure 19. Precipitation in the sample of 30 mM dodecyl glycinate with 0.42‰ $CaCl_2$ added.

Because of the precipitation, it was unmotivated to make further dilution series based on this stock solution.

5.2.6 Dodecyl glycinate and dodecyl glucoside mixtures

The results from surface tension measurements on the dodecyl glucoside mixtures with dodecyl glycinate can be seen in Table 6.

5.2.6.1 Molar ratio 1:1

The samples with surfactant mixture concentrations of 0.5 mM, 0.7 mM, 1 mM, 5 mM and 7 mM were clear directly after the dilutions were made but turned turbid after two days (they might have turned turbid earlier than that, but since the samples were shipped to another location for analysis during this time, it was not noted), Figure 21 and Figure 22. The surface tension as a function of surfactant concentration was evaluated anyway at a temperature of 22°C, three days after the solutions were prepared. The results are presented in Figure 20 and the CMCs and surface tension above CMC are presented in Table 5. From Figure 20 the CMC is approximately 0.25mM but it would be desirable to use conductivity to get at more accurate CMC. One should also consider that the surfactant mixture concentrations on the x-axis might not be accurate since precipitation occurred.

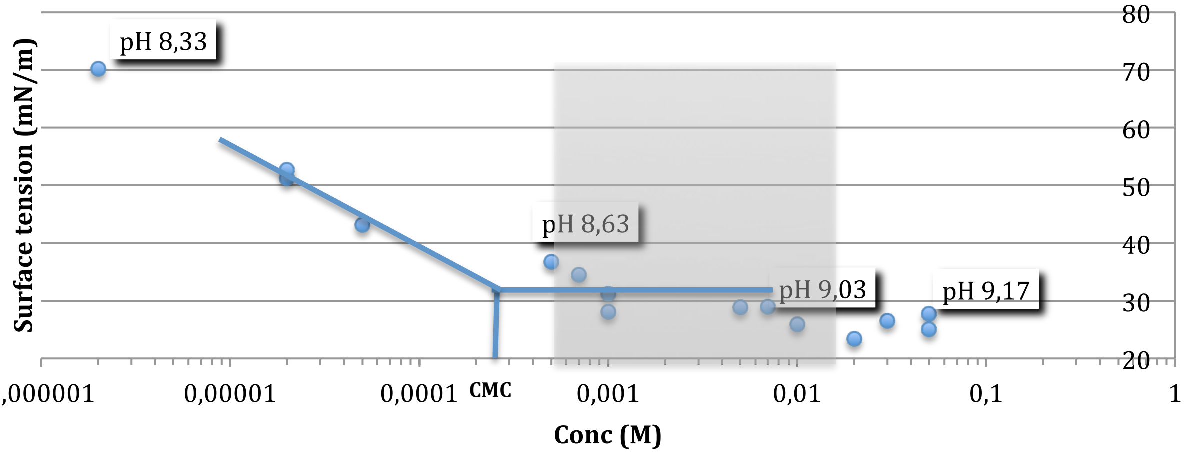


Figure 20. Surface tension vs. dodecyl glucoside/ dodecyl glycinate concentrations at a surfactant molar ratio of 1:1. The grey rectangle indicates the surfactant mixture concentration range where precipitation occurs. pH values are also presented at concentrations 0.002mM, 0.5mM, 10mM and 9.17mM. The line shows how the CMC is calculated.

Figure 21. From left to right: 50 mM, 30 mM, 20 mM, 10 mM, 7 mM and 5 mM of dodecyl glycinate + dodecyl glucoside mixture at a 1:1 molar ratio 1:1. As can be seen there is precipitation in the 7 and 5 mM samples.

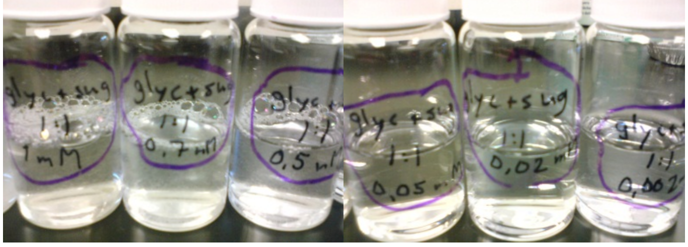


Figure 22. Lower concentrations of dodecyl glycinate + dodecyl glucoside mixtures. From left to right: 1 mM, 0.7 mM, 0.5 mM, 0.05 mM, 0.02 mM and 0.002 mM As can be seen there is precipitation in the 1 , 0.7 and 0.5 mM samples.

When a stock solution of dodecyl glycinate/glucoside of molar ratio 1:1 at 50 mM with 0.42‰ $CaCl_2$ was prepared precipitation occurred immediately. After 24 hours of magnetic stirring the precipitate remained, see Figure 23. Therefore no dilution series could be done for this solution.

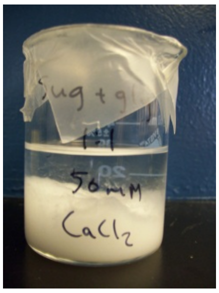


Figure 23.Precipitation in the dodecyl glycinate/glucoside stock solution (50 mM), mol ratio 1:1, 0.42‰ CaCl2. The picture was taken after the sample had been stirred with a magnetic stirrer for 24 hours and thereafter had been set to rest for two weeks.

5.2.6.2 Molar ratio dodecyl glycinate, dodecyl-glucoside 19:1

The mixtures with 95% dodecyl glycinate and 5% dodecyl glucoside were prepared three days before analysis was conducted, and analyzed at a temperatures between 21.9-22°C. A comparison of the surface behavior of the surfactant mixture with and without salt can be seen in Figure 24. From Figure 24, one can observe a CMC of 1.2 mM without NaCl and from those graphs it is difficult to evaluate a different CMC for the solutions with salt addition, however adding 3.5% NaCl indicates a lowering of the surface tension to 0.7mM. Adding 0.5% NaCl will give the same CMC as without salt addition. The surface tension above CMC is between 30-36mN/m with and without salt addition, Figure 24. All results can be found in Table 6.

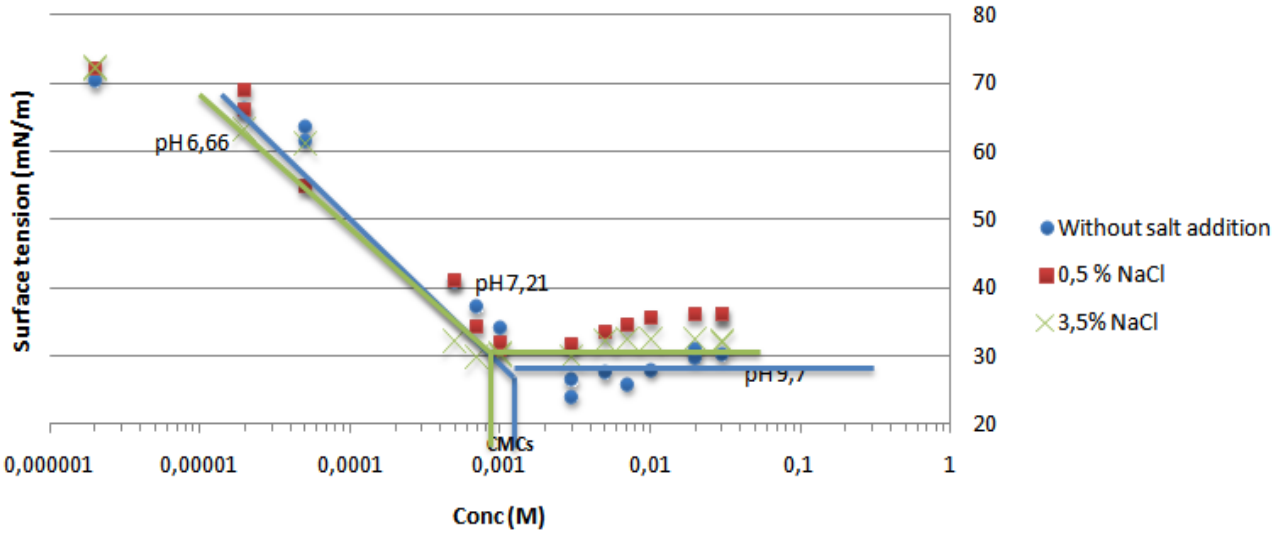


Figure 24. Comparision of surface tensions vs. surfactant concentration with and without salt addition for dodecyl glycinate/dodecyl gluatamte mixtures with a molar ration of 19:1. The lines indicates how the CMC was calculated. There is an obvious trend in pH increasing with concentration both with and without salt. The pH for no salt addition varied from 8.55 (the solution with the lowest conc) to 9.7 (the solution with the highest concentration). For 0.5% NaCl added the pH varied from 7.24 (the solution with the lowest conc) to 8.67 (the solution with the highest conc). And for 3.5% NaCl added the pH had a lowest value of 6.66 (the solution with the lowest conc) and a highest value of 7.89 (the sample with the highest concentration)

Precipitation was observed for a stock solution of dodecyl glycinate/glucoside with molar ratio 19:1 at 30 mM when 0.42‰ $CaCl_2$ was added, Figure 25. 24 hours of magnetic stirring did not contribute to dissolving the precipitation and no dilution series could be done for this solution.

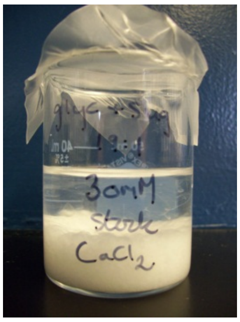


Figure 25. Precipitation in the dodecyl glycinate/glucoside stock solution (30 mM), mol ratio 19:1, 0.42‰ $CaCl_2$. The picture was taken after the sample had been stirred with a magnetic stirrer for 24 hours and thereafter had been set to rest for two weeks.

5.2.7 Table of results for dodecyl glycinate solutions

When using the calculated CMCs from the graphs and the Matlab program for the mixtures of glycinate and glucoside at the two different ratios and salinities the data in Table 5.

Table 5. β -parameters and Molar ratio of glycinate for different mixtures of glycinate and glucoside.

Mixture glycinate:glucoside	CMC of mixture (mM)	Molar ratio of glutamate in micelle	β-parameter
19:1 ratio, pH 7	1.2	0.41	-3.7
19:1 ratio, pH 7 0.5% NaCl	1.2	0.45	-2.8
19:1 ratio, pH 7, 3.5% NaCl	0.7	0.48	-3.5
1:1 ratio, pH 7	0.25	0.21	-4.4

In the following Table 6 the results from surface tension measurements of the different solutions of dodecyl glycinate are summarized.

Table 6. Summary Table for the results from the different experiments that were done for glycinate and its mixtures.

Glycinate solutions	**CMC (mM)**	**Plateau value of surface tension above CMC (mN/m)**	**Area per molecule (Å^2)**	**Interfacial tension with hexanes**	**Emulsification Capacity (EC) after 24 hours**	**Comments**
pH 7	10	40	569	-	-	
pH 9	6.7	36	54.8	10 (conc. 20 mM)	2.6% (conc. 20 mM)	
pH7, 0.5% NaCl	5	33	65.9	-	-	
pH7, 3.5% NaCl	2.2	28	43.7	5.8 (conc. 30 mM)	50% (conc. 30 mM)	
pH 7, 0.42 ‰ CaCl2	-	-	-	-	-	Precipitation of stock solution (30 mM)
MIXTURES **glycinate:glucoside**	**CMC (mM)**	**Plateau value of surface tension above CMC (mN/m)**	**Area per molecule (Å^2)**	**Interfacial tension with hexanes**	**Emulsification Capacity (EC) after 24 hours**	**Comments**
19:1 ratio, pH 7	1.2	30	94.2	-	-	
19:1 ratio, pH 7 0.5% NaCl	1.2	36	54	-	-	
19:1 ratio, pH 7, 3.5% NaCl	0.7	32	111	4.5 (conc. 30 mM)	53% (conc. 30 mM)	
19:1 ratio, pH 7, 0.42‰ CaCl2	-	-	-	-	-	Precipitation of stock solution (30mM)
1:1 ratio, pH 7	.25	30	76.8	0.04?! (conc. 30 mM)	53% (conc. 30 mM)	Precipitation at 0.5-7mM

5.2.8 Dodecyl sarcosinate

The surface tension and CMC of sarcosinate seems to be unaffected by an increase in pH, Figure 26, however an addition of NaCl seems to lower the CMC and also the surface tension above the CMC, Figure 28. A summary of the CMCs and surface tensions above the CMCs, calculated from the graphs below can be found in Table 8.

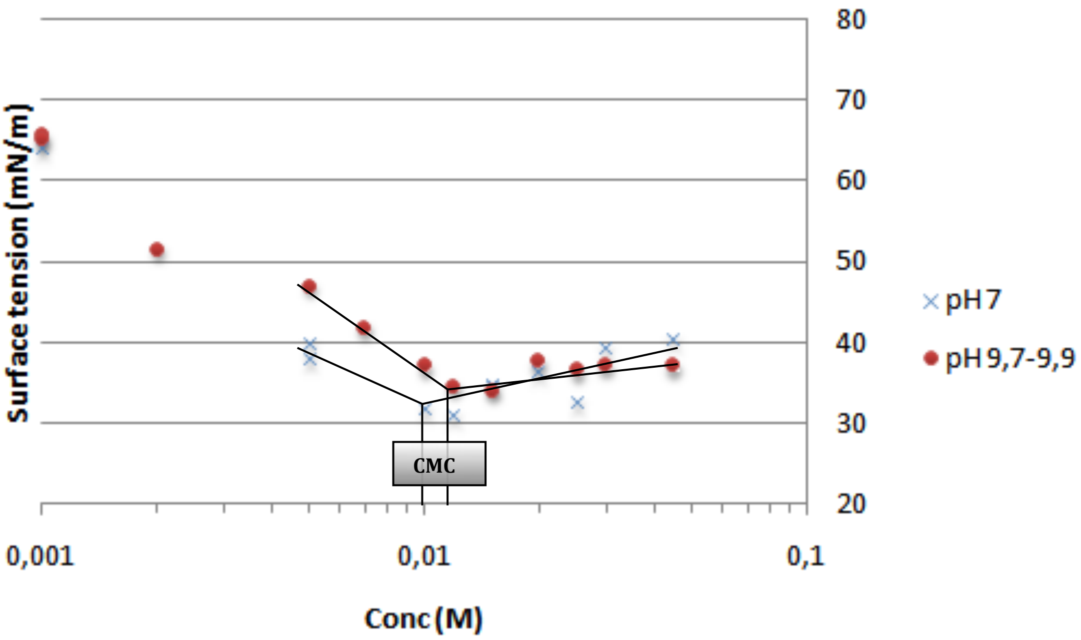


Figure 26. Comparison of a basic and a neutral solution of dodecyl sarcosinate. Surface tensions are plotted as a function of various concentrations of dodecyl sarcosinate. The lines show how the CMCs are calculated for a neutral and more basic solution. At pH 7 the CMC is about 10mM and at pH 9.7 the pH is about 12.5 mM.

When a concentration of 0.42 ‰ CaCl2 was added to the sarcosinate concentrations from 5-45mM, precipitation was observed and no dilution series and surface tension measurements were therefore performed, Figure. 27

Figure 27. 0.42 ‰ calcium chloride added to concentrations of dodecyl sarcosinate, 45-5mM.

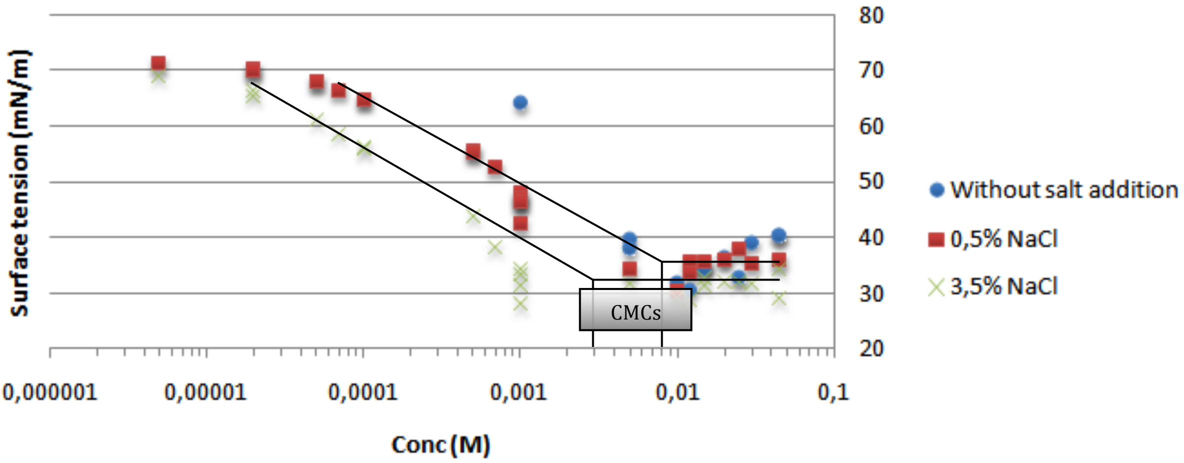


Figure 28. Comparison of surface tensions vs. various concentrations of dodecyl sarcosinate with and without salt. The lines indicate how the CMCs are determined for surfactant solutions with 0.5 and 3.5% NaCl. The CMC for 0.5% NaCl is, according to this graps, 8mM and 3.2 mM when adding 3.5% NaCl.

5.2.9 Dodecyl sarcosinate and dodecyl glucoside mixtures

5.2.9.1 Molar ratio 1:1

A dodecyl glucoside and dodecyl sarcosinate mixture at a 1:1 molar ratio was analyzed at a temperature of 21.1°C, one day after solutions were prepared. According to the results in Figure 29, the CMC reaches a very low value. From Figure 29 the CMC is approximately 0.25 mM. The surface tension above CMC also reaches a very low value, Figure 29, compared to what can be seen for the surfactants alone. A strange behavior was observed when the dilution series was prepared, concentrations of 0.7 mM, 1 mM, 2 mM , 5 mM , 7 mM and 10 mM were clear when they just had been diluted from the stock solutions but turned turbid after two days (they might have turned turbid earlier than that, but since the samples were shipped to another location for analysis during this time, it was not noted) See the photos below, Figure 30.

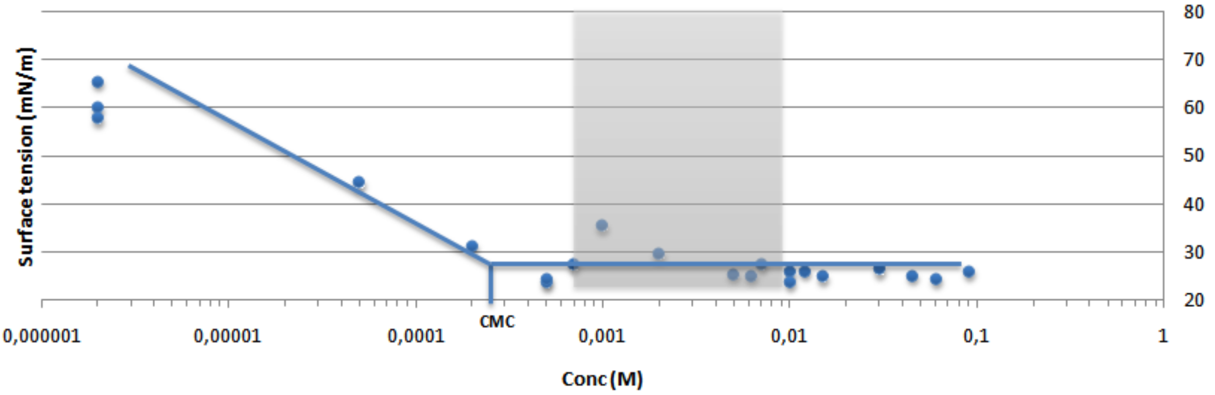


Figure 29. Surface tension as a function of surfactant mixture concentrations of a mixture of dodecyl glucoside and dodecyl sarcosinate at a molar ratio of 1:1. The pH was determined to be 7.96 during analysis. The grey area represents concentrations where precipitation emerged after two days. The line shows how the CMC was calculated.

When a stock solution of 30 mM was prepared with 0.42‰ $CaCl_2$ a somewhat turbid solution was the result, see Figure 31. The turbidity was either due to undissolved surfactant or precipitation. Since this turbidity occurred it was decided not to make a dilution series of this stock solution.

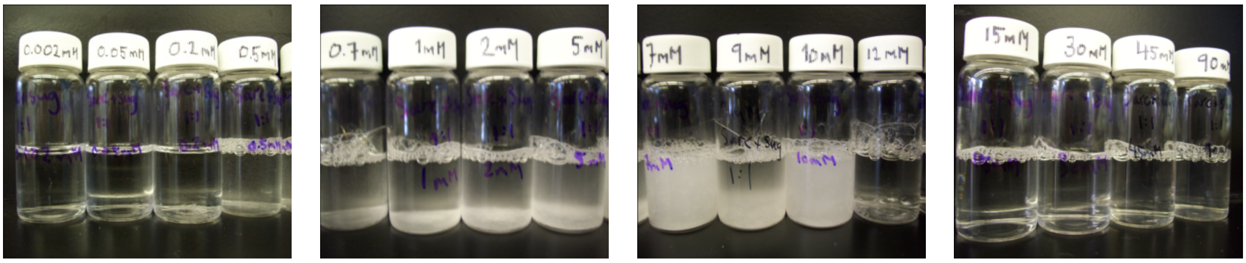


Figure 30. Photos of the samples of dodecyl sarcosinate + dodecyl glucoside mixtures at a ratio 1:1, ranging from 0.002 mM (left) to 90 mM (right). The photos were taken three days after the samples had been prepared. As can be seen there was precipitation from 0.7 to 10 mM

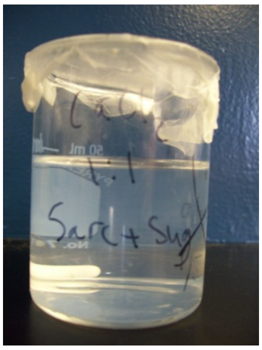


Figure 31. Precipitation in the dodecyl sarcosinate/dodecyl glucoside stock solution (30 mM), mol ratio 1:1, 0.42‰ $CaCl_2$. The picture was taken after the sample had been stirred with a magnetic stirrer for 24 hours and thereafter had been set to rest for two weeks.

5.2.9.2 Molar ratio 19:1

A mixture series of sarcosinate and glucoside with ratio 19:1 was analyzed at 21.5°C and prepared one day before analysis. The surface tension as a function of surfactant mixture concentrations is presented in Figure 32 and data from this graph is represented in Table 8. From interpreting Figure 32 one could find the CMC to be 1.6mM without salt addition. Addition of salt does not seem to have affected the CMC values very much, according to Figure 32, however a decrease in CMC when adding 3.5% NaCl is occurring. The CMC with addition of 3.5% NaCl is approximately 0.7mM from Figure 32. Adding 0.5% NaCl does not seem to affect the CMC value, it is the same as the CMC at neutral pH, Figure 32.

As in the case of preparing a sarcosinate/ dodecyl glucoside stock solution of molar ratio 1:1 a precipitation, Figure 33, was formed when the stock solution of sarcosinate/ dodecyl glucoside at a molar ratio of 19:1 was made at 30 mM with 0.42‰ $CaCl_2$ added. In the 19:1 case the stock solution was more prominent however. Also in this case it was decided not to proceed with a dilution series of this mixture.

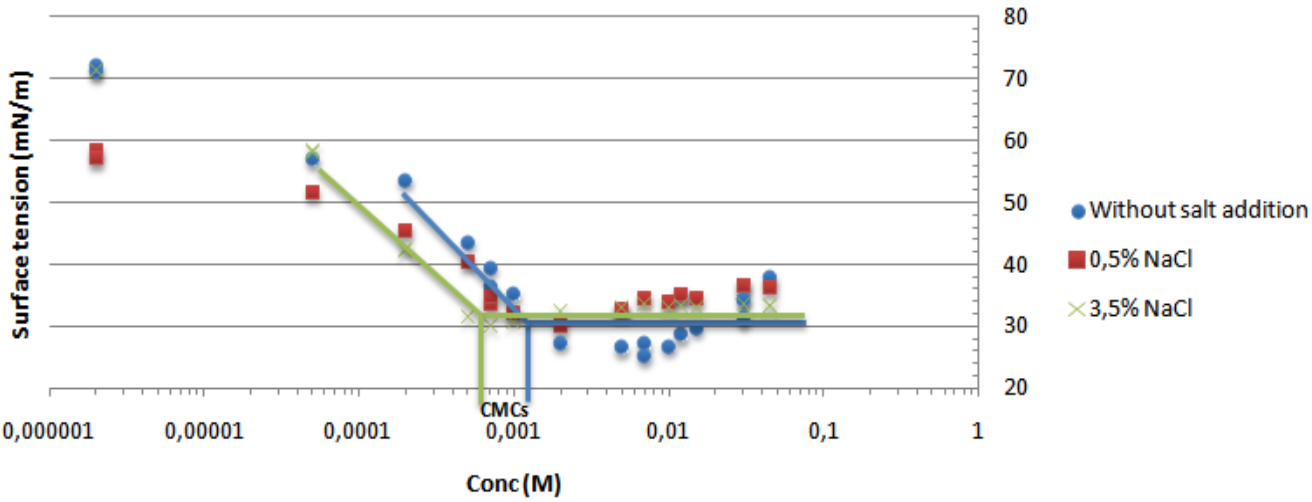


Figure 32. Comparison of surface tensions vs. surfactant mixture concentrations of a 19:1 molar ratio of dodecyl sarcosinate/dodecyl glucoside at with and without salt. The lines show how the CMC was calculated at neutral pH and how the CMC was calculated when 3.5% NaCl was added.

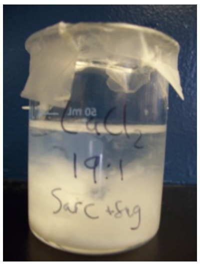


Figure 33. Precipitation in the stock solution of dodecyl sarcosinate and dodecyl glucoside at molar ratio 19:1 with with 0.42‰ CaCl2 added. The photo was taken after the sample had rested for two weeks from 24 hours of magnetic stirring.

5.2.10 Table of results for dodecyl sarcosinate solutions

The results in Table 7 are collected from when using the calculated CMCs from the graphs and the Matlab program for the mixtures of sarcosinate and glucoside at the two different ratios and salinities.

Table 7. β -parameters and molar ratio of dodecyl sarcosinate for different mixtures of dodecyl sarcosinate and dodecyl glucoside

Mixture sarcosinate:glucoside	CMC of mixture (mM)	Molar ratio of glutamate in micelle	β-parameter
19:1 ratio, pH 7	1.6	0.39	-2.5
19:1 ratio, pH 7 0.5% NaCl	1.6	0.42	-2.5
19:1 ratio, pH 7, 3.5% NaCl	0.7	0.51	-3.7
1:1 ratio, pH 7	0.25	0.21	-4.4

In the following Table 8 the results from surface tension measurements of the different solutions of dodecyl sarcosinate are summarized.

Table 8. Results from surface tension measurements for dodecyl sarcosinate and its mixtures with dodecyl glucoside under varying conditions

sarcosinate solutions	**CMC (mM)**	**Plateau value of surface tension above CMC (mN/m)**	**Area per molecule (Å^2)**	**Interfacial tension with hexanes (mN/m)**	**Emulsification Capacity (EC)**	**Comments**
pH 7	10	40	65.1	-	-	
pH 9	12.5	37	56.4	10 (conc. 30 mM)	48% (conc. 30 mM)	
pH 7, 0.5% NaCl	8	36	60.1	-	-	
pH 7, 3.5% NaCl	3.2	32	58.8	3.3 (conc. 30 mM)	31% (conc. 30 mM)	
pH 7,0.42 ‰ CaCl2	N.A	N.A	77	- (conc. 30 mM)	9% (conc. 30 mM)	Precipitation in entire series
MIXTURES **sarcosinate:glucoside**	**CMC (mM)**	**Plateau value of surface tension above CMC (mN/m)**	**Area per molecule (Å^2)**	**Interfacial tension with hexanes (mN/m)**	**Emulsification Capacity (EC)**	**Comments**
19:1 ratio, pH 7	1.6	38	63.1	2.55 (conc. 45 mM)	47% (conc. 45 mM)	
19:1 ratio, pH 7 0.5% NaCl	1.6	36	56.8	-	-	
19:1 ratio, pH 7, 3.5% NaCl	0.7	34	65.6	2.65 (conc 30 mM)	0% (conc. 30 mM)	
19:1 ratio, pH 7, 0.42‰ CaCl2	N.A	N.A	-	-	-	Stock solution precipitated
1:1 ratio, pH 7	0.25	25	65.4	- (conc. 60 mM)	50% (conc. 60 mM)	Precipitation at 0.7-10mM
1:1 ratio, CaCl2	-	-	-	-		Stock solution precipitated

5.4 Results from Conductivity measurements

The conductivity measurements were performed at the latest one week after the solutions had been prepared. The measurements at different salt conditions (0.5% NaCl and 3.5% NaCl) did not give good curves, this was probably due to that the salt was weighed up in every vial which sometimes gave a difference of ± 2 mg NaCl. This difference was probably due to the unlinearity in those measurements. Also, at higher salt concentrations the range for which where the conductometers are no longer accurate more than ± x mS/cm might be reached, this range was however not known for the instruments that were used. 10 ml surfactant solutions were also maybe a too small amount to measure since the electrode was just covered by the solution when it was immersed. The graphs below, Figure 34,35,36,37 are however those that gave the best curves and shows the desired shape for a conductivity measurement within the investigated range. That is, the curve shows a decrease in the slope for conductivity versus surfactant concentration once the CMC is reached. The CMC can therefore be taken from the conductivity curves where the intersection between the two curves are.

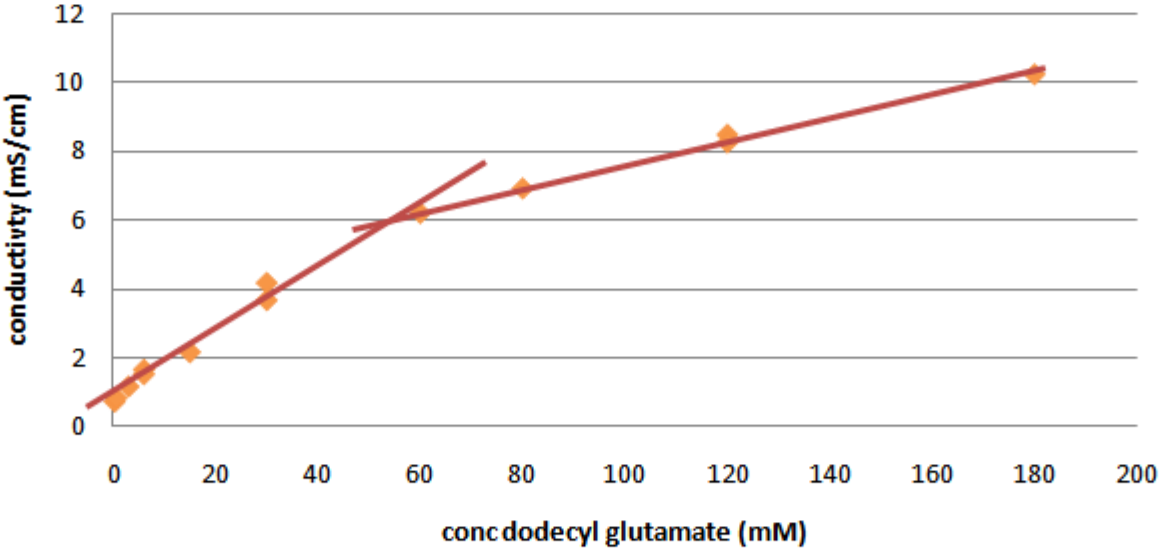


Figure 34. Results from conductivity measurements of dodecyl glutamate at pH 7 with 0.42 parts per thousand $CaCl_2$ added, the samples were measured one after they were prepared.

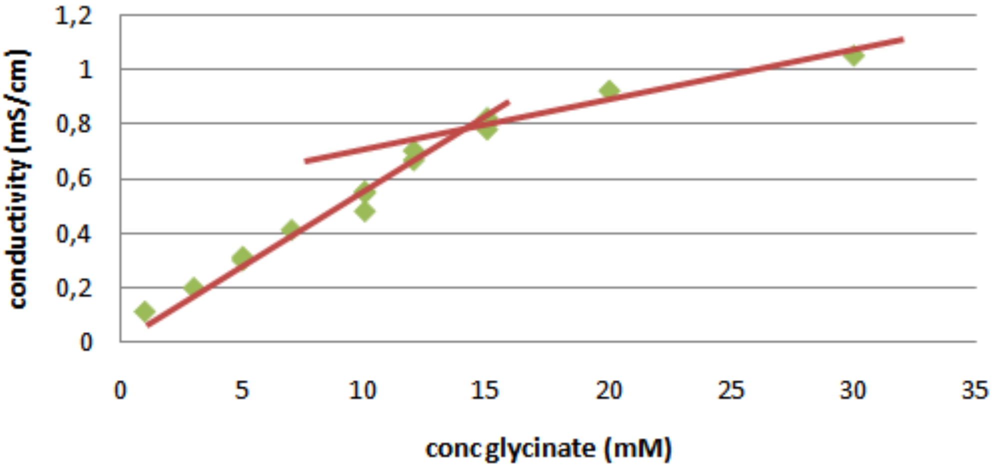


Figure 35. Results from conductivity measurements of dodecyl glycinate at pH 7, the samples were measured 1 day after they were prepared. From the intersection of the curves a CMC of 14.5 is given to the surfactant.

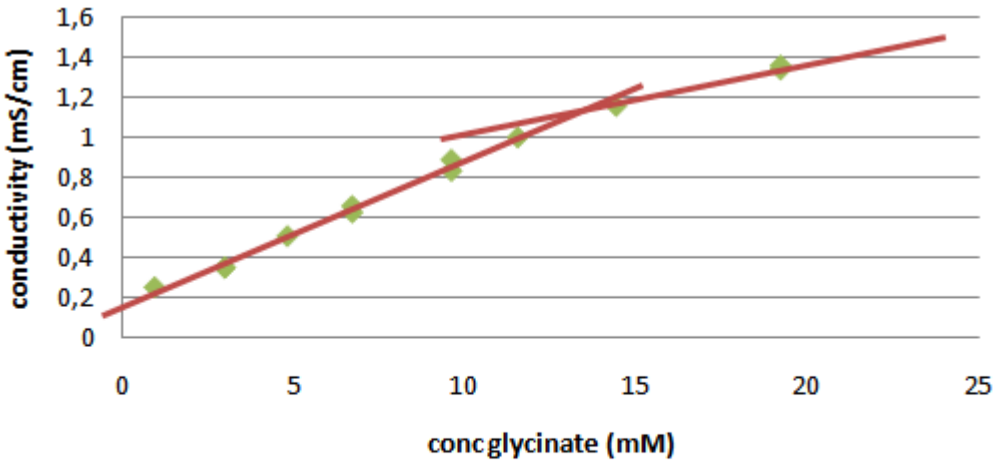


Figure 36. Results from conductivity measurements of dodecyl glycinate at pH 9.7, the samples were measured 1 day after they were prepared. From the intersection of the two curves a CMC of 13.7 is given to the surfactant.

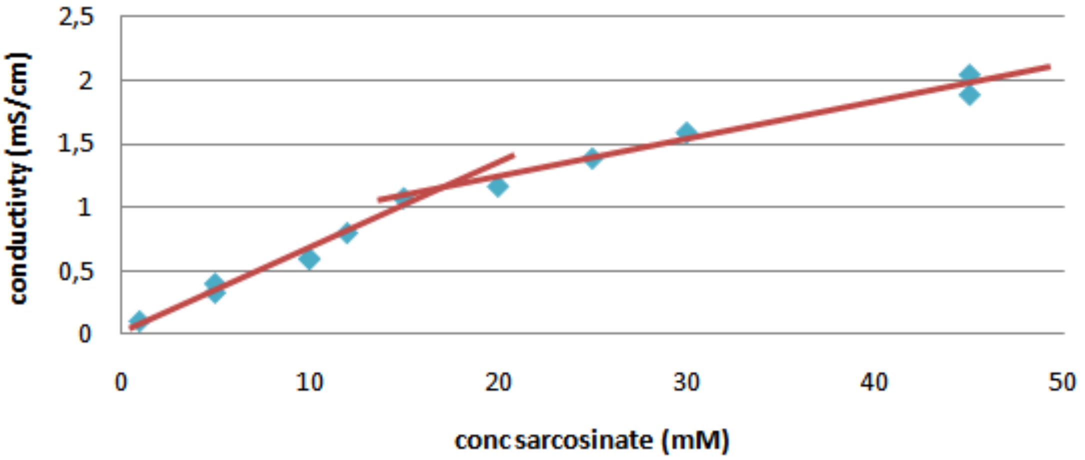


Figure 37. Results from conductivity measurements of dodecyl sarcosinate at pH 7, the samples were measured 1 day after they were prepared.

The degree of ionization of the micelles, α, can be calculated by dividing the slopes before and after the kink in the conductivity versus concentration curve. The CMC and ionization degrees of the surfactants above can be seen in Table 9 below.

Table 9. Results from conductivity measurements.

Surfactant	CMC according to Conductivity	Degree of ionization
Dodecyl glutamate pH 7, 0.42 parts per thousand $CaCl_2$	58	0.35
Dodecyl glycinate, pH 7	14.5	0.35
Dodecyl glycinate pH 9.7	13.7	0.49
Dodecyl sarcosinate, pH 7	17	0.51

5.5 Emulsion stability/Emulsification capacity

Below are the results from the emulsion stability tests presented. In, the emulsification capacity and foam height are presented and in Figure 38 the results of emulsification capacity can be seen. It is important to know that the emulsion tests were not tested after equal amount of time, which may have an effect to the results. Some samples were tested up to 6 weeks after they had been prepared and others only a couple of days after they had been prepared.

Table 10. Results from evaluating the emulsion capacity of the different surfactants.

Nr.	Sample	Emulsification capacity after 5 min (%)	Emulsification capacity after 30 min	Emulsification capacity after 24 hours	Foam height and other comments
1	glucoside 0.148 mM?!, pH 7, 3.5% NaCl	0	0	0	
2	glutamate, 120 mM, pH 7,3.5% NaCl	3	0	0	
3	glutamate, 180 mM, pH 7, 0.42‰ $CaCl_2$	49	54	2	5mm foam on 39 mm solution was observed after 5 min, then nothing
4	glutamate+glucoside 19:1 mol ratio, 110 mM, pH 7	50	50	52	20mm foam on 28mm solution after 5 and 30 min, then nothing
5	glutamate+glucoside 19:1 mol ratio, 110 mM, pH 7, 3.5% NaCl	48	23	0	
6	glutamate+glucoside, 1:1 mol ratio, 131 mM, pH 7	57	54	54	11 mm foam on 37 mm solution during all measurements
7	glutamate+glucoside, 1:1 mol ratio, 150 mM, pH 7, 0.42‰ $CaCl_2$	55	51	53	9 mm foam on 38 mm solutions was observed during all measurements
8	glycinate, 20 mM, pH 9	2.6	2.6	2.6	
9	glycinate, 30 mM, pH 7, 3.5% NaCl	47	47	50	17 mm foam on 32 mm solutions was observed after 5 and 30 min. Some bubbles were ”incorporated” in the emulsion layer after 24 hours
10	glycinate+glucoside, 19:1 mol ratio, 30 mM, pH 7, 3.5% NaCl	55	53	53	11 mm and 10 mm foam on 38 mm solution after 5 and 30 min. Some bubbles that were ”incorporated” in the emulsion after 24 hours
11	glycinate+glucoside, 1:1 mol ratio, 30 mM, pH7	55	50	53	11, 11 och 10 mm foam on 36 mm solutions after 5 min, 30 min and 24 hours
12	sarcosinate, 30 mM, pH 9	41	41	48	7, 5 and 0 mm foam on 27 mm after 5, 30 min and 24 hours
13	sarcosinate, 30 mM, pH 7, 3.5% NaCl	53	53	31	11, 5, 0 mm foam on 32 mm solutions after 5, 30 min and 24 hours
14	sarcosinate, 30 mM, pH 7, 0.42‰ $CaCl_2$	18	15	9	$CaCl_2$ in oil phase/the emulsion
15	sarcosinate+glucoside, 19:1 mol ratio, 45 mM, pH 7	45	45	47	17 mm foam on 31 mm solution after 5 and 30 min
16	sarcosinate+glucoside, 19:1 mol ratio, 30 mM pH 7, 3.5% NaCl	34	27	0	“clumpy/fluffy” emulsion
17	sarcosinate+glucoside, 1:1 mol ratio, pH 7	62.5	59	50	11, 11, 4 mm foam on 32, 32 and 38 mm solutions at 5, 30 min and 24 h

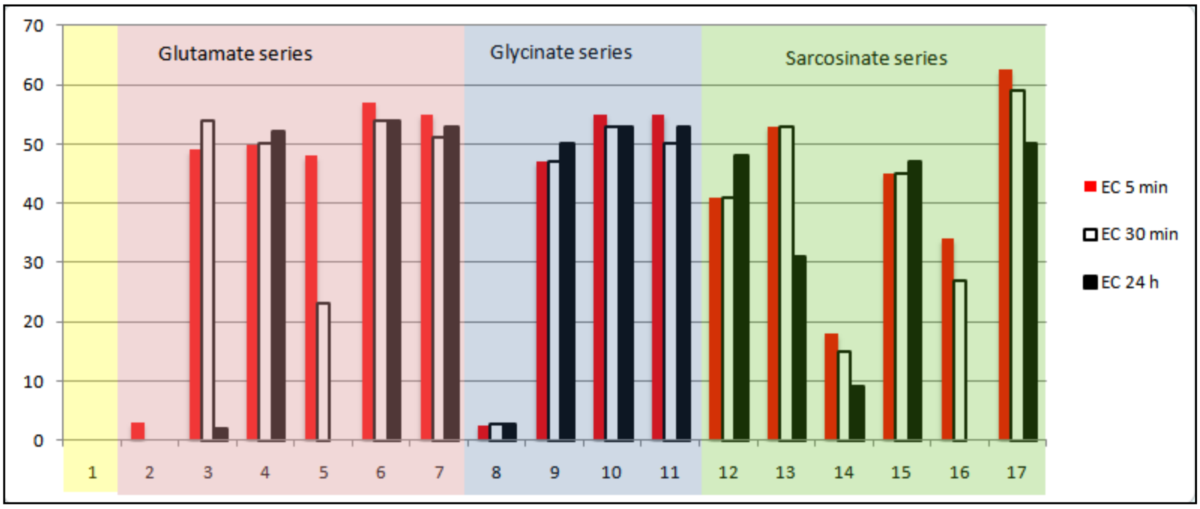


Figure 38. Emulsification Capacity test results after 5 min, 30 min and after 24 hours for the different surfactants series. The y-axis represents the emulsification capacity in % and the x-axis shows the number of the emulsion, collected from Table 10.

The results from the emulsion stability tests that can be seen in Table 10 and Figure 38 indicates that the emulsions that are the most efficient, by having the highest emulsion capacity over time, seems to be the mixtures of the anionic surfactants and the nonionic glucoside. Glycinate alone with 3.5% NaCl also shows a high emulsification capacity. The emulsions that had the higher emulsification capacities also formed foam on the top of the emulsions. The reason for that the emulsification capacity can go up after some time, as in number 4 for example, is that the foam settles and thereby increases the total height of the emulsion, which gives a lower percentage of the emulsion (see method for calculating emulsion capacity, section 4.6) It is interesting to see that the emulsion of glucoside alone did not show any emulsification capacity at all. This is probably due to the solubility problems that occurred when making the samples of glucoside alone which resulted in a solution that not had a concentration of 3*CMC, as in the case of the other samples.

5.6.2 Dodecyl glucoside solubility with dodecyl sarcosinate

Literature indicates that mixing two surfactants can enhance dissolution [28]. This also seems to be the case in our study where a concentration of 45mM sarcosinate could dissolve more than 0.5469 g n-dodecyl-β-D-glucoside in 35ml aqueous solution, which is a significant larger amount of n-dodecyl-β-D-glucoside in comparison to what could be dissolved in pure water.

6. Discussion

6.1 Effect of salt addition to the different biosurfactants

In this section it is discussed what effects the NaCl addition does to the CMC of the surfactants alone and to the mixtures.

6.1.1 Effect of NaCl addition

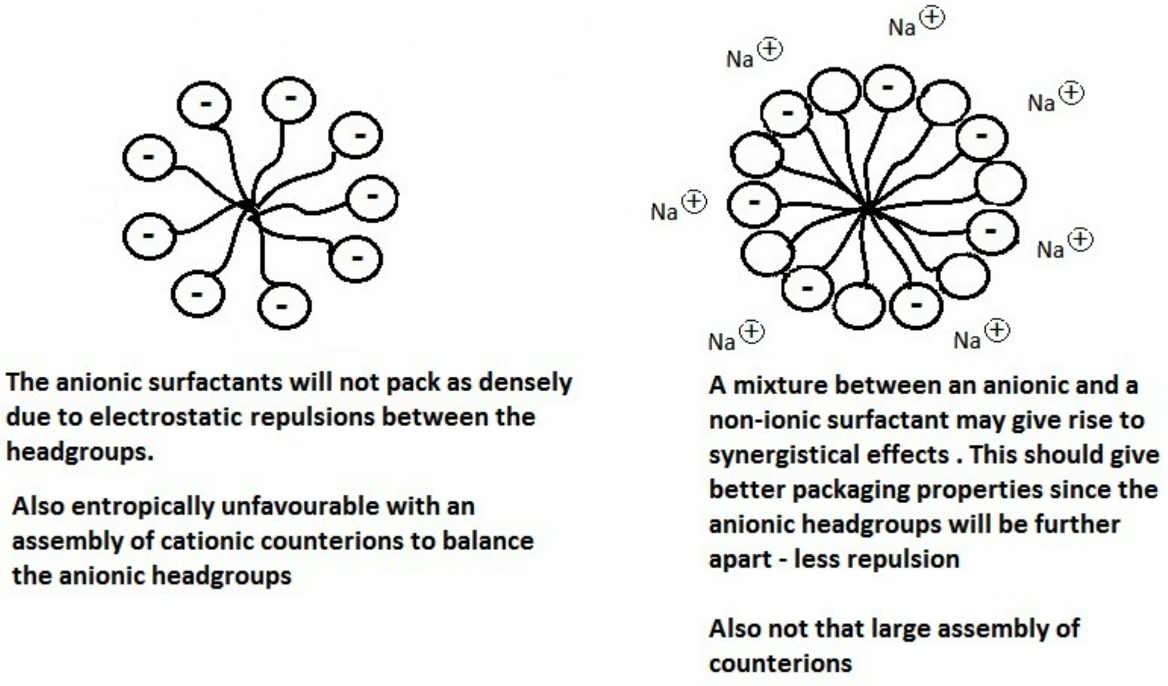
6.1.1.1 Dodecyl glucoside

Somasundaran et al. have shown for the nonionic surfactant n-dodecyl-β–D-maltoside, which is similar to dodecyl glucoside, results that are similar to those from Figure 7. Somasundaran et al. studied the surface tension of a nonionic surfactant similar to dodecyl glucoside, n-dodecyl-β-D-maltoside. This surfactant was studied when salt was added and a reduction in CMC could be observed. Suggested in the article by Somasundarans et al., this occurrence is due to hydration of salt ions, which occurs in the aqueous solution. This affects the solvent properties of the nonionic surfactant by decreasing the water activity and increasing the effective concentration of the surfactant, hence decreasing both surface tension and CMC [29]. The process when an organic phase is separated from an aqueous phase in the presence of salt is known as the salting out effect. Glucoside was very hard to dissolve to make an entire dilution series. This problem resulted in a stock solution that not had a concentration of 3*CMC, as in the case of the other samples. When making the mixtures of glucoside and the anionic surfactants the solubility of glucoside increased. Literature indicates that mixing two surfactants can enhance dissolution [28]. This also seems to be the case in our study where a concentration of 45mM sarcosinate could dissolve more than 0.5469 g glucoside in 35ml aqueous solution, which is a significant larger amount of glucoside in comparison to what could be dissolved in pure water.

6.1.1.2 Overall effects on the anionic surfactants

The effect of NaCl addition is different for every surfactant. Some trends can be seen when comparing the three anionic surfactants to each other though:

The lowering of CMC as NaCl is added is a phenomenon known from literature about ionic surfactants. Addition of NaCl would screen the repulsion from the ionic head group of the surfactant (see section "2.3.1 General facts regarding CMC"), which should result in a denser packing of the surfactants in the micelles and at the surfaces -hence a reduction of the CMC which can be seen for all the surfactants when used alone. One would also expect that the plateau value of surface tension above CMC would decrease, using the same reasoning as for the lowering of the CMC, but this is not the case for all the surfactants actually. The effects that are present when salt is added and when making a mixture between surfactants are summarized in the Figure below, which was the hypothesis through the entire study.



The anionic surfactants will not pack as densely due to electrostatic repulsions between the headgroups.

Also entropically unfavourable with an assembly of cationic counterions to balance the anionic headgroups

A mixture between an anionic and a non-ionic surfactant may give rise to synergistical effects . This should give better packaging properties since the anionic headgroups will be further apart - less repulsion

Also not that large assembly of counterions

Figure 39. The hypothesis that was used through the entire study, that a better packaging of the surfactants in the micelles and at the surfaces should be obtained once the repulsions between the head groups were shielded, either by the addition of counter ions or by making mixed micelles.

However, the effect of salt addition seems to vary to some extent between the three anionic surfactants, and an evaluation of every surfactant might therefore be appropriate:

6.1.1.3 Dodecyl glutamate

Looking at the results from NaCl addition to the solutions of glutamate where pH is neutral some trends can be seen (Table 4 and Figure 9). The CMC is decreasing with addition of NaCl but there is no significant effect on the plateau value of surface tension. The interfacial tension values also follow this trend, no big difference of the interfacial tension as NaCl is added, which is very reasonable. The reason for that the plateau value of surface tension is hard to evaluate. It is almost impossible to get 100% purity of a surfactant and our dodecyl glutamate had a purity of 98%. The nature of the impurities is not known but is expected to be small amounts of a hydrophobic byproduct, fatty alcohol [30].

When comparing Figure 9 with Figure16, NaCl addition to glutamate alone to NaCl addition to glutamate /glucoside mixture, the following can be seen. In the case with the mixtures with ratio 19:1 of glutamate/glucoside one can see that the effect of NaCl addition is not as abundant as compared to the solution with only glutamate with NaCl addition it looks as 3.5% NaCl addition might give a slightly lower CMC but the same CMC is calculated from all the three curves since no significant difference could be seen. As mentioned above, the NaCl addition does not seem to support or disturb the interaction between the two surfactants in this case.

6.1.1.4 Dodecyl glycinate

From Table 6 and Figure 17 it can be seen that the NaCl addition to glycinate alone has an effect on both CMC and plateau value of surface tension. The CMC is decreasing with NaCl addition and so does the plateau value of surface tension.

In the case of adding NaCl to the mixtures with 19:1 ratio the effect of CMC was not so large, see Figure 24. But here, the NaCl addition at 3.5% gave a β-parameter with a higher magnitude which indicates stronger interaction when theory actually goes against this behavior (see above). Also the plateau value of surface tension shows an increase from 30 mN/m to 36 mN/m with 0.5% salt addition and then a decrease with 3.5% NaCl addition to 32 mN/m. However the differences are still quite small and can also have to do with uncertainties in the measurements.

6.1.1.5 Dodecyl sarcosinate

When studying Table 8 and Figure 28 it can be seen that in this case the CMC and plateau value of surface tension is decreasing with NaCl addition for sarcosinate alone. For the mixtures with glucoside at ratio 19:1 the CMC is very little affected as NaCl is added but actually seems to decrease a little bit when the NaCl concentration is 3.5% These differences are small however, but also shows a somewhat stronger interaction between the anionic and nonionic surfactant as NaCl is added, same effect as for glycinate, which is a bit surprisning.

6.1.1.6 Effect of NaCl addition to the mixtures

The lowering of CMC as NaCl is added to the mixtures of nonionic and anionic surfactants is not as large as when adding NaCl to the anionic surfactant solutions alone. But the effect seems to have a somewhat positive effect on the CMC on all of the anionic surfactants (by making it lower). When looking at the results for NaCl-addition to the 19:1 mixing ratio for all three mixtures, the same trend can be seen in the β-parameter values, see Table 3, 5 and 7. At first the β-parameter is negative and when 0.5% NaCl is added the β-parameter becomes less negatively charged but then turns out to be more negatively charged again when the NaCl concentration increases to 3.5%. A more negatively charged β-parameter would indicate a stronger interaction. The results for 3.5% NaCl addition then actually goes against theory, see section "2.3.2 CMC and mixtures", where it is explained that NaCl addition should make the β-parameter decrease in magnitude due to that the net interaction between the surfactants should decrease when NaCl screens the negative charges of the anionic surfactant. However the effects are quite small in these results and one could maybe instead blame the differences to be due to vague measuring results which then gave errors when finding the CMC:s from the curves.

6.1.2 Effect of an addition of 0.42‰ of CaCl2

The overall effect of adding $CaCl_2$ to the anionic surfactants and their mixtures was that it in most cases resulted in precipitation. Either precipitation of the stock solutions at 3*CMC concentrations or precipitation for some concentration interval. This concentration interval actually included the entire series after a couple of days in some cases. Unfortunately these effects were not fully evaluated.

Glutamate was the only surfactant that did not precipitate at a concentration of ~3*CMC when used both alone, Figure 40, and in mixtures (except for the mixture with 19:1 ratio of glutamate and glucoside) The addition of 0.42‰ $CaCl_2$ lowered the plateau value of surface tension with about 10 mN/m. Previous work at Chalmers [16] has already confirmed these effects with glutamate and $CaCl_2$ addition and that the effect is due to that the two carboxylate groups on the glutamate head group would form an intermolecular bonding with the calcium ions in the solution, instead of an intramolecular bonding that would result in precipitation. The intermolecular bonding will thereby trigger a closer packaging of the glutamate surfactants at the surface and this would therefore lower the surface tension.

Figure 40. Concentrations of dodecyl glutamate with 0.42‰ calcium chloride, from left to right: 0.3 mM, 0.6 mM, 3 mM, 6 mM, 15 mM, 30 mM, 60 mM, 64.7 mM, 100 mM, 120 mM and 180 mM

The reason for the precipitation with glycinate and sarcosinate solutions is due to that calcium is a divalent ion and an explanation for the precipitation could be that the divalent ion forms a complex with the carboxylic groups of two-sarcosinate/glycinate molecules. It is possible to calculate the theoretical amount of sarcosinate that has formed complexes with the calcium ions and then determine what concentration of sarcosinate that is left in solution.

Colloidal systems may coagulate when an electrolyte is added to the system. According to literature, all electrolytes are capable of causing coagulation. When a system has a concentration of an electrolyte that is sufficiently high to compress the electric double layer of particles and thereby lower the energy barrier that hinders the particles from sticking together under a collision, coagulation might occur. This was discovered by Hardy. The minimum concentration of an electrolyte that must be reached for coagulation to occur due to the compression of the electric double layer until it is no longer the energy barrier which protects the particles from sticking together by means of the molecular forces of attraction is called the coagulation threshold. The coagulation power was found to be different for different ions according to the Schultze-Hardy rule/ valency rule, which in short shows that the coagulation power grows as the valency of the ion is increased. This in not a direct dependence, the coagulation power grows much more rapidly than the valency [17] .

According to these facts our study of the solutions with NaCl would probably show this behavior at a certain point of concentration too. This was however not found. It would have been interesting to find this and see if the Schultze-Hardy rule would have applied.

Some experiments with Zeta pulse were however carried out in an attempt to try and find the coagulation threshold of $CaCl_2$, or at least find an interval of this threshold. The results from these experiments did unfortunately not give any reasonable results to publish.

6.2 Synergism/Antagonism of anionic biosurfactants when mixed with nonionic dodecyl glucoside

When looking at the overall results for the three different mixtures, glucoside + glutamate/glycinate/sarcosinate at the two mixing ratios 19:1 and 1:1, it can be seen that by doing a mixture between an anionic amino acid based surfactant and a nonionic sugar based surfactant a lower surface tension and CMC can be reached, which indicates a synergistic behavior between the two surfactants, see further section 2.3.2. The higher the molar ratio of the nonionic surfactant in the solution, the lower the CMC of the mixture. This can be compared with a study of a similar system that is presented in the book “Surfactants and polymers in aqueous solution” by Holmberg et al. p. 122 The Figure can be seen below in Figure 41 and it shows the same behavior for another system that also contains an anionic surfactant and a nonionic surfactant.

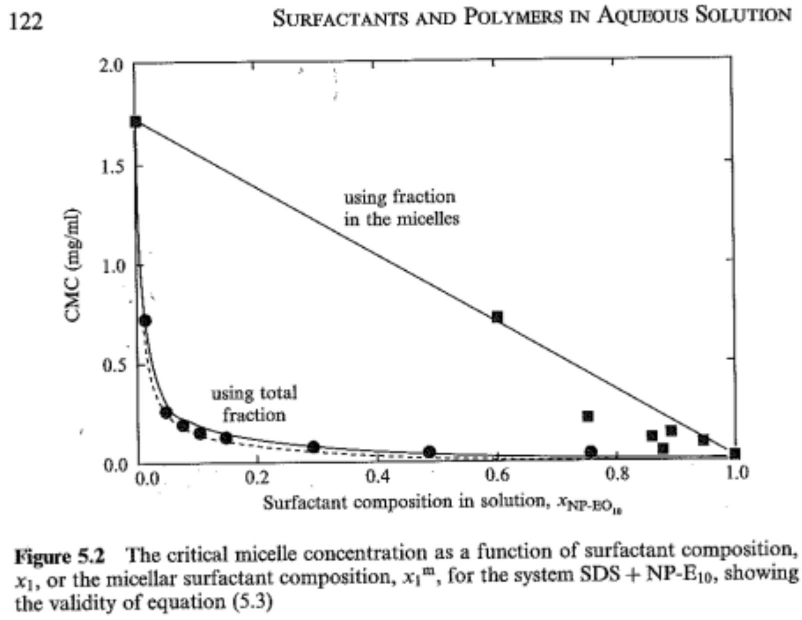


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Figure 5.2 The critical micelle concentration as a function of surfactant composition, x_1, or the micellar surfactant composition, x_1^m, for the system SDS + NP-E_{10}, showing the validity of equation (5.3)

Figure 41. The CMC as a function of surfactant composition in solution and in micelle for a mixture of an anionic surfactant (SDS) and anon-ionic surfactant (NP-E10)

It is also clear that the molar fraction of the anionic surfactants in the mixed micelles is less than the nonionic surfactant. This was the case for all the evaluated surfactants. The low CMC of the nonionic surfactant represents a more hydrophobic surfactant which therefore is more prone to be in a micelle structure. Therefore the results from molar ratios in the micelles are very reasonable. This can also be seen from other results of the same study, mentioned above, where it can be seen that for their system of anionic and nonionic surfactant, the nonionic surfactant with the lower CMC is present in a larger fraction in the micelle as the ratio of this surfactant is increased in the solution. This can be seen in Figure 42, which is also collected from the book “Surfactants and polymers in aqueous solution” by Holmberg et al p. 123.

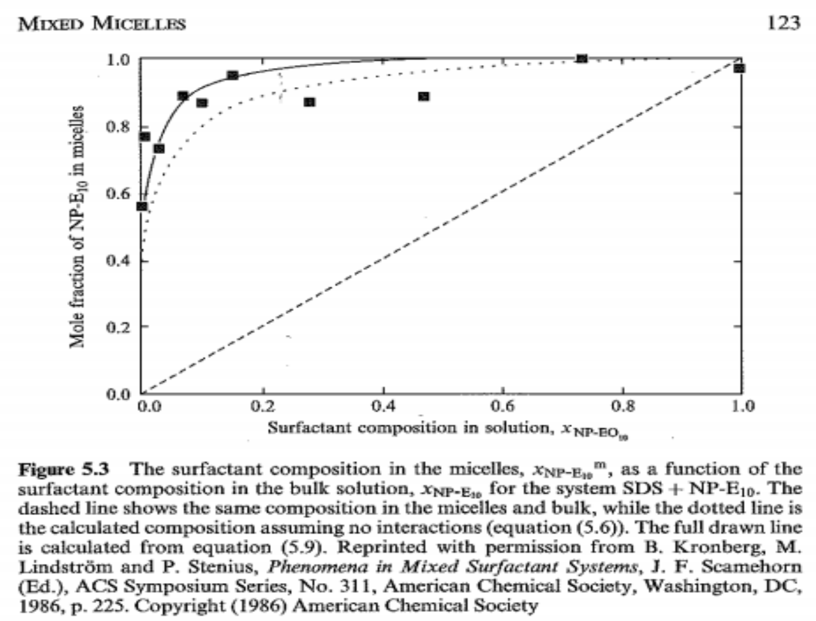

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Figure 5.3 The surfactant composition in the micelles, $x_{NP\text{-}E_{10}}{}^{m}$, as a function of the surfactant composition in the bulk solution, $x_{NP\text{-}E_{10}}$ for the system SDS + NP-E$_{10}$. The dashed line shows the same composition in the micelles and bulk, while the dotted line is the calculated composition assuming no interactions (equation (5.6)). The full drawn line is calculated from equation (5.9). Reprinted with permission from B. Kronberg, M. Lindström and P. Stenius, *Phenomena in Mixed Surfactant Systems*, J. F. Scamehorn (Ed.), ACS Symposium Series, No. 311, American Chemical Society, Washington, DC, 1986, p. 225. Copyright (1986) American Chemical Society

Figure 42. The graph shows the surfactant composition in the micelles as a function of surfactant composition in the solution for the system of SDS with NP-E10

The β-parameters and molar ratios of anionic surfactants in micelles should probably not be seen as very accurate reliable results. To get more significant values more measurement points should be added and it would also be good to look for the CMC using another technique too, like conductivity or bubble pressure. The variations one get in the CMC for the mixtures is very much dependent on the β-parameter, only small variations like 1 mM difference in the CMC-value gives a large effect to the β-parameter. However according to the experimental results for the evaluated surfactants the β-parameter is in the range -2 - -4.4, the expected values for a system like this should maybe have been be somewhat lower, see further section 2.3.2. The increase in magnitude for the β-parameter when salt is added to some of the mixtures is further discussed in section 6.1.1.6 Comparing Tables 3,5 and 7 The surfactants that shows the largest synergistic effect, according to our results, is glycinate followed by sarcosinate that has very similar results in both β-parameters and molar ratio of these surfactants in the micelles. This is also reasonable since these molecules are very similar, differing with only one methyl group. Glutamate does not participate as much in the mixed micelles as glycinate and sarcosinate this could be due to the divalency of this surfactant. The anionic hydrophilic head takes up more volume which might make it harder to fit in a larger fraction into the micelles.

6.3 Effect of pH on biosurfactants performance

As can be interpreted from surface tension measurements results, there is an unexpected and undesirable increase in pH with increasing surfactant solution concentration. The explanation to this could be found when observing what species of the surfactant that are present in a surfactant solution. There will always be some free hydrogen atoms present in the surfactant solutions containing carboxylic groups, since the carboxylic groups can be either protonated or deprotonated. The increase in pH by adding more surfactant is probably explained by the fact that adding surfactant means adding base. At approximately pH 7 the deprotonated form of the surfactant is available, and is characteristic to a base.

6.3.1 Dodecyl glutamate

When titrating with acid, the carboxylate groups of the anionic dodecyl glutamate are being protonated and will start to form complexes of either inter- or intramolecular acid soaps (acid soaps are held together by hydrogen bonds between protonated and deprotonated carboxyl groups), this will then precipitate in the solution. To calculate at which pH the precipitation will start the Henderson-Hasselbach formula (8) could be used.

$$pH = pKa + \log\left(\frac{[\mathrm{A}^-]}{[\mathrm{HA}]}\right) \qquad (8)$$

The pKa-values for glutamic acid are according to literature: 2.10, 4.07 and 9.47 for the first carboxyl, the second carboxyl and the ammonium group [16]. However, when the surfactants of dodecyl-glutamate are in self-assembled structures the carboxylic groups will probably have higher pKa-values. The higher pKa value can be expected since an added proton could be shared between several head groups in a micelle or the surfactants that are lined up at a surface. This would explain why precipitation occurs at a higher value than the expected (the pKa of the second carboxyl, around 4.07).

When the sample of 15 mM dodecyl glutamate was titrated with 0.1M HCl a precipitation started to form already at a pH as high as pH 6, see Figure 43-46 below. The precipitate turned into smaller "flakes" as the beaker was shook vigorously.

15 mM sample:

Figure 43. A solution of 15 mM dodecyl glutamate at pH 6.14, small precipitates can be seen.

Figure 44. A solution of 15 mM dodecyl glutamate at pH 5.55, the precipitates are getting larger.

Figure 45. A solution of 15 mM dodecyl glutamate at pH 5.25 the precipitation is very clear when the solution is shaken.

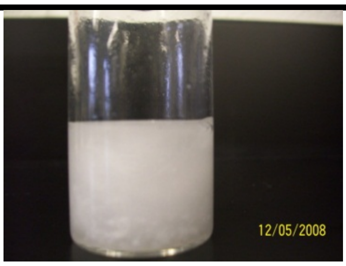

Figure 46. A solution of 15 mM dodecyl glutamate At pH 5.12, the solution is now very cloudy from the precipitation.

The CMCs of dodecyl glutamate solutions are different when the pH of the surfactant solution changes from neutral to more basic. At pH 7 the CMC is 57mM and at pH 9.7 the pH is 67mM. The explanation for the differences in CMC from the surface tension measurements could be that at pH 9.7 a formation of species has taken place, Figure 47, which are not present at pH 7. The variation of different species as a function of pH can be found in Figure 47, calculated on a total surfactant concentration of 120mM. To construct the species distribution graph, Figure 47, a Matlab program was used, Appendix 4. Figure 47 is a theoretical model from (5) and (6) and is constructed based on the pka values for the amino acid, glutamate. For the surfactant, a fully deprotonated specie will not be present even though this is true for the amino acid and not for the surfactant. According to Figure 47, the highest concentration present at pH 7 would be C12NHCH(CH$_2$)2(COO-)2. At a higher pH there is still a high concentration of C12NHCH(CH$_2$)2(COO-)2 present and no other specie available. The deprotonated species prefer to be present in the hydrophilic environment, in this case the solution, and not so easily go to the surfaces. The protonated species would go to the surfaces easier since they are less ionic. The species distribution, Figure 47, shows an increase in deprotonated species at a higher pH and after pH about 3.5, there would only be deprotonated species left in the aqueous solution. Hence there would be less surfactant at the surface, which could increase the CMC. One would, from Figure 47, not assume that there would be a significant difference in CMCs since the dominating specie is C12NHCH(CH$_2$)2(COO-)2 at both pH 7 and pH 9.7.

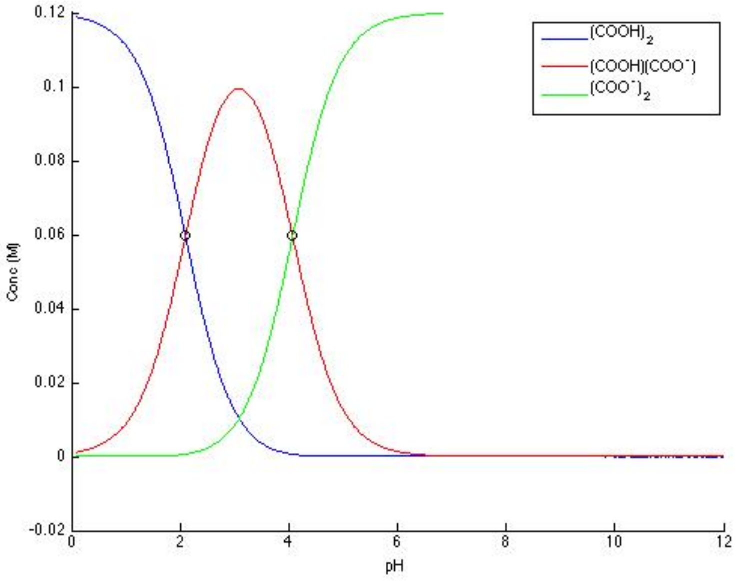

Figure 47. Species distribution for dodecyl glutamate as a function of pH.

6.3.2 Dodecyl glycinate

From the conductivity curves it can be determined that the CMC at higher pH seems to be slightly lower than the CMC at neutral pH which is a CMC of 13.7 mM at pH ~9 compared to CMC 14.5 mM at pH ~7. According to the surface tension curves they show the same trend, a CMC of 10mM at the neutral pH compared to a CMC of 6.7mM at pH ~9. At a higher pH the carboxylic group on glycinate is theoretically prone to be more deprotonated. The plots from a species distribution constructed in Matlab, Appendix 5, Figure 48, with a total surfactant concentration of 20mM, shows that at pH ~9 there is no significant difference in the presence of the ionic specie, C12NHCH$_2$(COO-) compared to at pH 7. The plot in Figure 48 is based on the pka value for the amino acid glycine, which is 2.35. Following the same reasoning with dodecyl glutamate, a higher or lower amount of the ionic specie could alter the CMC.

Figure 48 is based on the equilibrium constant for the deprotonation of the carboxylic group on the amino acid glycine and the charge and mass balances for the surfactant in aqueous solution. From Figure 48 it is not possible to explain why there would be a difference in CMCs, since the ionic specie concentration is almost constant between pH ~7 and pH ~9. The pH was only assumed to be 7 for the neutral solutions and not controlled, hence is difficult to determine exactly what species were present when the CMCs from the surface tension/conductometry measurements were performed. The change in CMCs is probably due to measuring errors, which is likely since the variation in CMCs is not major.

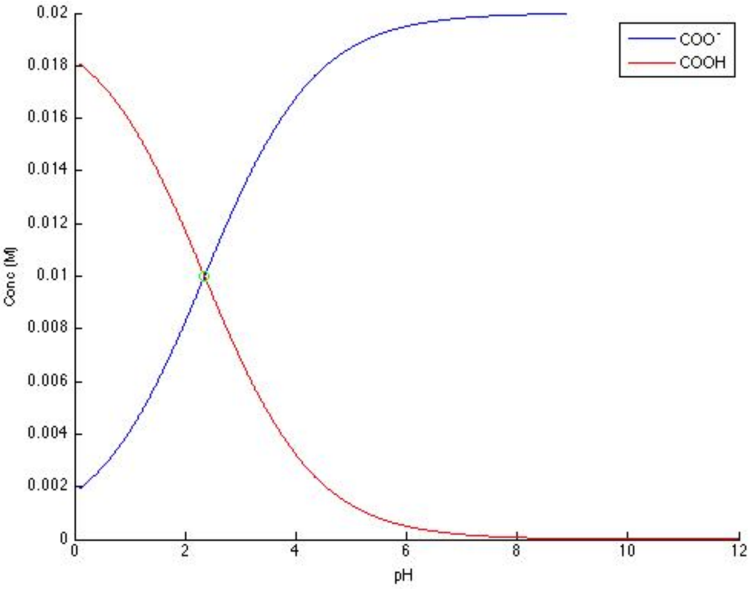

Figure 48 Species distribution of dodecyl glycinate as a function of pH.

6.3.3 Dodecyl sarcosinate

The CMC of sarcosinate is increasing from 10 mM to 12.5 mM when pH changes from ~7 to ~9. The distribution of species for sarcosinate at various pH should not be very different from the species distribution for glycinate since the different surfactants only differ with one methyl group and the pka value for sarcosine is 2.23. This implies that there should not be any difference in CMCs at pH ~7 and pH ~9, considering Figure 48. Since there theoretically should be a slight increase in the amount of deprotonated species at a higher pH, it seems reasonable that the CMC is slightly higher since there would be less species going to the surface. The difference in CMC is only minor and could also be due to errors when calculating the CMCs from the graphs.

6.4 Evaluation of emulsion stability

First of all one need to consider that the emulsification tests were done to systems that not had been prepared at the same time, some samples differs with weeks from each other. The emulsification capacity is very different between the different surfactants and their mixtures. It can clearly be seen from Table 10 and Figure 38 that the emulsions that are in mixtures with glucoside shows the best results. The reason for that glucoside did not show a high emulsification capacity alone is probably due to the solubility problems that occurred when making the samples of glucoside (see further discussion of glucosides dissolution problems in section 6.1.1.1).

From looking at the results from emulsification capacity in Table 10 and Figure 38 it cannot be seen that the 19:1 mixing ratio would give better results than the 1:1 ratio or vice versa, maybe differences can be seen when making a study over a longer time period.

When looking at the summary Tables for each of the anionic surfactant systems some trends can be found. For all the series of the three surfactants the best results in emulsification capacity are obtained when the CMC, plateau value of surface tension and interfacial tension are having the lowest values. Some exceptions from this trend can be seen however. To add NaCl seems to have an effect on some of the emulsions but not to others. The effect can be seen when comparing the mixtures of dodecyl glutamate and dodecyl glucoside with a 19:1ratio and sarcosinate and dodecyl glucoside 19:1 ratio with and without NaCl (see Table 10 sample number 4, 5 and sample number 15 and 16). Here one can see that the NaCl addition eliminates the emulsification capacity after 24 hours. When comparing the emulsification capacity the first 30 minutes the results are however quite similar. This NaCl effect, with the lowering of emulsification capacity with time, can also be seen for the sarcosinate sample with added NaCl, nr 13. For dodecyl glycinate this effect is not present either in mixture (sample nr 10)or when dodecyl glycinate is used alone (see sample number 9), so it seems as dodecyl glycinate is not as effected by the NaCl additions.

The addition of CaCl2 to the dodecyl glutamate and dodecyl glucoside mixture, 19:1 ratio, does not have the same negative effect as adding NaCl to the same mixture, compare sample nr 5 with nr 7. The emulsion with the mixture of dodecyl glutamate and glucoside actually shows very promising results when CaCl2 is added since this gives an emulsion, which is stable over time.

6.4 Comparison of surfactants, structure-property relationship

Here the values of area per molecule, CMC, and ionization degree for both NaCl addition and mixtures are compared and the effects of the structural differences are evaluated.

Table 11. Comparsion Table where some of the results for the different biosurfactants are compared to emphasize on the differences due to structural variations

Surfactant	Approx. Area per molecule alone (Å^2)	CMC (mM)	Plateau value of surface tension (mN/m)	Interfacial tension with hexanes	Ionization degree
glucoside	**27**	**0.15 – 0.3** (lit.)	N.A.	pH 9: **10.5**	-
glutamate	**100**	pH 7: **57** 3.5% NaCl: **6** 1:1 mix: **0.3**	pH 7: **53** 3.5% NaCl: **51** 1:1 mix: **31**	pH 7: **18.6** 3.5% NaCl: **17.2** 1:1 mix: **2.8**	CaCl2: **0.35**
glycinate	**60**	pH 7: **10** 3.5% NaCl: **2.2** 1:1 mix: **0.25**	pH 7: **40** 3.5% NaCl **28** 1:1 mix: **30**	pH 7: **- (10 for pH9)** 3.5% NaCl: **5.8** 1:1 mix: **0.04**	pH 7: **0.35** pH 9: **0.49**
sarcosinate	**60**	pH 7: **10** 3.5% NaCl: **3.2** 1:1 mix: **0.25**	pH 7: **40** 3.5% NaCl: **32** 1:1 mix: **25**	pH 7: **- (10 for pH 9)** 3.5% NaCl: **3.3** 1:1 mix: **N.A**	pH 7: **0.51**

The glycinate and glutamate only differs in structure with an extra carboxylic group on the glutamate that sits on a spacer with two CH2-groups, see Figure 2 and Figure 1. But this difference seems to give very different results. From Table 11 it can be seen that glycinate shows overall much more promising results, except for the mixtures, where the results are quite similar.

The difference between the glycinate and sarcosinate is an extra methyl group on the amine-nitrogen. From the Table above it can be seen that the differences between all glycinate and sarcosinate results are very small, if any. The only actual difference between these two surfactants seems to be in the degree of ionization. The glycinate has a lower degree of ionization at pH 7 than sarcosinate. This could be due to the extra methyl group on sarcosinate, which thereby would make the hydrophilic head more hydrophobic and not so dense with negative charge as the glycinate head. Sarcosinate should therefore be more prone to be ionized compared to glycinate since it can lose a hydrogen more easily.

From the Table above it can also be seen that glutamate with its larger area per molecule compared to glycinate and sarcosinate shows higher values of CMC and plateau values of surface tension. A smaller area per molecule probably packs better both in micelles and at surfaces.

7.Conclusions

The evaluated factors pH, salinity and mixtures all have an effect on the systems of the amino acid based surfactants. The effects are different between the different surfactant but an overall trend can be seen for some of the factors. The NaCl addition to the pure surfactant follows, in most of the cases, results presented in literature very well, that NaCl addition gives lowering of CMC and plateau value of surface tension which indicates a better packaging behavior. But when NaCl is added to the mixed systems the results do not agree with literature in all cases. Comparing zero addition of NaCl to 0.5% concentration of the added NaCl the results are in line with literature and gives a β-parameter with a smaller magnitude. But by increasing the NaCl concentration to 3.5% the opposite effect can be seen. Here an increasing salt concentration increases the magnitude of the β-parameter slightly, instead of going in the opposite direction proposed by literature.

The CMC increases with an increase in pH for glutamate and sarcosinate, but the opposite occurred for glycinate. The effect of pH could be explained by species distribution graphs and they show that an increase in pH will give an increase in the amount of deprotonated species. The deprotonated species are more prone to stay in the solution, surrounded by water molecules instead of going to the surfaces and therefore a slight increase in CMC should be expected with a higher pH. Why this is not true for glycinate could be explained by the fact that after pH 7 there is no significant variation in the concentration of deprotonated species, and the CMC should therefore not be different at pH 7 compared to at pH 9.7.

Regarding the mixtures of the surfactants the results from the experiments in this thesis are in a very good agreement with literature also. Increasing the ratio of the more hydrophobic surfactant, in this case the glucoside, gives a larger lowering of CMC and a higher ratio of this surfactant in the micelles.

The emulsion stability/emulsification capacity experiments show that a low interfacial tension not necessary means a stable emulsion. They also show that glycinate gives a very stable emulsion when used by itself. The majority of the most stable emulsions are however the emulsions that are produced of the mixtures of an anionic surfactant and a nonionic surfactant. The emulsion tests also show that several of the surfactant samples lose their emulsification capacity after 24 hours when they are emulsified in the presence of NaCl.

Dodecyl glutamate seems to be a surfactant useful for oil spill remediation in the ocean since it is the only surfactant compatible with the $CaCl_2$ content that is part of the sea. However, the emulsion stability is poor when the glutamate is used by itself, but when glutamate is in a mixture with glucoside the emulsion stability shows much more promising results. Though the results for that mixture does not show a stable emulsion after 24 hours in the presence of NaCl.

Glycinate and sarcosinate varies with one methyl group on the amine nitrogen in the hydrophilic headgroup, this difference does not show when comparing CMC and surface tension plateau values under different conditions, but does show a difference in the degree of ionization. Also Emulsion stability tests show somewhat different results between these two surfactants, with the most important difference probably being that glycinate seems somewhat more stable when it is in a solution with NaCl present.

Structure property results also show that a larger area per molecule, as for glutamate, gives higher values of CMC and surface tension plateau compared to glycinate and sarcosinate that both have smaller area per molecule values.

8. Future Work

For coming experiments to evaluate these surfactants capability as oil spill remedies it would of course be very interesting to see how they would behave with real crude oil and sea water. It would also be interesting to evaluate the emulsions at a lower temperature, to see how effective these surfactants might be if there was an oil spill in the north sea or Baltic sea for example.

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Appendix

Appendix 1 - Solubility of n-dodecyl-β-D-glucoside

The initial assumption was that this surfactant would cooperate in a way that we would have expected for an nonionic surfactant, that is, to not be affected by salt and rather easy to dissolve. This was however not true since we only could dissolve 0,08mM but would have desired to dissolve 0,9mM. To improve the solubility, heating was conducted on a 0,9mM solution and at 40°C it was possible to see that the large crystals of n-dodecyl-β-D-glucoside powder had disappeard, however a turbid solution emerged at this temperature, see Figure 1.

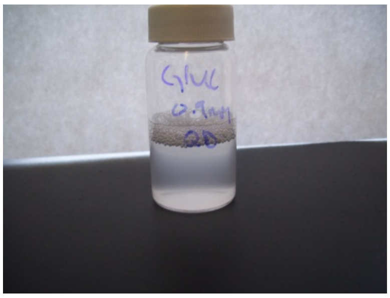

Figure 1. A 0.9 mM solution of dodecyl glucoside at 40°C

The turbidity of the solution is probably due to undissolved crystals of n-dodecyl-β-D-glucoside. Heating the solution further, to 95°C, did not contribute to a better solubility, see Figure 2.

Figure 2. A 0.9 mM solution of dodecyl glucoside at 95°C

The same behavior, a turbid solution at about 40°C, was observed for 0,6mM and 0,3mM. However, the sample with a concentration of 0,3mM became clear at a temperature of 78,5°C. When temperature dropped from 78,5°C, the 0,3mM sample became turbid again. The conclusion from this experiment is that the analysis with dodecyl glucoside alone must be performed at room temperature since otherwise temp needs to be set at 78,5°C throughout all experiments, which is too difficult in this study.

Appendix 2 – Matlab script for the calculation of β-parameters

The following Matlab script was used for the calculations of the β-parameters and the molar ratio of the anionic surfactant in the micelle for the different surfactant solutions. The data in the script below was collected from the surface tension measurements of the surfactants solutions at different ratios and salinity. This script can be used for every solution by activating or deactivating the data in the script. The script below is activated for the surfactant mixture between dodecyl sarcosinate and dodecyl glucoside at a 19:1 ratio with 3.5% of NaCl in the solution.

```
%%Implementation of the Newton-Raphson iteration for mixed micelles
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%Calculate the proportion of surfactant in the micelle and the beta
%%parameter.

%%am = molratio anionic surfactant in micelle;
%%al = molratio  anionic surfactant in solution;
%%nm = molratio glucoside in micelle;
%%nl = molratio glucoside in solution
%%CMCA = CMC hos anjon;
%%CMCN = CMC hos nonjon;
%%CMCM = CMC hos mixture;
clear all

n = 0; % reset iteration

%19:1
al = 0.95;
nl = 0.05;
% %1:1
% al=0.5;
% nl=0.5;

% GLUCOSIDE
CMCN = 0.19e-3;

%%SARCOSINATE SARCOSINATE SARCOSINATE SARCOSINATE SARCOSINATE

%no NaCl no NaCl no NaCl no NaCl no NaCl no NaCl no NaCl
% CMCA = 10e-3;
%%1:1
% CMCM = 0.25e-3;
%%19:1
% CMCM=0.7e-3;

% 0.5% NaCl 0.5% NaCl 0.5% NaCl 0.5% NaCl 0.5% NaCl
% CMCA=7.4e-3;
% %1:1
% % CMCM= no measure
% %19:1
% CMCM=1.4e-3;

% 3.5% NaCl 3.5% NaCl  3.5% NaCl 3.5% NaCl 3.5% NaCl
CMCA=3.2e-3;
```

```
%1:1
%CMCM= no measure
%19:1
CMCM=0.7e-3;

% %%GLUTAMATE GLUTAMATE GLUTAMATE GLUTAMATE GLUTAMATE GLUTAMATE

%no NaCl no NaCl no NaCl no NaCl no NaCl no NaCl
% CMCA=57e-3;
%1:1
% CMCM=0.34e-3;
%%19:1
% CMCM=2.9e-3;

% 0.5% NaCl 0.5% NaCl 0.5% NaCl 0.5% NaCl 0.5% NaCl
% CMCA=18e-3;
% %1:1
% CMCM= no measure
%19:1
% CMCM=2.3e-3;

% 3.5% NaCl 3.5% NaCl 3.5% NaCl 3.5% NaCl 3.5% NaCl
% CMCA=12.7e-3;
% %1:1
% % CMCM=no measure
% % 19:1
% CMCM=1.9e-3;

%%GLYCINATE GLYCINATE GLYCINATE GLYCINATE GLYCINATE GLYCINATE

% no NaCl no NaCl no NaCl no NaCl no NaCl no NaCl
% CMCA=10e-3;
% %1:1
% % CMCM=0.25e-3;
% % %%19:1
% CMCM=1.2e-3;

% 0.5% NaCl 0.5% NaCl 0.5% NaCl 0.5% NaCl 0.5% NaCl
% CMCA=6e-3;
% % %1:1
% % %CMCM= no measure
% % %19:1
% CMCM=1.2e-3;

% 3.5% NaCl 3.5% NaCl 3.5% NaCl 3.5% NaCl 3.5% NaCl
% CMCA=2.8e-3;
% %1:1
% %CMCM=no measure
% %19:1
% CMCM=0.7e-3;


% Starting conditions to solve the equations - x1m should be
% between 0 and 1!!!!
```

```
x1m = 0.5; %x2m=1-x1m
error = 1e-8; % accuracy aimed at

fonc = (x1m^2)*log(al*CMCM)-(x1m^2)*log(x1m*CMCA)-((1-
x1m)^2)*log(nl*CMCM)+((1-x1m)^2)*log((1-x1m)*CMCN); % Equation 5.22 p138
Krister's book

% Derivative
% dif=1+2*(1-x)*(log(nl*CMCM)-log((1-x)*CMCN))+2*al*(log(al*CMCM)-log(x*CMC
% A))

Figure
hold on

% Newton-Raphson loop
while abs(fonc) > error && n<10000
 n = n+1 ;
 fonc = (x1m^2)*log(al*CMCM)-(x1m^2)*log(x1m*CMCA)-((1-
x1m)^2)*log(nl*CMCM)+((1-x1m)^2)*log((1-x1m)*CMCN);
 dif=1+2*(1-x1m)*(log(nl*CMCM)-log((1-x1m)*CMCN))+2*al*(log(al*CMCM)-
log(x1m*CMCA)); %derivative
 if dif ~= 0
     xn = x1m - fonc/dif;% Formule calculate the new starting point
     else  disp('Derivative =0');
 end
 plot([n-1 n],[x1m xn],'-k*',...
 'MarkerSize',6,'LineWidth',1)
  x1m = xn;
end;
grid
x1m
x2m=1-x1m

beta=1/(x1m^2)*log(nl*CMCM/(x2m*CMCN))

beta2=1/(x2m^2)*log(al*CMCM/(x1m*CMCA))
```

Appendix 3 – Calculations for preparations of surfactant solutions

The solutions of surfactants at different concentrations were made in 20 ml vials from a stock solution containing a concentration of 3*CMC of the surfactant/surfactants. The calculations for the dilutions were then made from $C = n/V$ and $C_1V_1 = C_2V_2$ in an excel-file. The following examples shows the excel-file for a single surfactant with 0.42‰ $CaCl_2$ and for a mixture that NaCl was added to.

Example 1: preparation of stock solution of single surfactant with 0.42‰ of $CaCl_2$

Glycinate

pH 7 $CaCl_2$

OBS! All solutions are diluted with a solution of 0.42 parts per thousand of $CaCl_2$ This solution is prepared according to:
För 1 l = 1000 ml = 1000 g, x/1000 = 0.00042 → x = 0.42 g $CaCl_2$ i 1 l deionized water.

Mw = 279,356 g/mol
CMC = 10 mM

*For dilution series with start at 3*CMC = 3*10mM = 30mM:*

Dilution from stock solution			
C1, stock (M)	***C2(solution to be prepared) (M)***	***V1 (amount to take from stock, C1)(l)***	***Amount of water to be added (l):***
3,00E-02	3,00E-02	1,00E-02	0,00E+00
V2	2,00E-02	6,67E-03	3,33E-03
1,00E-02	1,50E-02	5,00E-03	5,00E-03
	1,20E-02	4,00E-03	6,00E-03
	1,00E-02	3,33E-03	6,67E-03
	0,007	2,33E-03	7,67E-03
	0,005	1,67E-03	8,33E-03
	0,003	1,00E-03	9,00E-03
	0,001	3,33E-04	9,67E-03

SUMMARISED	**3,43E-02**
Prepare of stock (ml):	**4,00E-03**
Amount of glycinate to be added to stock (g):	**3,35E-01**

Example 2: Preparation of dilution series of a mixture between dodecyl glutamate and dodecyl glucoside at two ratios and two different salinities

Dodecyl-glutamate + dodecyl glucoside

CMC dod-glu: ca 50 - 60 mM

CMC dod-sug: ca 0.15 – 0.30 mM

Startkonc: 3*CMC of both surfactants

Tabell 2 Amounts and volumes of the surfactants at the two different ratios

dod-glu+dod-sug

RATIO	**19:1**	**1:1**
mw sug	348,5	348,5
mwglu	373,45	373,45
Vtot	3,50E-02	3,50E-02
conc i stock solution	1,10E-01	1,80E-01
moltot	3,85E-03	6,30E-03

Tabell 3 Amount in moles and grams and concentratio of the glucoside and glutamate for the two ratios

ratios	**part glutamate**	**part glucoside**	**mol glutamate (mol)**	**mol glucoside (mol)**	**glutamate (g)**	**glucoside (g)**	**conc glutamate (M)**	**conc glucoside (M)**
19:1	0,95	0,05	3,66E-03	1,93E-04	1,37E+00	6,71E-02	1,05E-01	5,50E-03
1:1	0,5	0,5	3,15E-03	3,15E-03	1,18E+00	1,10E+00	9,00E-02	9,00E-02

Dodecyl-glutamate + dodecyl-glucoside

ratio 19:1 (95% glutamate, 5% glucoside)

version 1: neutral pH, no salt → 0,5% → 3,5% NaCl

ratio 19:1 (95% glutamate, 5% glucoside)

Starting from a stock solution			
C1(M)	***C2(baker to be prepared) (M)***	***V1 (to take from stock solution C1)(l)***	***Amount of water that is to be added (l):***
1,10E-01	(110 mM) 1,10E-01	(10 ml) 1,00E-02	0,00E+00
V2	(80 mM) 8,00E-02	(7,27 ml) 7,27E-03	2,73E-03
1,00E-02	(60 mM) 6,00E-02	(5,45 ml) 5,45E-03	4,55E-03
	(30 mM) 3,00E-02	(2,73 ml) 2,73E-03	7,27E-03
	(10 mM) 1,00E-02	(0,909 ml) 9,09E-04	9,09E-03
	(5 mM) 5,00E-03	(0,455 ml) 4,55E-04	9,55E-03
	(1 mM) 1,00E-03	(90,9 mikrol) 9,09E-05	9,91E-03
	(0,7 mM) 7,00E-04	(63,6 mikrol) 6,36E-05	9,94E-03
	(0,5 mM) 5,00E-04	(45,5 mikrol) 4,55E-05	9,95E-03
	(0,05 mM) 5,00E-05	(4,55 mikrol) 4,55E-06	1,00E-02
	(0,002 mM) 2,00E-06	(0,182 mikrol) 1,82E-07	
	SUMMARISED	**2,70E-02**	
	Volume to be prepared of stock solution (l):	**(35 ml) 3,50E-02**	
	Amount of glucoside for stock solution (g):	**(0,0671 g) 6,71E-02**	
	Amount of glutamate for stock solution (g):	**(1,37 g) 1,37E+00**	

Measure the surface tension of the solutions with no salt and then add salt to get 0.5 % salinity according to:
For 10 ml vials = 10 g, x/10 = 0,005 → x = 0,05 g = 50 mg NaCl in each vial.

Measure the surface tension of the solutions with no salt and then add salt to get 3.5 % salinity according to:
For 10 ml vials = 10 g, (x+0,05)/10 = 0,035 → x = 0,30 g = 300 mg NaCl in each vial.

Appendix 4 – Matlab code for constructiong species distribution curves for dodecyl glutamate

m –file 1:

```
clear all
y=[];
pKa=2.35; % pka of the acid
Concentration=0.02; %Concentration of surfactant
param=1e-5;
threshold_a=0.02-1e-9;

for pH=0.1:0.01:12 % pH range
    answer=NaN;
    while isnan(answer)==1
        answer=fzero(@(x) mypka(x,pH, pKa, Concentration),param);
        if param<threshold_a
            param=param+0.00001;
        end
    y=[y; answer];
    end
end
pH=0.1:0.01:12; % pH range - copy as in the loop
A=y(~isnan(y)); % A is the concentration in deprotonated form

%plotting

Figure
hold on
plot(pH,A)
AH=Concentration-A; % AH is the concentration in protonated form
plot(pH,AH, 'color','red')
legend('COO^-','COOH')

plot(2.35,Concentration/2,'o','color','green')

m-file 2:
function F = mypka(x, pH, pKa, Concentration)
% pKa=2.35;
% C=0.02;
% pH=2;
F=log(x/(Concentration-x))-pH+pKa;
```

Appendix 5 – Matlab code for constructing species distribution curves for dodecyl glycinate

m-file 1:

```
clear all
y=[];
pKa1=2.1; % pka of the acid 1
pKa2=4.07; % pka of the acid 2
C=0.12; %Concentration of surfactant

param=1e-5;
threshold_a=0.02-1e-9;
x0=[0.001 0.001 0.001];


for pH=0.1:0.01:12 % pH range
    y=[y; fsolve(@(x) mypkaa(x, pKa1, pKa2, C, pH), x0)];
end
% fsolve is much more powerfull and give easy answers to process!

pH=(0.1:0.01:12)'; % pH range - copy as in the loop
% y(1) is the concentration of
% y(1) is the concentration in deprotonated form
% y(1) is the concentration in deprotonated form

%plotting

Figure
hold on
plot(pH,y(:,1))
plot(pH,y(:,2),'color','red')
plot(pH,y(:,3),'color','green')

legend('(COOH)_2','(COOH)(COO^-)','(COO^-)_2')

plot(pKa1,C/2,'o','color','black')
plot(pKa2,C/2,'o','color','black')
```

m-file 2:

```
function f = mypkaa(x, pKa1, pKa2, C, pH)
% pKa1=2.1;
% pKa2=4.07;
% C=0.12;
% pH=2;
f(1)=x(1)*10^(-pKa1)-x(2)*10^(-pH);
f(2)=x(2)*10^(-pKa2)-x(3)*10^(-pH);
f(3)=x(1)+x(2)+x(3)-C;
% f(1)=x(1)*10^(-2.3)-x(2)*10^(-2);
% f(2)=x(2)*10^(-4.6)-x(3)*10^(-2);
% f(3)=x(1)+x(2)+x(3)-0.02;
```

Appendix 6 – List of chemicals

Chemical	**CAS-number**	**Molecular weight (g/mol)**	**Manufacturer,** **Purity**
95% n-Hexane	110-54-3	86.18	JT Baker
Dodecyl glycinate	90387-74-9 (Sodium-cocoyl-glycinate)	279.36	Romain Bordes
Dodecyl glutamate	3397-65-7	373.40	Romain Bordes 98% (NMR estimation
N-Lauroyl Sarcosine Sodium Salt	137-16-6	293.40	MP biomedicals, LLC 90 - 100%
N-dodecyl-B-D-Glucopyranoside	59122-55-3	348.48	Affymetrix ≥ 99% by HPLC analysis
Deionized water			Nanopure diamond (Barnstead)