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Synthesis and Characterization of Amide Compounds from Amidation of Some Fatty Acids with Sodium Glisinic and Sodium Glutamate along Their Utilization as Surfactants and Antibacterials

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Abstract: *Synthesis of Sodium N-Acyl Glycinate and Sodium N-Acyl Glutamate syntheses have been carried out by the amidation process of various types of Fatty Acid Methyl Esters with two types of amino acid salts, namely Sodium Glisinic and Sodium Glutamate. Amidation is carried out in a methanol solvent using a sodium methoxide (NaOCH3) catalyst and refluxed for 6-8 hours at 80-90 oC. The reflux product is then crystallized with Benzene: ethanol in a ratio of 1: 1. Then testing the crystals formed were FT-IR analysis, Du Nuoy Ring Surface Stress analysis and Anti-Bacterial Analysis of Staphylococcus Aureus and Escherichia Coli Bacteria. Of the 8 types of Amide synthesized, Sodium N-Capraloil Glisinate has the highest CMC value of 1.75 mol / L and with HLB value of 5.56. This shows that the synthesized surfactant is included in the water-oil emulsifier surfactant category. Based on the anti-bacterial activity value of 8 amides synthesized against Staphylococcus Aureus and Escherichia Coli bacteria, the highest value of anti-bacterial activity was obtained by Sodium N-Capraloil Glycinate against Escherichia Coli bacteria with inhibition zones of 15.3 mm at 5% solution concentration. This shows that the synthesized amide has strong antimicrobial activity and tends to be more anti-bacterial against gram-negative bacteria than gram-positive bacteria.*

Keywords: ***Amidation, Sodium N-Acyl Glycinate, Sodium N-Acyl Glutamate, Surfactant, Anti-Bacterial***

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I. Introduction

Surfactants are a group of organic compounds that continue to attract great interest from researchers because of a variety of applications can be used as detergents, emulsifiers, corrosion inhibitors, oil recovery and medicine. Surfactants are the most representative chemicals of products that will be consumed in large quantities every day and globally and in the past caused adverse effects on the aquatic environment. Many previous studies have revealed the adverse effects of the widespread use of conventional surfactant chemicals on the environment (Ivancovic and Hrenovic, 2010).

Biosurfactants are a class of green and sustainable chemistry of active substances that are naturally synthesized from microorganisms such as bacteria, fungi and yeast or are extracellular excreted. Synthesis which is equivalent to biosurfactant can be carried out by designing molecules that mimic the structure of natural amphibians such as phospholipids, alkyl glucosides and amino acid acyl. Amino acid surfactant is one type of surfactant that can generally be derived from animals or raw materials derived from agriculture. Amino acid surfactants have gained great interest from scientists over the past two decades as new surfactants because they can be synthesized using renewable sources and their ease of degradability and harmless byproducts make them safer for our environment (Kango, 2010).

The use of chemical synthesis materials for medicinal purposes as antimicrobials, especially for food, must be in safe doses. Materials that are commonly used as antimicrobials are fatty acids, esters of glucose or glycerol containing fatty acids. The activity of these materials was reported by Conley and Kabara (1973). In the research results it was explained that the fatty acid esters from polyhydroxy alcohol are more effective as antimicrobial agents in gram-positive bacteria compared to gram-negative bacteria. Arginine-based surfactants have been studied in detail having antimicrobial properties. Where gram-negative bacteria are found to be more resistant to arginine-based surfactants than Gram-positive bacteria. Antimicrobial activity of surfactants is usually increased in the presence of hydroxyl groups, cyclopropane or double bonds and acyl chains (Tripathy et al, 2017).

N-acyl arginine methyl ester is another type of cationic surfactant which has antimicrobial properties with broad spectrum activity and is easily biodegradable and has no toxicity properties at all. Studies on the

interaction of N-acyl arginine surfactant with 1,2-dipalmitoyl-sn-glycero-3-phosphocoline and 1,2-dimiristoyl-snglycero-3-phosphocoline, with living organisms with or without external inhibition also show good antimicrobial activity (Mhatre and Singare, 2012). There are several N-Acyl amino acids that can be used as anti-microbial, one of which is N-Palmitoil piroline and N-Acetyl Cysteine. Both types of amides are proven to inhibit the growth of gram-positive and gram-negative bacteria (Takehara, 1989).

From the description above, the researcher is interested in synthesizing amides as surfactants from liquid unsaturated fatty acids (caprylic acid and capric acid) and solids (palmitic acid) at room temperature and using unsaturated fatty acids, oleic acid, where fatty acids are applied first form the fatty acid methyl esters. The fatty acid methyl ester is then reacted with two types of amino acid salts namely glycine and glutamic acid to form amides. The amide produced was then tested for its ability as a surfactant by testing the value of the Critical Concentration Micelle (CMC) as well as an antibacterial test to see the different abilities of various types of amides as inhibitors of bacterial growth.

II. Material and Research Methods

Ingredients: pa absolute methanol, absolute ethanol pa, anhydrous caCl2 pa, 98% sulfuric acid pa, n-hexane pa, sodium methoxide pa mixed oleic acid from PT.Socimas, caprilic acid from PT.Socimas, capric acid from PT.Socimas, Palmitic Acid from PT.Socimas, Sodium sulfuric anhydrous pa, Glycine pa, Glutamic acid pa, Petroleum Benzene pa, NaOH pellet pa, Nutrient agar (NA) pa, Mueller Hinton Agar (MHA) pa, Nutrient Broth pa, Mcfarland Standard Solution, Staphylococcus aureus and Escherichia Coli bacteria.

Research Methods: The research conducted is a laboratory experiment. Fatty acids are esterified with absolute methanol to form methyl esters of fatty acids. Fatty acid methyl esters are then reacted with sodium salts from amino acids namely sodium glycinate and sodium glutamate using reflux for 6-8 hours where the reaction results are then recrystallized with petroleum benzene and ethanol in a ratio of 1: 1. The amide formed was tested by FT-IR to see the amide group formation, the determination of Critical Critical Values (CMC) by the Du iNuoy Ring Method from the sample and anti-bacterial testing to see the ability of the amide as a surfactant as well as an antibacterial.

III. Results and Discussion

3.1 Manufacture of Fatty Acid Methyl Esters from Various Types of Fatty Acids

Preparation of methyl fatty acid esters (MEAL) by esterification of various types of fatty acids with methanol in benzene solvents using concentrated sulfuric acid catalyst at 80-90^{o}C to produce fatty acid methyl esters. FTIR spectroscopic analysis provides a spectrum with absorption peaks at specific wave numbers (Figure 1). The results of FT-IR spectroscopic analysis showed the absorption peak at the wave number region 1745 cm^{-1} which is a typical absorption band of the C = O ester group and supported by the absorption band at wave number 1255-1246 cm^{-1} which shows the typical absorption of CC (= O)-O esters. The C-C (= O)-O band is asymmetric vibration which is coupled from the C-O stretching vibration. The presence of absorption bands in the area of 1100 cm^{-1} indicates that this compound is a long-chain Methyl Esther Fatty Acid, in which the bands in the wave number area 1171 cm^{-1} are the strongest. This spectrum is supported by the non-emergence of the absorption peak which widens in the region of the wave number above 3000 cm^{-1} which is the peak of the absorption absorption typical OH group. The absorption peak at the wave number region 2928 cm^{-1} is a typical absorption of CH sp^3 asymmetric stretching vibrations and the wave number region 2857 cm^{-1} is a typical absorption of the CH sp^3 metric stretching vibration supported by the CH sp^3 asymmetric bending vibrations in the wave number region 1460 cm^{-1} and CH sp^3 symmetric buckling vibrations in the wave number region 1365 cm^{-1}. The spectrum in the region of wave number 726 cm^{-1} is the rocking vibration of the methylene group (CH_2) n from a straight chain alkane consisting of seven or more carbon atoms (Silverstein, 1963).

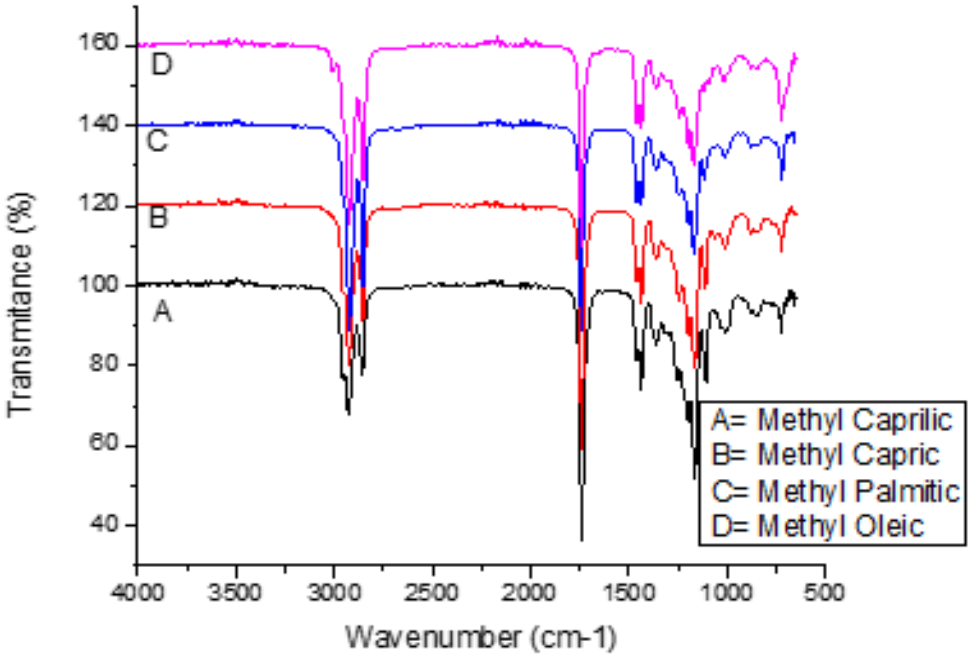

Figure 1. FT-IR Spectrum of Synthesized Fatty Acid Methyl Esters

With the FT-IR spectrum of the Fatty Acid material used in Figure 2.

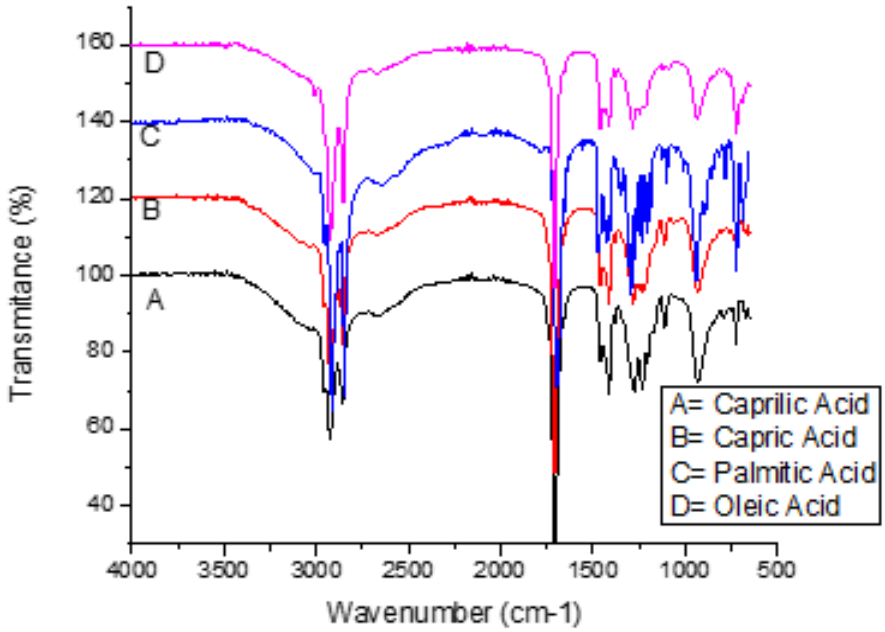

Figure 2. FT-IR spectrum of various fatty acids used

3.2 Amidation of Methyl Esters of Fatty Acids (MEAL) with Sodium Salt from Glycine Using Sodium Metoxide Catalyst

Sodium N-Acyl Glycinate compound can be produced from the results of amidation between Methyl Esther Fatty Acid (MEAL) with salt from glycine in methanol solvents with the help of sodium methoxide catalyst at a temperature of 80-90^{o}C. Based on the principle of HSAB, the amidation of Methyl Esters of Fatty Acid can produce amide compounds where the H^+ from NH_2 derived from Glycine is a hard acid which is easy to react with $^-OCH_3$ which is a hard base and N^- from glycine which is a soft base which will react with the acyl group $R\text{-}C^+{=}O$ which is a soft acid. The results of FT-IR spectroscopic analysis gave the peak of strong

absorption in the region of the wave number in the region of 3000 which is around 3378 - 3287 cm^{-1} which shows the vibration stretching N-H and. In addition, the wave number 1562 -1557 cm^{-1} shows the secondary tacyclic amide band where this band is produced by the interaction between NH bending and CN-H group CN extension. The secondary amide support band appears at wave number 1176-1160 cm^{-1} (weaker peak) which is also produced by bending NH and CN stretches. The absorption peak at the wave number region 2956-2922 cm^{-1} is a typical absorption of CH_3 asymmetric stretching vibrations and the wave number region 2853 cm^{-1} is a typical absorption of CH sp^3 symmetric buckling vibrations supported by the CH3 asymmetric vibration in the wave number region 1480 - 1422 cm^{-1} and CH sp^3 symmetric buckling vibrations in the wave number area 1277-1160 cm^{-1}. The spectrum in the wave number region of 800 cm^{-1} is the rocking vibration of the methylene group (CH_2) n from a straight chain alkane consisting of seven or more carbon atoms (Figure 3) (Silverstein, 1963).

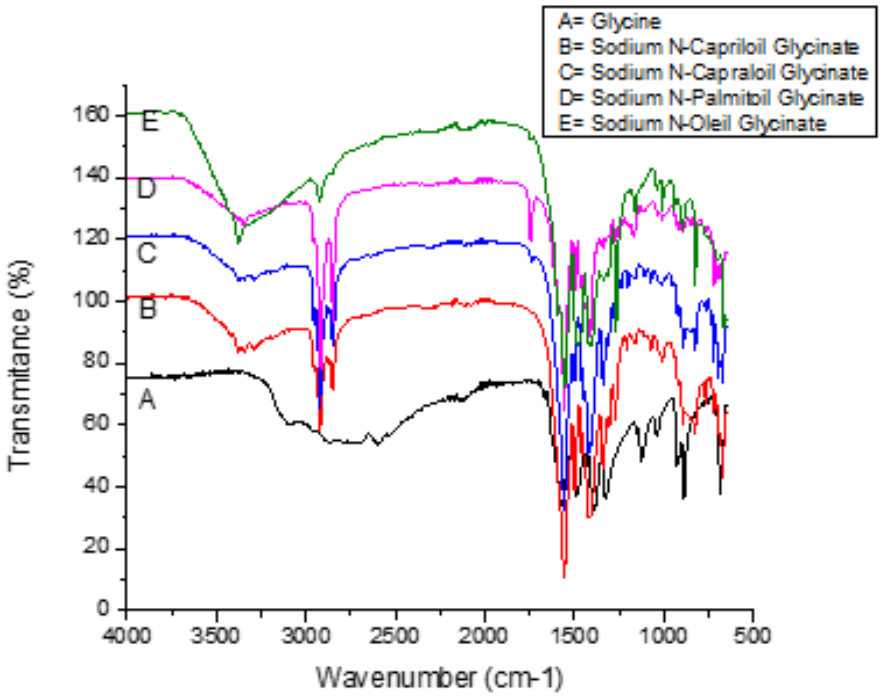

Figure 3. FT-IR Spectrum of Various N-Acyl Glycinate Amides Formed

3.3 Amidation of Methyl Esters of Fatty Acids (MEAL) with Sodium Salt from Glutamic Acid Using a Sodium Metoxide Catalyst

Sodium N-Acyl Glutamide Compounds can be produced from the amidation between Methyl Esters of Fatty Acids (MEAL) with salts of glutamic acid in methanol solvents with the help of a sodium methoxide catalyst at 80-90^oC. Synthesis of Sodium N-Acyl Glutamate Compound uses the same principle as the synthesis of Sodium N-Acyl Glutamate which is the HSAB principle. The results of FT-IR spectroscopic analysis provide strong absorption peaks in the region of wave numbers in the region of 3000 which is around 3371 - 3343 cm^{-1} which shows the vibration stretching N-H. In addition, the wave number 1662 -1562 cm^{-1} shows the secondary tacyclic amide band where this band is produced by the interaction of bending NH with CN CN stretch. H. The secondary amide support band appears at wave number 1176-1160 cm^{-1} (weaker peak) which is also produced by bending NH and CN stretches. The absorption peak at the wave number region 2924-2916 cm^{-1} is a typical absorption of CH sp^3 asymmetric vibrations in the wave number region 2857-2851 cm^{-1} is a typical absorption of CH sp^3 symmetric buckling vibrations supported by CH sp^3 asymmetric vibrations in the wave number region 1449-1404 cm^{-1} and CH sp^3 symmetric buckling vibrations in the wave number area 1277-1160 cm^{-1}. The spectrum in the region of the wave number 851- 782 cm^{-1} is the rocking vibration of the methylene group (CH2) n from a straight chain alkane consisting of seven or more carbon atoms (Figure 4) (Silverstein, 1963).

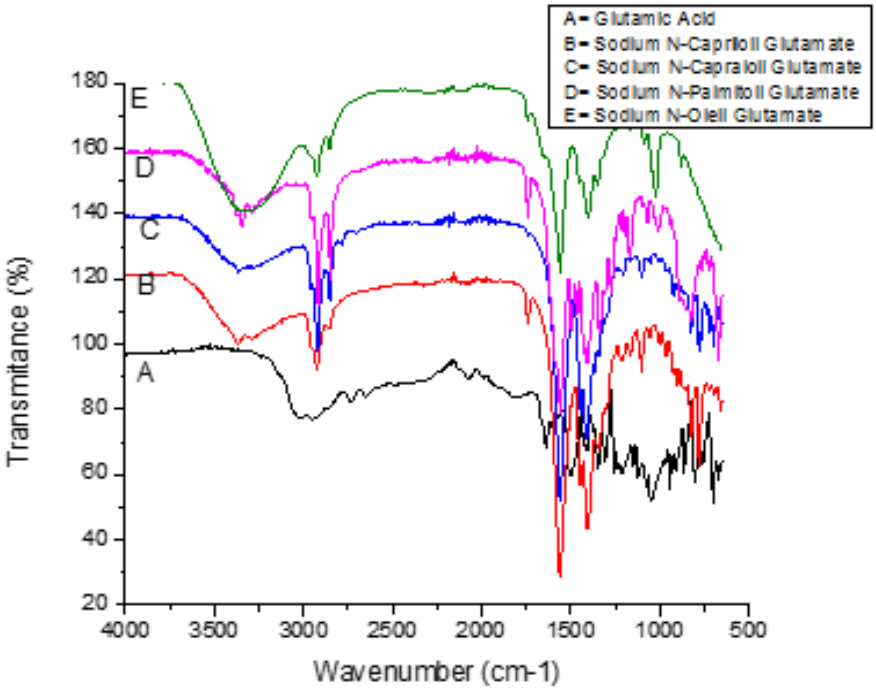

Figure 4. FT-IR Spectra of Various N-Acyl Glutamate Amides Formed

3.4 Result of Alkanolamide Critical Micelle Concentration (CMC) using Du Nuoy Ring Tensiometer

The determination of CMC was carried out on amides from the results of the amidation of fatty acid methyl ester compounds with sodium salt from Glycine and sodium salt from Glutamic Acid. The alkanolamide obtained was diluted with a concentration variation of 1% - 6% where an increase in the concentration of surfactant in aqueous solution caused the surface tension of the solution to drop to a certain concentration until it reached a constant point. The addition of surfactants which exceeds this concentration will aggregate to form micelles. The concentration at which these micelles are formed is called the critical micelle concentration (CMC).

Based on the CMC value of the synthesized amide, we can calculate the HLB value in practice based on the equation:

HLB = 7- 0.36 ln (Co / Cw) …… .. (Equation 1) (Swern, 1979)

Where Co = CMC Price

Cw = 100 - Co

Table 1. List of CMC Values of Formed Amides

No.	Amide Compounds	Surface Tension Value (Dyne/CM)	CMC Value (mol. L)	HLB Value
1	Sodium N-Capriloil Glycinate	41.43	1.785	5.56
2	Sodium N- Capraloil Glycinate	38.17	1.195	5.41
3	Sodium N-Palmitoil Glycinate	39.70	0.299	4.91
4	Sodium N-Oleoil Glycinate	33.36	0.277	4.88
5	Sodium N- Capriloil Glutamate	46.62	0.946	5.33
6	Sodium N- Capraloil Glutamate	40.38	1.154	5.40
7	Sodium N-Palmitoil Glutamate	38.7	0.233	4.82
8	Sodium N-Oleoil Glutamate	31.42	0.220	4.80

The following graph shows the value of surface tension vs. logarithm of compound concentration

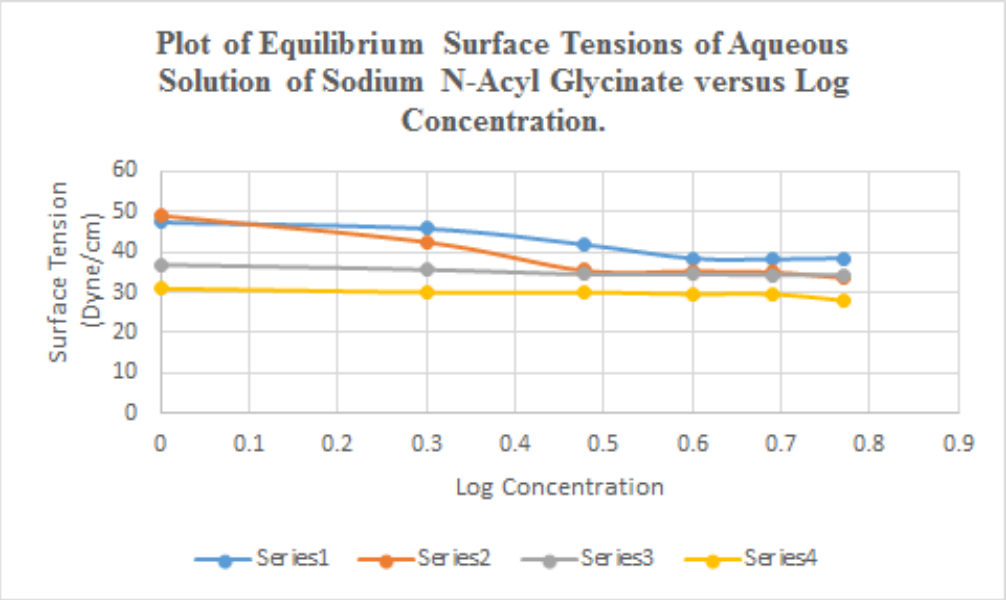

Figure 5. Graph of Surface Tension Values (Y) vs Logarithm of The Concentration of Sodium N-Acyl Glycinate Compound. Series 1 = Sodium N-Capriloil Glycinate; Series 2 = Sodium N- Kapraloil Glycinate; Series 3 = Sodium N-Palmitoil Glycinate; Series 4 = Sodium N-Oleoil Glycinate

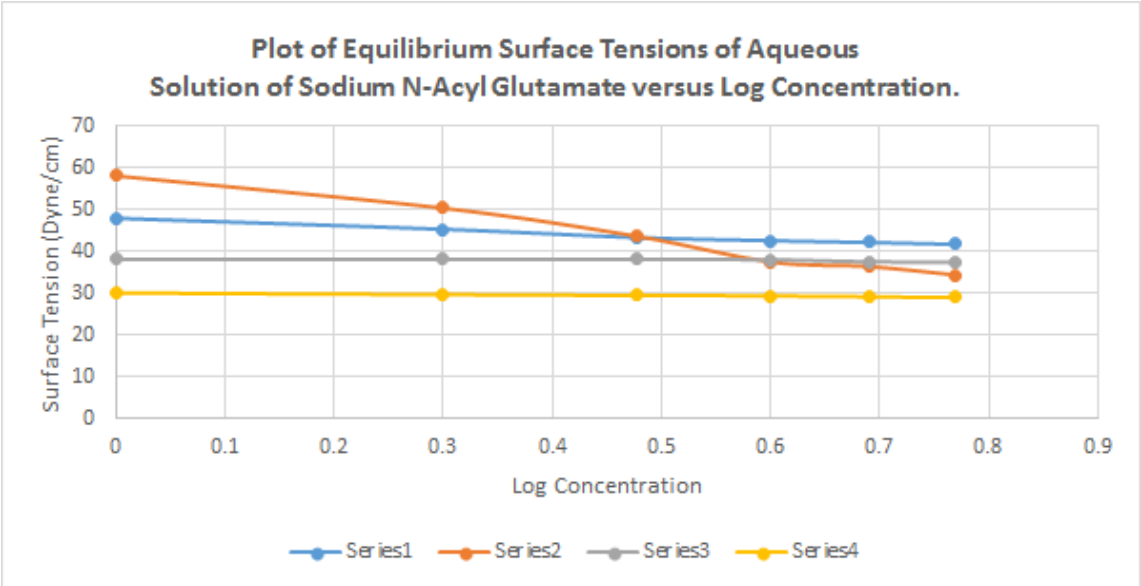

Figure 6. Graph of Surface Tension Values (Y) vs Logarithm of The Concentration of Sodium N-Acyl Glutamate Compound. Series 1 = Sodium N-Capriloil Glutamate; Series 2 = Sodium N- Kapraloil Glutamate; Series 3 = Sodium N-Palmitoil Glutamate; Series 4 = Sodium N-Oleil Glutamate

It can be seen that the greater the surfactant concentration, the greater the ability to reduce surface tension. This is because the surfactant concentration affects the formation of micelles. The greater the concentration of the surfactant, the lower the surface tension so that the more micelles are formed so that the surface tension produced is constant at a concentration.

In Sodium N-Acyl Glycinate Amide synthesized, There is a decrease in surface tension in Sodium N-Capriloil Glisinate up to a concentration of 4% while at a concentration of 5 to 6% the value of surface tension is constant. Likewise with Sodium N-Capraloil glycinate, the surface tension decreases to a concentration of 3%, whereas at concentrations of 4-6% the surface tension value tends to be constant. Different things happen to Sodium N-Palmitoil Glycinate and Sodium N-Oleoil Glycinate. Surface tension values in the two amides did not decrease even though the surfactant concentration in water had been increased. This is because the surfactant solution forms a gel making it difficult to work to reduce surface tension.

In the synthesized N-Asyl Glutamate Amide, there is a decrease in the surface tension of Sodium N-Capriloil Glutamate to a concentration of 2% while at a concentration of 3 to 6% the value of the surface

tension is constant. Likewise with Sodium N-Capraloil glycinate, the surface tension decreases to a concentration of 4%, whereas at a concentration of 5-6% the surface tension value tends to be constant. Different things happen to Sodium N-Palmitoil Glutamate and Sodium N-Oleoil Glutamate. Surface tension values in the two amides did not decrease even though the surfactant concentration in water had been increased. This is because the surfactant solution forms a gel making it difficult to work to reduce surface tension. This is consistent with research from Li (2011), that the value of CMC will decrease with increasing carbon fiber length of the amide formed. This is because the hydrophobic group of carbon chains contributes to Gibbs energy to form micelles. Based on the HLB values in Table 1 above, it can be concluded that the surfactants formed are water emulsifiers in oil. In this case, surfactants are more easily dissolved in oil than in water so that these surfactants can be used in the butter and margarine industry.

3.5 Antibacterial Activity Testing Results

The method used in this study is the agar diffusion method by measuring the diameter of the inhibition zone. Based on the diameter of the inhibitory zone in the two test bacteria which increases with increasing amide solution concentration. This proves that the increase in the concentration of amide solution has a positive correlation with the increase in the inhibition zone in the growth of the bacteria Staphylococcus aureus and Escherichia Coli.

From the anti-bacterial analysis conducted on 8 types of amide formed, it can be seen in the table that the amide solution at concentrations of 3 and 5% is effective in inhibiting bacterial growth. The synthesized amide tends to be effective in inhibiting the growth of Escherichia Coli colonies compared to Staphylococcus Aureus bacteria. This is in line with Tripathy et al (2018), where gram-negative bacteria were found to be more resistant to amino acid-based surfactants than Gram-positive bacteria. Antimicrobial activity of surfactants usually increases by the presence of hydroxyl groups, cyclopropane or double bonds and acyl chains. The table also shows that surfactant concentrations can affect the ability to inhibit bacterial growth activities.

The ratio of the clear zone diameter produced by the surfactant solution is directly proportional to the increase in the concentration of the surfactant solution. The greater the concentration of surfactant given, the greater the clear zone formed. This proves that the synthesized amide compound can affect anti-bacterial activity.

From the antibacterial testing of 8 types of amides against Staphylococcus Aureus and Escherichia Coli bacteria, it can be seen that Sodium N-Capraloil Glycinate has the most powerful antibacterial strength against Escherichia Coli bacteria compared to other amides. Based on the amino acid source used, there is a clear difference between amides from glycine and amides from glutamic acid. The anti-bacterial value of glycine amide is greater than the amide value of glutamic acid.

Table 2. Amide Inhibitory Zone Diameter Data for Escherichia Coli and Staphylococcus Aureus Bacteria with Distilled Water

No.	Sample	%	Bacteria	
			Staphylococcus Aureus (Positive Gram)	Escherichia Coli (Negative Gram)
1	Sodium N-Capriloil Glycinate	3%	6.4	6.2
		5%	6.9	8.3
2	Sodium N-Capraloil Glycinate	3%	9	13.1
		5%	9.5	15.3
3	Sodium N-Palmitoil Glycinate	3%	8.3	8.5
		5%	10.2	10.1
4	Sodium N-Oleoil Glycinate	3%	10.5	11
		5%	10.9	13.3
5	Sodium N-Capriloil Glutamate	3%	6.3	6.5
		5%	7.95	7.6
6	Sodium N-Capraloil Glutamate	3%	8.7	6.2
		5%	10	9.5
7	Sodium N-Palmitoil Glutamate	3%	8.3	8.2
		5%	9.4	9.8
8	Sodium N-Oleoil Glutamate	3%	8.2	7.3
		5%	10	10.75

The strengths of antibacterial activity are: a) Inhibition zone <= 5mm = Weak, b) Inhibition zone 5-10 mm = Medium, c) Inhibition zone 10-20 mm = strong, d) Inhibition zone => 20 mm = Very Strong.

IV. Conclusion

Based on the CMC critical micelle concentration value of 8 types of amides synthesized, the highest CMC value is N-Capriloil Glisinate which is 1.75 mol / L with HLB value of 5.56. This shows that the amide has the highest ability as a water-in-oil emulsifier surfactant. Based on the value of anti-bacterial activity of 8

amides synthesized against Staphylococcus Aureus and Escherichia Coli bacteria, the highest value of anti-bacterial activity was obtained by Sodium N-Capraloil Glycinate against Escherichia Coli bacteria with inhibition zones of 15.3 mm at a concentration of 5% solution. This shows that the synthesized amide has a strong antimicrobial activity and tends to be more anti-bacterial against gram-negative bacteria than gram-positive bacteria.

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