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A RESPONSE SURFACE METHOD FOR THE PREPARATION OF N-LAUROYL LYSINE FROM MEDIUM CHAIN FATTY ACID CATALYZED BY SODIUM METOXIDE

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ABSTRACT

N-lauroyl lysine is a non-ionic surfactant obtained from the synthesis of medium-chain fatty acids with lysine. This study aims to obtain data on the effect of the molar of a substrate, solvent ratio, and catalyst concentration on the fact of lauric acid (LA) conversion by compiling and observing a mathematical model with Response Surface Methodology (RSM). Lauric acid is a medium-chain fatty acid amidized with lysine and sodium methylate catalyst in a solvent mixture of 2-propanol and n-hexane. In the optimization stage, with the variables of lysine to a fatty acid molar ratio (2-4 M), solvent ratio (1-3 v/wLA), catalyst concentration (3-7 w/wLA), with a temperature of 55^{o}C and reaction time 2 hours, the maximum conversion is 85.94%. The results showed that the independent variables affected the conversion with an R^2 value of 84.85%. Analysis of the sample results using Fourier Transform Infrared Spectroscopy (FTIR) to confirm the formation of N-lauroyl-lysine.

Keywords: amidation, lauric acid, sodium methylate, optimization, N-lauroyl lysine.

INTRODUCTION

The response surface method is closely related to factorial experiments. A factorial experiment is an experiment whose treatment consists of all possible combinations of levels of several factors. The primary purpose of factorial experiments is to see the interactions between the factors being tested. The advantage of using the Response Surface Method is that it makes it easier to find the optimum area [1, 2, 3, 4].

Surfactants play a crucial role in the development of technologies such as nanomaterials and materials. Surfactants can be found in detergents, personal care products, additives to paints and coatings, dyes, biocides, materials science, organic synthesis, pharmaceuticals, textiles and leather, and agrochemicals plastics, food processing, and environmental protection (oil coating dispersion). Surfactants are also used to replace traditional solvents, providing lower risk, and reduced environmental impact. The increase in surfactant use is driven by higher demand for personal care products, detergents, cleaners, industrial products, anti-corrosion, and biocides. This is expected to lead to the introduction of innovative and effective surfactant-based products [5, 6, 7, 8].

Palm oil, which contains many medium-chain fatty acids, is an inexpensive and abundant source of raw materials to synthesize amino acid surfactants. Amino acid-based surfactants have attracted great interest because they are more biodegradable and less harmful to the environment [9, 10]. It was found that this class of surfactants exhibits lower toxicity when compared to conventional surfactants, making them a promising alternative to anionic and non-ionic surfactants, which are commercially available as pharmaceutical and cosmetic formulations [11, 12].

Amino acids are substances with an amine group and a carboxylic group in a molecule and are constituents of proteins. Today, high purity amino acids are available at relatively low cost and are used in feed additives and pharmaceuticals. Research has recently been carried out regarding the use of amino acids in the fields of cosmetics, surfactants, and polymers [13, 14]. Many standard amino acids offer a wide variety of chemical structures that allow fine adjustment of the surfactants' physical-chemical properties. Amino acids can be linked via various types of breakable bonds, such as amide bonds for N-acyl amino acid-based surfactants [15, 16].

Lauric acid with the highest concentration was found in palm kernel oil (PKO) and coconut oil, namely 53.3% and 45.90%, respectively. The carboxyl and methyl groups in lauric acid cause derivatives of this compound are often used in the washing industry such as shampoo and the cosmetic industry. Thus, lauric acid is very suitable for making compounds for cleaning products [17, 18].

The basic form of amino acids has two functional groups: an amino group and a carboxyl group. In this research, surfactant N-lauroyl lysine will be synthesized by the amidation reaction between lauric acid and lysine with a sodium methylate catalyst, which is reactive and affordable in a solvent mixture of 2-propanol and n-hexane. Based on the solubility of raw materials and products against the solvent, it is known that the amide group of lysine is more soluble in polar alcohol solvents. Simultaneously, pure fatty acids are more or less soluble in non-polar solvents such as hexane [19, 20]. Thus, the mixture of alcohol and hexane is expected to be the best solvent for the intermediate reaction. It can dissolve all the required components and produce the most product [21, 22].

Sodium methylate functions as a catalyst to speed up the surfactant's chemical reaction process by reducing the energy required to start the reaction. It is successfully

used in large-scale production of all primary raw materials such as refined vegetable oil, animal fat, and yellow fat. The main advantage of sodium methylate is that it is almost water-free. This results in higher yields, lower purification costs, and more consistent surfactant quality [23].

Based on the studies that have been described, it is necessary to research the synthesis of surfactants based on amino acids: N-lauroyl lysine from lauric acid using chemical catalysts to obtain important information regarding research variables such as molar of a substrate, catalyst concentration, and solvent ratio using the Response Surface Method.

MATERIALS AND METHODS

Chemicals

The chemicals used were lauric acid ($C_{12}H_{24}O_2$), lysine ($C_6H_{14}N_2O_2$), sodium methylate ($NaOCH_3$), n-hexane (C_6H_{14}), and 2-propanol (C_3H_8O), obtained from E. Merck. Purification materials in the form of citric acid and acetone and analytical materials in potassium hydroxide (KOH), Phenolphthalein, 2-propyl alcohol, and hydrochloric acid (HCl) were also obtained from E. Merck.

Experimental Section

Three grams of lauric acid (LA) were put in a three-neck flask. Lysine was put into a beaker glass with variations in lysine ratio to lauric acid 2, 3 and 4 Molar. Sodium methylate was added as much as 3%, 5%, and 7% by weight of lauric acid into the beaker glass together with lysine. The mixed solvent with various solvent ratios to lauric acid (v/w) 1, 2, and 3 times was added to dissolve lysine and sodium methylate. The solution is stirred until homogeneous, then put into a three-neck flask, heated to a temperature of 55^{o}C, and 2 hours. A sampling of 2 grams was performed and titrated with 0.1 N KOH solution. The mixture in a three-neck flask was then purified for further analysis.

The synthetic product is purified by adding 5 ml of 10% citric acid so that the catalyst will settle, and then the precipitate is separated by filtration. The filtrate is then evaporated at 90oC, and the evaporation product is washed with acetone to dissolve excess lysine. N-lauroyl lysine will be obtained as a lower layer, and as an upper layer, excess lysine and acetone will be obtained.

Experimental Design

This run selection is carried out using Design Expert Version 10.0.1.0 with the response surface method. RSM contains mathematical and statistical collections that are useful for problem modeling and analysis, in which several variables affecting the response will be optimized [1, 24]. The optimization result will be a mathematical model composed of independent variables [25].

The independent variables and levels in this study include molar of lysine substrate against lauric acid, denoted by A, with a range between 2 to 4 Molar. The variable solvent to lauric acid ratio, denoted by B, ranges from 1 to 3 (v/wLA). Moreover, the third variable is the catalyst concentration, denoted by C, with a range between 3 and 7 (w/wLA). Meanwhile, as the response variable, it is denoted by R1, which is the percent conversion of lauric acid.

RESULTS AND DISCUSSIONS

Amino acid-based surfactants can be synthesized by adding a hydrocarbon chain to an amino acid functional group [13, 21]. The linkages formed are of three different types, which are determined by the amino acid structure. The three types are ester, amino, and amido related. Next is to investigate the effects of the various related types on the properties of the solution. Moreover, specific amino acids have two carboxyl groups. The length of the distance between the two carboxyl groups is different. Therefore, next is to investigate how distance plays a role in surfactant aggregation behavior [6, 7].

FTIR Analysis

The chemical structure of the surfactants can be seen using FTIR spectroscopy. The results of this analysis are to indicate the type and structure of the surfactants produced. FT-IR spectroscopy is a tool for detecting the functional groups with an infrared spectrum of organic compounds that have unique physical properties. Infrared radiation energy will be absorbed by organic compounds so that the molecules will undergo rotation or vibration. Each different chemical bond, such as C-C, C-H, C = O, O-H, has a different vibrational frequency. The results of the spectrum of lauric acid, lysine, and N-lauroyl lysine obtained are shown in Figures 1 to 3, respectively.

Figure-1 shows the results of the FT-IR spectrum analysis of the lauric acid raw material. In Figure-1, it found the carbonyl absorption at 1692.06 cm^{-1} with reasonably strong intensity. The carbonyl absorption is in the range 1700-1725 cm^{-1}. At 2911.35 cm^{-1}, the OH stretching absorption of lauric acid was found, which is a typical absorption of C-H sp3 stretching vibrations. This absorption is strengthened by the bending vibration of C-H sp3 in the area of wave number 1426.29 cm^{-1}. At the same time, the C-O stretch at 1288.35 cm^{-1} shows the lauric acid fingerprint area. The infrared spectrum of the lysine raw material is shown in Figure-2. Lysine shows the NH stretch band found at 2903.83 cm^{-1}. The NH bending was found at 1616.88 cm^{-1}, and the OH bending at 1486.67 cm^{-1}. The C-N stretch also expresses the vibration for the C-N bond at 1137.76 cm^{-1}. At the same time, the absorption of C-C was at 1003.80 cm^{-1}.

Figure-3 shows the results of N-lauroyl lysine FT-IR spectrum analysis at the molar ratio of lysine to 4 Molar lauric acid, solvent to substrate ratio 3 (v/wLA), and catalyst concentration of 5% by weight of lauric acid. The vibration for the C-N bond for amides is in the wavenumbers 1350-1000 cm^{-1}. From the results of the FTIR spectrum test of the sample, it shows that the absorption peak in the wavenumber area of 1298.11 cm^{-1} indicates the presence of an N-H group in this wavenumber region, which indicates the sample contains an amide group. While the absorption at wave number

2918.38 cm^{-1} indicates the presence of an OH group. The OH group is present in lauric acid compounds. The absorption peak at wave number 988.90 cm^{-1} indicates a C-H group. A wave absorption 1170.15 cm^{-1} indicates a C-O group for aliphatic ester compounds, a side reaction of the amidation reaction.

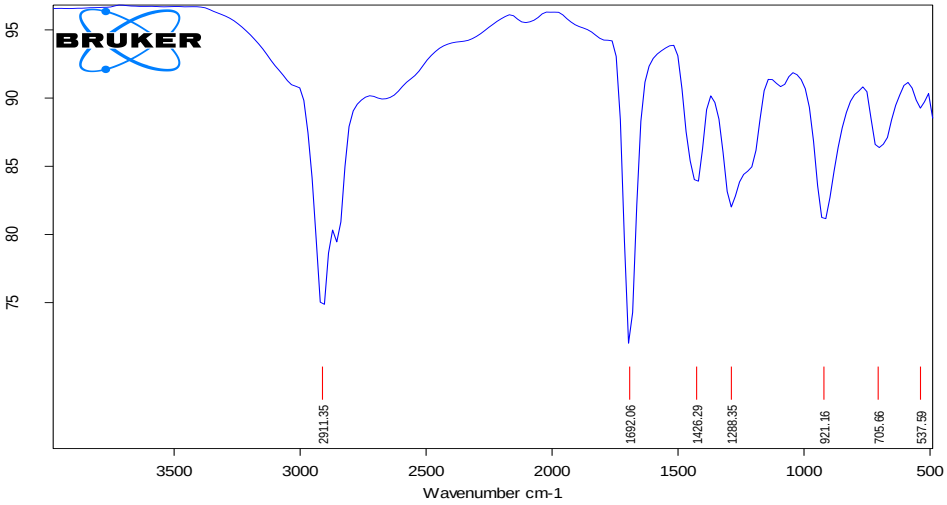

Figure-1. Results of FT-IR spectrum analysis of lauric acid raw material.

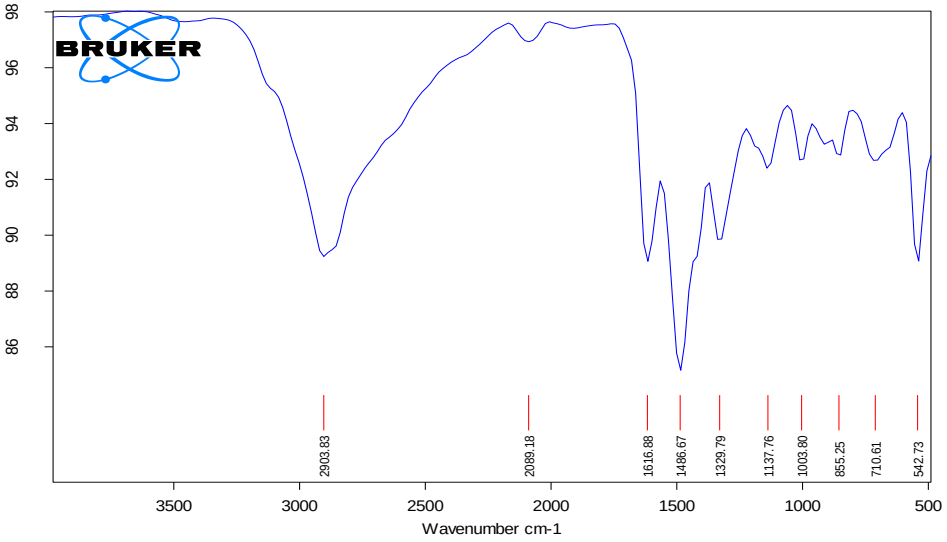

Figure-2. Results of FT-IR spectrum analysis of lysine raw material.

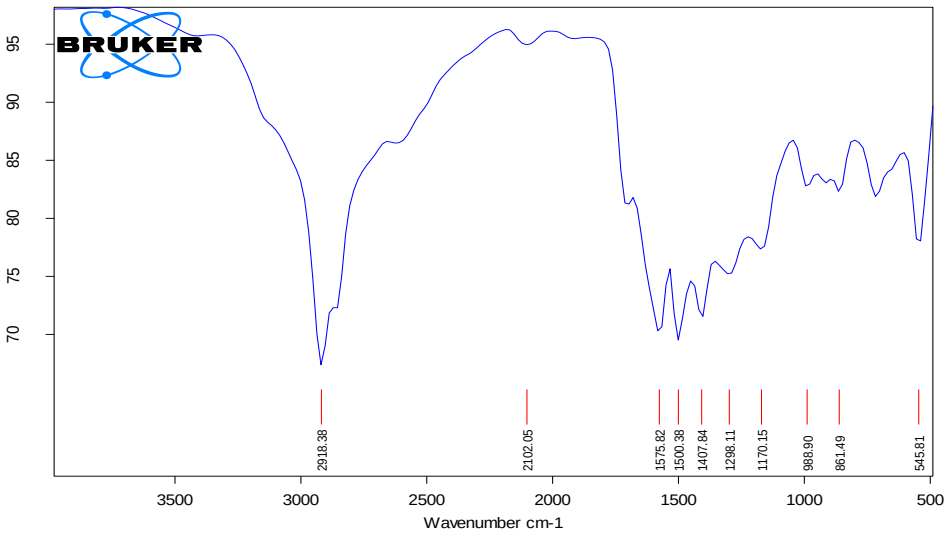

Figure-3. Results of N-lauroyl lysine FT-IR spectrum analysis.

Optimization Results

Optimization of research conditions aims to simultaneously observe the three experimental variables simultaneously arranged in Box-Behnken Design and determine the optimum conversion of surfactant N-lauroyl lysine synthesis [1, 3]. The optimization results of surfactant N-lauroyl lysine synthesis in the percentage conversion value are shown in Table-1.

The design experiment in Table-1 shows that the best optimization results are in the ninth study, with the substrate ratio conditions of 4 Molar (L/AL), the solvent ratio is 3 (v/wLA), and the catalyst concentration is 5%, which results in a percent conversion of 85.94%. The extensive conversion reaction is due to an increase in the substrate ratio, which corresponds to an increase in the solvent ratio and an increase in the weight of the catalyst. A high solvent ratio will dissolve more substrate, and in the presence of a catalyst, it can reduce the activation energy and speed up the reaction rate [4, 25].

The ratio of reactants, temperature, and reaction time affects the number of fatty amides produced. In the amidation process, the use of amines derived from lysine must be excessive. At least the required amine to methyl ester mole ratio is 1.1 / 1. This is because the higher the mole ratio of amines, the better the process is. However, the use of excess amines allows the presence of unreacted amines to form products. To remove residual lysine, a purification process can be carried out [9, 16, 24].

Analysis of the most influential independent variables in determining the optimum response conditions is carried out by optimization using the Box Behnken Design (BBD) method. This study chose BBD as a form of experimental design because the run is less, so it is considered to save money and study time [2, 3].

The research data obtained from these 15 treatments, then analyzed using the RSM. The analysis aims to establish a relationship between variables, determined through regression analysis, models, and residuals. The ANOVA results for responses to the quadratic surface model are given in Table-2. The results of the prediction of the regression coefficients carried out to compile the regression model are shown in Table-3.

Furthermore, an equation model representing the relationship between the three variables is obtained and is arranged as follows.

$$\text{Acid Conversion} = 79.3 + 1.48*A + 2.85*B - 0.64*C + 0.78*AB + 0.25*AC - 1.52*BC - 0.40*A2 + 0.20\ B2 - 2.51*C2 \quad (1)$$

From Table-3 and the equation above, it is found that the molar of the substrate and the solvent ratio gives a positive effect of 1.48 and 2.85, respectively. Meanwhile, the catalyst concentration has a negative effect of 0.64.

Table-1. Optimization results of surfactant N-lauroyl lysine in the percentage value of the lauric acid conversion.

Run	A-Molar of substrate (Lysine/Lauric Acid)	B-Solvent Ratio (v/$w_{Lauric\ Acid}$)	C-Catalyst Concentration (w/$w_{Lauric\ Acid}$)	R1-Acid Conversion (%)
1	3	3	3	82.03
2	2	3	5	80.08
3	4	2	3	76.52
4	3	1	7	75.00
5	3	3	7	75.56
6	2	2	3	75.39
7	4	2	7	77.89
8	4	1	5	76.56
9	4	3	5	85.94
10	2	2	7	75.78
11	3	2	5	79.30
12	3	2	5	79.30
13	3	1	3	75.39
14	2	1	5	73.83
15	3	2	5	79.30

Table-2. Analysis of variance for response surface quadratic model.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob>F
Model	121.80	9	13.35	3.11	0.1121
A-Molar of Substrate	17.49	1	17.49	4.02	0.1012
B-Solvent Ratio	65.15	1	65.15	14.98	0.0118
C-Catalyst Concentration	3.25	1	3.25	0.75	0.4268
AB	2.45	1	2.45	0.56	0.4868
AC	0.24	1	0.24	0.055	0.8236
BC	9.24	1	9.24	2.12	0.2047
A^2	0.59	1	0.59	0.13	0.7284
B^2	0.15	1	0.15	0.034	0.8602
C^2	23.19	1	23.19	5.33	0.0690
Residual	21.75	5	4.35		
Lack of Fit	21.75	3	7.25		
Pure Error	0.000	2	0.000		
Cor Total	143.55	14			

Table-3. The prediction results of the regression coefficients to construct a regression model.

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	79.30	1	1.20	76.20	82.40	
A-Molar of Substrate	1.48	1	0.74	-0.42	3.37	1.00
B-Solvent Ratio	2.85	1	0.74	0.96	4.75	1.00
C-Catalyst Concentration	-0.64	1	0.74	-2.53	1.26	1.00
AB	0.78	1	1.04	-1.90	3.46	1.00
AC	0.25	1	1.04	-2.44	2.93	1.00
BC	-1.52	1	1.04	-4.20	1.16	1.00
A^2	-0.40	1	1.09	-3.19	2.39	1.01
B^2	0.20	1	1.09	-2.59	2.99	1.01
C^2	-2.51	1	1.09	-5.30	0.28	1.01

From Table-2 and Table-3, it is found that the molar of a substrate (A) has a positive effect of 1.48 and is less significant on product formation. However, the solvent ratio gave a positive effect of 2.85 and was significant in product formation. The catalyst concentration variables, both in the singular form, the interaction with the molar of the substrate and the quadratic form, have adverse effects, respectively, -0.64, -1.52, and -2.51 on product formation, although this effect was not significant. The existence of positive and negative effects on these three interactions indicates that the optimum limit of the effects of the three in the formation of N-lauroyl lysine was found.

The equation's negative sign shows an inversely related relationship with the response variable (R1) [24]. The most significant influence on product yield is the solvent ratio variable, with the solvent ratio coefficient value of 2.85. This coefficient is positive with a P-value <0.05, which means that the solvent ratio has a significant positive effect on N-lauroyl lysine formation.

Interaction Effects of Molar of Substrate, Solvent Ratio, and Catalyst Concentration

The effect of the molar interaction of the substrate and the solvent ratio is shown in Figure-4. Based on the observations, it was found that the N-lauroyl lysine conversion would increase with the increase in the substrate ratio and the solvent ratio. The contour surface shows that the maximum conversion can be obtained if the substrate's molar ratio is 4 Molar while the solvent ratio is three v/wLA. In this reaction condition, 85.94% conversion of N-lauroyl lysine can be obtained. This contour plot also shows that the increase in N-lauroyl lysine conversion is more significant when the substrate ratio increases.

The use of hexane / 2-propanol mixed solvent will produce a better yield [14]. Mixed solvents with different polarities significantly increase yield because lysine, like other amines, is more soluble in alcohol solvents such as 2-propanol. At the same time, lauric acid is more soluble in non-polar n-hexane solvents [22].

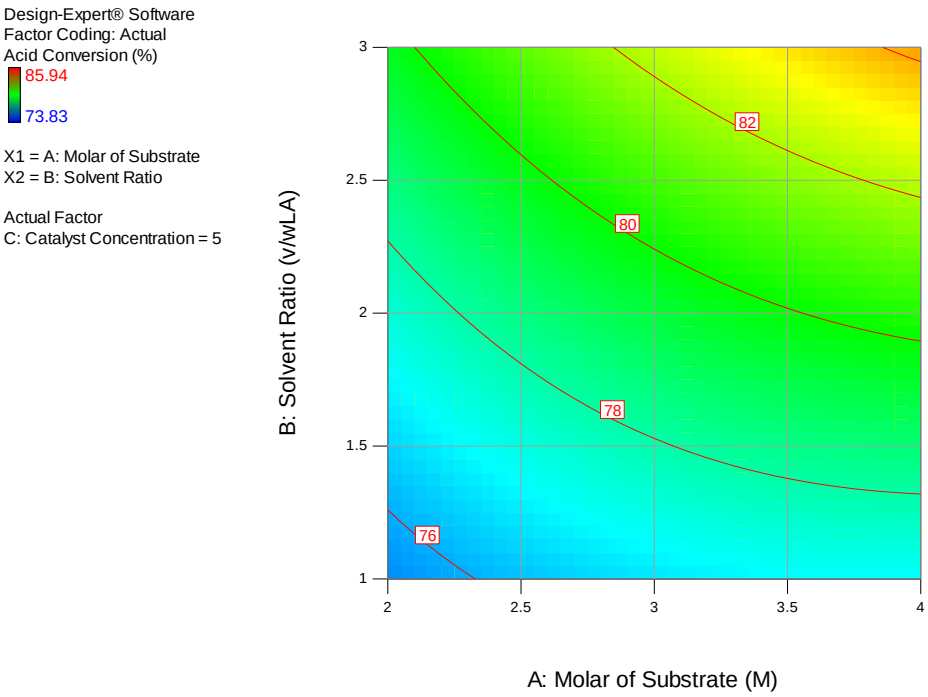

Figure-4. Contour plots effect of interactions between molar of substrate and solvent ratio.

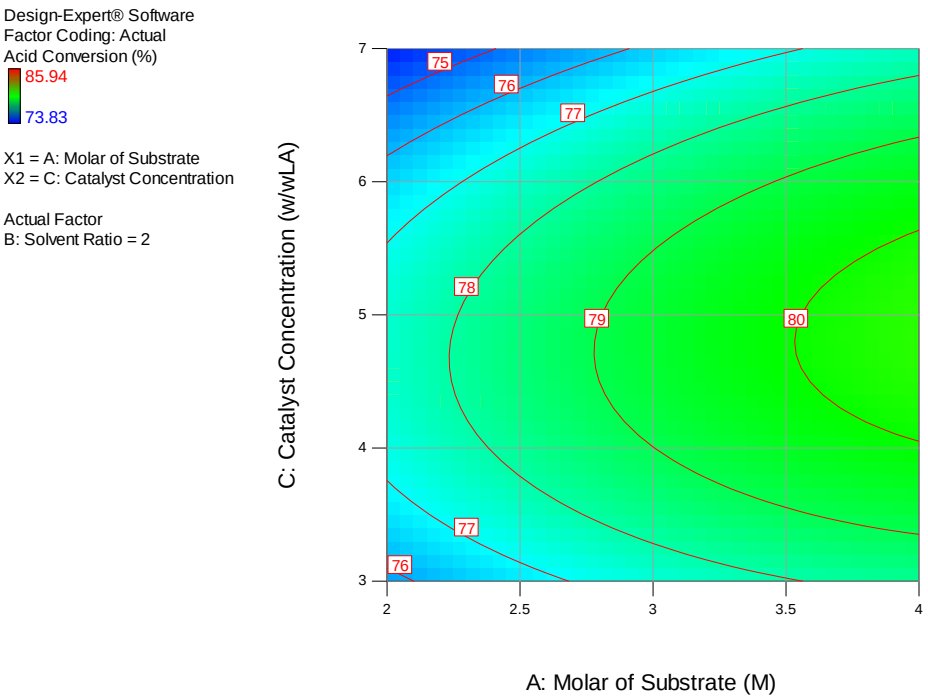

Figure-5. Contour plot of the interaction effect between molar of substrate and catalyst concentration.

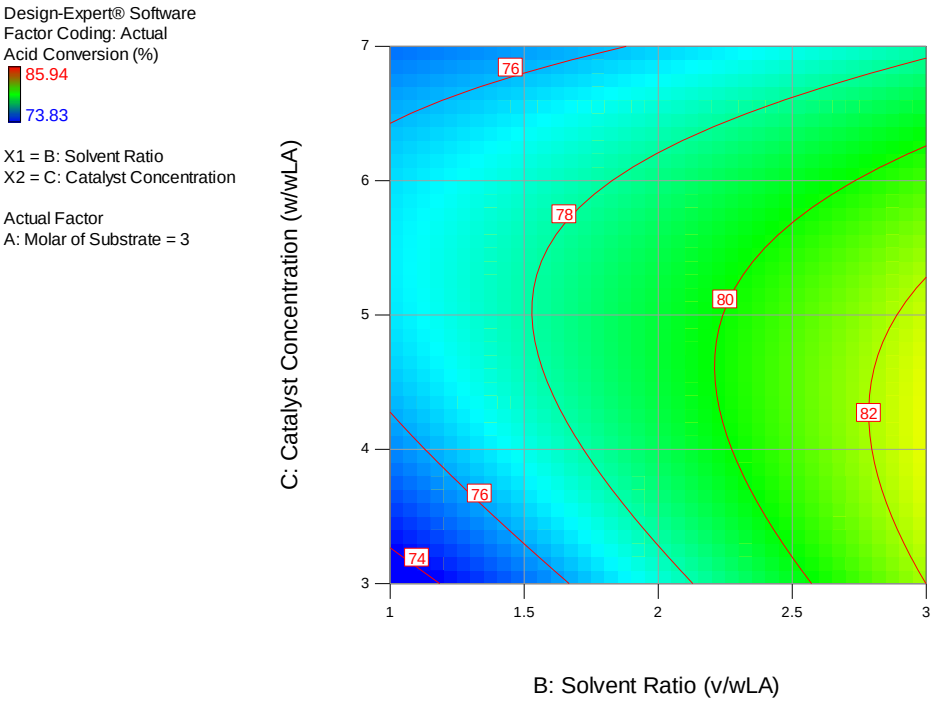

Figure-6. Contour plot of the effect of the interaction between solvent ratio and catalyst concentration.

The contour plot in Figure-5 shows the yield of lauric acid conversion on the interaction between substrate ratio and catalyst weight. In general, the conversion of N-lauroyl lysine will increase, along with the increase in substrate ratio and catalyst weight. In particular, it was found that at a substrate molar ratio of 2 Molar, the increased use of catalyst alone was not able to increase the conversion of lauric acid to acyl lysine. However, suppose the substrate ratio is increased to 3 Molar. In that case, the catalyst weight increase will increase the lauric acid conversion, although it decreases slightly as the further increase of the catalyst weight increases. The maximum yield of lauric acid conversion was obtained using a maximum substrate ratio and a catalyst weight of 5 w/wLA. The optimum field will be obtained in this range, as shown in Figure-5, with a lauric acid conversion of more than 84%.

High conversion of lauric acid characterizes the high N-lauroyl lysine recovery. To observe this gain and its effect on the variable solvent ratio and catalyst weight, the contour plot obtained can be seen in Figure-6. The observations show that the conversion of N-lauroyl lysine will also increase with increasing the ratio of solvent and catalyst weight. Based on Figure 6, it can be seen that the optimum conditions when the ratio of the solvent to lauric acid is 3 v/wLA and the concentration of the CH3ONa catalyst are 5% (w/wLA), resulting in a conversion of 85.94%. The contour plot shows that the increase in the conversion of N-lauroyl lysine when the catalyst weight is 0 is more significant than the maximum catalyst weight, namely 7%. The same trend as FIG. 5 is also found in FIG. 6, wherein if the solvent ratio is minimal, using the weight of the catalyst overall ranges does not increase the conversion. The conversion of lauric acid at a solvent ratio of 2 v/wLA is less than 80%, while in reverse; the conversion can be more than 84%.

If the solvent ratio increases while the catalyst concentration is maintained at a certain amount, the percentage conversion decreases. However, if the solvent ratio is maintained at a certain amount while the catalyst concentration increases, it does not significantly increase the percent conversion [11]. This is consistent with research conducted by Masyithah *et al.* (2020), where there was no significant increase in alkanolamide content on the addition of the amount of catalyst [4].

Comparison of Actual Conversions with Model Conversions

The lauric acid conversion can be calculated to be compared between the studies carried out with the lauric acid conversion of the compiled models. The calculation results are shown in Figure-7, showing the comparison of the experimental conversion value with the model conversion value, where there is an insignificant deviation in the resulting conversion value. The deviation is obtained from the standard deviation value of the model.
The smaller the difference in the acquisition between the predictive conversion data and the experimental conversion data, the smaller the resulting equation will be the better. This shows that the equation model, generated by statistical methods, is acceptable because the resulting deviation is small with a small error. It is concluded that the lower the standard deviation, the closer the data is to the average. In contrast, if the standard deviation value is higher, the more comprehensive the range of data variations is [1, 3].

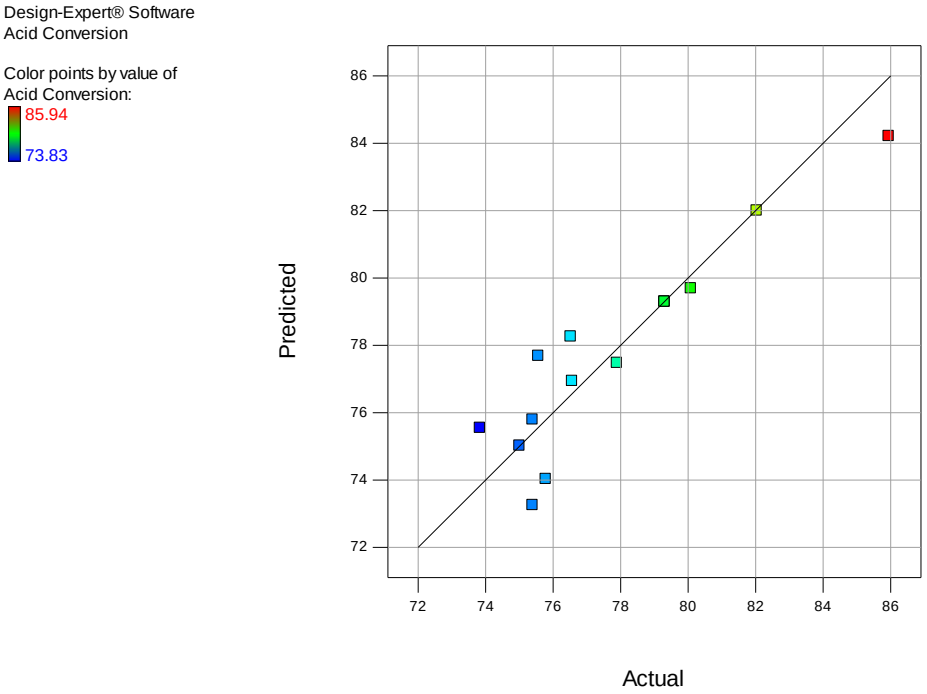

Figure-7. Comparison of actual conversion results with the model conversion.

Analysis of Acid Numbers

The The acid number states the percentage reduction in fatty acids, which is an indicator of the best value. The high acid number will affect the polarity and foam, thereby reducing the final product's quality [26]. The acid number is determined by a standard solution of potassium hydroxide using the phenolphthalein indicator. It is known that the higher the KOH acid number used for the titration process, the higher the acidity value. The results showed that the acid number of the best product was 50.49.

Analysis of Saponification Numbers

The saponification number is expressed as the number of KOH needed to lather one gram of oil or fat. The alcohol in KOH functions to dissolve fatty acids from hydrolysis and facilitate alkalis reactions to form soap. The greater the number of saponification, the smaller the fatty acids, and the better the oil quality. On the other hand, if the soaping number is small, the fatty acids are large, and the quality decreases. A saponification number is a number that shows the number of milligrams of KOH needed to lather 1 gram of oil. The amount of soaping number depends on the molecular mass of the oil. The greater the molecular mass, the lower the soaping number. The result of measuring the saponification number of surfactant N-lauroyl lysine is 29.4525.

Analysis of Hydrophile - Lipophile Balance

To determine the usefulness of the surfactant, the HLB value is usually determined first [27]. In this study, the surfactant N-lauroyl lysine product's HLB value was measured using the acid number and saponification number approach. The HLB value of the surfactant products obtained was 8.333. This HLB's value shows that the surfactant N-lauroyl lysine can be used as a mixture of cosmetic products.

CONCLUSIONS

The RSM method with Box Behnken Design can be used to optimize the N-lauroyl lysine surfactant Synthesis. The parameters that statistically have the most significant effect on the surfactant synthesis process are the solvent ratio of the hexane-2-propanol mixture. The P-value for the solvent ratio is 0.0118, with a coefficient of determination (R^2) of 84.85%. The surfactant N-lauroyl lysine characteristics have an acid number of 50.49 and an acid number of 29.4525. With an HLB value of 8.333, the resulting surfactant can be used as a mixture for cosmetic products.

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