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Green Synthesis Optimization of Glucose Palm Oleate and Its Potential Use as Natural Surfactant in Cosmetic Emulsion

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Abstract: This study aimed to optimize the green synthesis of glucose palm oleate catalyzed by *Carica papaya* Lipase (CPL) through transesterification in a solvent-free system. Palm olein was used as a fatty acid donor for transesterification reactant and was also employed as a reaction medium. Reaction optimization was performed by using response surface methodology (RSM). Seventeen synthesis conditions were generated by a Box–Behnken design and the products were further determined by ultra-performance liquid chromatography (UPLC). Fatty acid compositions of palm olein identified by gas chromatography-mass spectrometry (GC-MS) found that oleic acid (51.77 ± 0.67%) and palmitic acid (37.22 ± 0.48%) were major components. The synthesis variable factors of 50 °C, 45 h reaction time, and 1400 U of CPL were predicted by the RSM to be optimum conditions and thus provided the highest glucose palm oleate of 0.3542 mmol/g. Conjugation between palm olein fatty acids and glucose via transesterification resulted in glucose palm oleate being obviously verified by UPLC, Fourier-transform infrared spectroscopy (FTIR), and thin-layer chromatography (TLC) analyses. The synthesized sugar fatty acid ester revealed an HLB value of 6.20 represented by the lowest % creaming index (%CI) of 35.40 ± 3.21%. It also exhibited a critical micelle concentration (CMC) of 3.16×10^{-5} M. This study is the first report to reveal the transesterification of glucose and palm olein catalyzed by CPL in a system without using any solvent. Glucose palm oleate has been shown to be derived from an environmentally friendly synthesis process and would be promising as a potential alternative natural surfactant for cosmetic application.

Keywords: glucose palm oleate; *Carica papaya* Lipase; green synthesis; natural surfactant; transesterification

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1. Introduction

Surfactants are amphiphilic molecules that are widely used in the cosmetic, food, agricultural, and pharmaceutical industries. In cosmetic products, surfactants may serve as emulsifiers, detergents (cleansing agents), wetting agents, foaming agents, and so on. Sugar fatty acid esters are non-ionic, non-toxic, and biodegradable surfactants and have good emulsifying, stabilizing, conditioning, detergency, and other properties [1,2]. Generally, sugar esters can be produced by either chemical or enzymatic methods. Most commercial surfactants on the market are manufactured by chemical syntheses that cause environmental impacts, high energy consumption, and human health risk concerns [3]. It is based on high-temperature esterification in the presence of an alkaline catalyst and organic solvents used as media that are toxic and not readily biodegradable [2]. This problem leads to the use of biological catalysts such as the lipase enzyme. Enzymatic sugar synthesis provides mild reaction conditions with less energy consumption, and the denaturation of reactants and products can be avoided. In addition, the enzyme can also offer high regioselectivity leading to a high production yield [4].

Lipase, EC 3.1.1.3, typically catalyzes the hydrolysis of lipids in aqueous media, but this equilibrium reaction shifts towards synthesis in non-aqueous solvents in the presence

of small amounts of water [5]. In the synthesis of sugar fatty acid esters, lipases are almost used in immobilized form, typically bound to porous spheres [5]. Immobilization of lipase has benefits in enzyme recycling, scaling up of production, and stabilizing the enzyme activity. *Carica papaya* lipase (CPL) is obtained from papaya latex which is tightly attached to the dry matter present in the latex and subsequently is insoluble in water. Therefore, it has been considered a "natural immobilized" biocatalyst that is easy to prepare and can be reused [6,7]. It has been employed in various esterification and transesterification reactions [8–12]. For transesterification, the CPL was reported to be relatively cheap and has potential as a catalyst [8].

It is noticed that even using the enzymatic method for sugar ester synthesis, the reaction still needs organic solvents to force the reaction equilibrium to undergo ester synthesis rather than hydrolysis. Hence, the problem of organic solvent use still remains. For this reason, other alternative methods have been searched for the development of environmentally friendly processes [13]. There have been reports of the use of ionic liquids or deep eutectic solvents [14,15], supercritical carbon dioxide [16], and solvent-free systems [17].

Glucose fatty acid ester is a typical sugar ester. Its synthesis was previously described by employing glucose and fatty acids as reactants and using solvents such as t-butanol [1] and eutectic mixture [14,15] as a reaction medium. Its synthesis from natural oil such as palm olein has not been reported. Moreover, for green process consideration, the use of natural oil as a dual function for both reactant and reaction medium has also been unpublished. To our knowledge, more information on the non-solvent enzymatic synthesis of glucose fatty acid esters is still needed. In addition, transesterification of glucose and palm olein fatty acids catalyzed by CPL has not been studied. Therefore, this study aimed to synthesize glucose palm oleate from glucose and palm olein catalyzed by CPL and utilize palm olein as both a reactant and reaction medium. The reaction condition was also optimized by using response surface methodology (RSM).

2. Materials and Methods

2.1. Materials

Refine palm olein (Morakot brand) was purchased from a supermarket in Chiang Rai Province, Thailand. Palmitic acid, molecular sieve, *p*-Anisaldehyde, and pyridine were purchased from Sigma (St. Louis, MO, USA). Oleic acid was purchased from PenReac (Barcelona, Spain). Castor oil was purchased from QReac (New Zealand). D-glucose was purchased from Merck (KGaA, Darmstadt, Germany). Acetic acid and formic acid were purchased from Thermo Fisher Scientific (Waltham, MA, USA).

2.2. Preparation of Carica papaya Lipase (CPL)

Carica papaya latex was obtained by making a longitudinal incision on the unripe fruit (70–100 days) on papaya trees (Kaeg-Dum variety in which the taxonomy was determined by Mae Fah Luang University Botanical Garden) in papaya plantation, Khuntan district, Chiang Rai Province, Thailand, according to the method of Chaiwut et al. [18]. The latex was then collected and mixed with DI water (ratio 1:3 w/v). The mixture was then stirred for 15 min and centrifuged at 10,000$\times$ g rpm, 4 °C for 20 min. The precipitate was lyophilized and referred to as *C. papaya* lipase (CPL) [9]. The lipase activity of CPL was assayed by using *p*-nitrophenyl palmitate (*p*NPP) as a substrate. One unit of enzyme was expressed as the amount of enzyme releasing 1 mmol *p*-nitrophenol per min at the assayed condition [19].

2.3. Determination of Moisture Content

The moisture contents of CPL and substrates were determined by using an Ohaus MB45 moisture analyzer (Shah Alam, Selangor, Malaysia). The result was expressed as a percentage of moisture content (%).

2.4. Determination of Palm Olein Fatty Acids Composition

Besides being used as a reaction medium, palm olein was also used as a substrate in the synthesis of glucose palm oleate in which the fatty acids comprising palm olein play an important role in the transesterification reaction with glucose. Therefore, the fatty acid compositions of palm olein were analyzed according to the method modified by Pintathong et al. [20]. Fatty acid methyl esters (FAMEs) were prepared prior to analysis by GC-MS. Three grams of the oil sample was weighed accurately in a round bottom flask and 3.00 mL of 0.9 M sulphuric acid in methanol and 1.00 mL of toluene were added. The solution was refluxed for 3 h. Then, the extract was concentrated in a rotary evaporator at 50 °C for 30 min. The sample pH was adjusted to pH 2.0 with 0.1 M NaOH. Then, the product was analyzed by GC-MS (19091S-433, Agilent). The injector and detector temperature was set at 215 °C and 250 °C, respectively. The utilized column was an HP-5MS (30 m × 0.25 mm, with a film thickness of 0.25 µm). Helium and nitrogen of ultrahigh purity grade were used as carrier gases at flow rates of 11.07 and 31.24 mL/min.

2.5. Green Synthesis of Glucose Palm Oleate Catalyzed by CPL

Usually, transesterification reactions use organic solvents as a reaction medium in order to eliminate the water content and render ester bond formation instead of ester bond hydrolysis. In this study, palm olein was used as a substrate and reaction medium to promote a solvent-free system. Palm olein fatty acids were expected to be transesterified with D-glucose via CPL catalysis. The reaction mixture consisted of a palm olein:glucose ratio of 10:1 mmol, various CPL amounts, 1 g of 4 Å molecular sieve, and 10% (w/w) of total water content. A small amount of water is essential for the enzyme's active site function. The amount of CPL, temperature, and reaction duration varied. The reaction was performed in a 1.2 oz. vacuum-capped glass vial. The sample was shaken at 170 rpm in a water bath shaker at desired temperatures and times. A control without the enzyme addition was also performed for each reaction mixture. At the end of the reaction, the molecular sieve was removed by a spatula. The mixture was then centrifuged at 25 °C for 15 min in which the CPL and the excess palm olein appeared at the bottom and top layer, respectively. The middle layer of glucose palm oleate was collected and rinsed with water to remove the remaining glucose (sample-to-water ratio of 1:2 w/v). The glucose ester was dried in a desiccator for 24 h and analyzed by UPLC, TLC, and FTIR. Standard glucose palmitate and glucose oleate were used as references to calculate the amount of synthesized glucose palm oleate.

2.6. Optimization of Glucose Palm Oleate Green Synthesis

Response surface methodology (RSM) using Design-Expert® Software (Stat-Ease Inc., Minneapolis, MN, USA, trial version 11) was employed to study the optimization, levels of significant parameters, and the interaction between variables that influence the synthesis of glucose fatty acid esters. A Box–Behnken design (BBD) was also used for the experimental design. The three independent variables considered remarkable factors for the response function of glucose palm oleate synthesis were A: CPL amount (1200–3600 unit), B: temperature (40–50 °C), and C: time of reaction (36–60 min). The experimental design consisted of 17 trials (Table 1), and the independent variables were studied at three different levels of low (−1), medium (0), and high (+1) as shown in Table 2. All the experiments were performed in triplicate. The predicted response value Y in each trial of the quadratic model was expressed as:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_1\,\beta_1 A^2 + \beta_2\beta_2 B^2 + \beta_3\beta_3 C^2 + \beta_1\beta_2 AB + \beta_1\beta_3 AC + \beta_2\beta_3 AC \quad (1)$$

where Y is the measured response, β_0 is the intercept, $\beta_1\beta_2\ \beta_3$ are the linear coefficients, $\beta_1\ \beta_1$, $\beta_2\beta_2$, and $\beta_3\beta_3$ are the quadratic coefficients, $\beta_1\beta_2$, $\beta_1\beta_3$, and $\beta_2\beta_3$ are the interactive coefficients, and A, B, and C are the independent variables. Second-order polynomial coefficients were calculated and analyzed using the Design Expert statistical software (Trial Version 11, Stat-Ease, Minneapolis, MN, USA).

Table 1. Experimental run from the Box–Behnken design of the 3 variables.

Run	A: CPL Activity (Unit)	B: Temp. (°C)	C: Time (h)
1	2400	45	48
2	1200	40	48
3	3600	40	48
4	3600	45	60
5	1200	45	36
6	2400	40	36
7	2400	45	48
8	2400	45	48
9	1200	45	60
10	1200	50	48
11	2400	50	36
12	2400	40	60
13	3600	45	36
14	3600	50	48
15	2400	45	48
16	2400	50	60
17	2400	45	48

Table 2. Independent variables and their level for Box–Behnken design.

Factor	Independent Variables	Code Level		
		−1	0	+1
A	CPL activity (Units)	1200	2400	3600
B	Temperature (°C)	40	45	50
C	Time (h)	36	48	60

2.7. Analyses of Glucose Palm Oleate

2.7.1. UPLC Analysis

The synthesized glucose fatty acid esters were analyzed by using UPLC (Waters Acquity UPLC H-Class system) equipped with an ACQUITY UPLC PDA detector using BEH phenyl 1.7 µm columns (2.1 × 100 mm). The reference and sample glucose fatty acid esters were dissolved in pyridine and filtered through a 0.45 µm nylon filter. The separation was performed by a gradient mobile phase consisting of acetonitrile (A) and 0.1% (*w*/*w*) of formic acid (B). The elution was programmed as follows: 0 min; 50% A and 50% B, 10 min; 100% A, 15 min; 50% A and 50% B and 20 min; 50% A and 50% B at a flow rate of 0.3 mL/min. The glucose fatty acid esters were detected at 205 nm.

2.7.2. FTIR Analysis

The dry glucose palm oleate was mixed with KBr by grinding with a mortar until homogenous. The sample amount was 0.1–2% of KBr. Subsequently, the mixture powder was transferred to a 7 mm collar pellet die and then put into hydraulic pressure to prepare a pellet form of the sample powder. Then, the sample was analyzed by using FTIR (LS-55, Perkin Elmer) from 4000–400 cm^{-1} with a resolution of 4 cm^{-1}.

2.7.3. Thin Layer Chromatography (TLC)

One milligram of sample was dissolved with 0.10 mL of pyridine. Ten micrograms of the sample solution was applied as spots on an analytical silica gel 60 TLC plate (10 × 20 cm, film thickness 0.25 mm). The spots on the plate were developed in a mobile phase consisting of chloroform:methanol:acetic acid:water (ratio 60:25:13:2 by volume) [21]. The sample components were visualized by spraying with a visualization spray reagent consisting of methanol:sulfuric acid:p-anisaldehyde (ratio 50:50:0.5 by volume) followed by heating at 140 °C for about 10 min. Then, the spot of the sample was observed visually.

2.8. Determination of Glucose Palm Oleate Properties

2.8.1. Determination of Hydrophilic-Lipophilic Balance Value (HLB)

The HLB values of glucose fatty acid esters were determined by using the creaming index (CI) method which was modified from a previous report [22]. Emulsion composing of 15% oil phase (mixture of oleic acid and castor oil), 80% water, and 5% of glucose palm oleate emulsifier was prepared at different required HLB values ranging from 1.0–14.0. Each emulsion formula was prepared in a test tube by weighing all ingredients and then heated to 70 °C. The mixture was mixed with a vortex mixer for 5 min. Then, it was cooled to room temperature and phase separation was observed. The creaming index was calculated from the percentage of the height of the serum layer at the bottom over the height of the total emulsion sample as the equation below.

$$\text{Creaming index } (\%) = \left(\frac{H_S}{H_T}\right) \times 100 \quad (2)$$

where:

H_S is the height of a serum layer

H_T is the total height of an emulsion sample

The less % creaming index showed the best emulsion forming [22] and was chosen to expand the HLB scale by using the same method described above.

2.8.2. Microscopic Observation of Emulsion

Microscopic observation of the emulsion from the HLB determination section was carried out and photographed using an optical microscope (BA300, Motic) to show the particular structure of emulsion systems. The type of formulation (O/W or W/O) could be observed by this method. The blue color solution was used to dye the water phase, while the oil phase of the emulsion was stained with the red oil soluble color. The microscopic samples were examined at room temperature under ×10–×40 magnification.

2.8.3. Surface Tension Analysis

A series of aqueous solutions (50 mL) at various concentrations ranging from 1×10^{-8} to 1×10^{-2} M of glucose palm oleate in Milli-Q ultrapure water were prepared. The solution was left at room temperature for 24 h in order to reach absorption equilibrium at the surface. The surface tension of the sample ester was determined at room temperature with force tensiometers (Dataphysics Instruments, Filderstadt, Germany) by the du Noüy ring method. Measurements were repeated three times, and the results were given as mean values ± SD.

2.9. Statistical Analysis

All experiments were performed in triplicate ($n = 3$). The measured values of each analysis were analyzed using SPSS version 20. p-values < 0.05 were regarded as statistically significant.

3. Results and Discussion

3.1. Fatty Acid Compositions of Palm Olein

Palm olein is a liquid fraction derived during the fractionation of palm oil. The palm olein fatty acid composition analyzed by GC-MS is summarized in Table 3. Ten fatty acids were found, and four prominent peaks corresponded to oleic acid, palmitic acid, stearic acid, and myristic acid, respectively, which accounted together for approximately 97.20%. It was noticed that oleic acid and palmitic acid were the major components and appeared to be 88.99%. This result agreed with previous reviews in which palm olein was composed of 39.8–46.0% oleic acid and 38.0–43.5% palmitic acid [23]. Therefore, it was expected that the glucose palm oleate produced via CPL catalysis in this study would be represented by glucose oleate and glucose palmitate, while glucose stearate and glucose myristate were minor compounds.

Table 3. Fatty acids composition of palm olein (Morakot brand).

Fatty Acids	% *w*/*w*
Lauric acid (12:0)	0.74 ± 0.07
Myristic acid (14:0)	2.20 ± 0.29
Pentadecylic acid (15:0)	0.11 ± 0.01
Palmitoleic acid (16:1)	0.47 ± 0.10
Palmitic acid (16:0)	37.22 ± 0.48
Margaric acid (17:0)	0.22 ± 0.05
Oleic acid (18:1)	51.77 ± 0.67
Stearic acid (18:0)	6.01 ± 0.61
Linoleic acid (18:2)	0.55 ± 0.17
Arachidic acid (20:0)	0.67 ± 0.21
Saturated fatty acids (SFAs)	47.17 ± 0.57
Monounsaturated fatty acids (MUFAs)	52.24 ± 0.67
Polyunsaturated fatty acids (PUFAs)	0.55 ± 0.17

3.2. Moisture Content of CPL, Glucose and Palm Olein

Moisture content is a major factor in lipase-catalyzed esterification or transesterification reactions in which a minimal amount of water is necessary for the enzyme's active site to retain its active structure. However, an excess of moisture in the reaction medium results in the lipase catalyzing a competitive hydrolysis reaction [24]. Accordingly, water existing in the system can affect ester synthesis, and the moisture content of the reactants would then be known. The moisture contents of CPL and substrates were determined by using a moisture analyzer and the results are shown in Table 4. All reactants contained less moisture contents (less than 10%) which could proceed with the ester synthesis. A moisture content less than about 18% w/w or water activity lower than 0.6 in the system can proceed with the conversion yield and initial reaction rate of sugar ester syntheses [5,25]. Moreover, Turon et al. [26] reported that the moisture content can vary up to values as high as 11% in an ester synthesis system catalyzed by microbial lipase. Though, water is also a by-product of the transesterification reaction. An accumulation of water produced by the glucose palm oleate reaction can force the reaction equilibrium towards hydrolysis rather than ester synthesis, and the use of a 4 Å molecular sieve to remove water in the system is one of the acceptable methods [5]. The presence of a 9.0 g molecular sieve in xylitol oleate synthesis in *t*-butanol composed of 8 mmol of reactants provided a conversion of up to 98% when compared with 40% of the non-molecular sieve added [2]. The 0.50 g molecular sieve added in the system of glucose palmitate synthesis in hexane which is composed of 0.50 mmol of each reactant exhibited the highest conversion at 41.18% [1]. In this study, the solvent-free synthesis of glucose palm oleate which employed palm olein as both reactant and reaction medium used a 1.0 g molecular sieve for the system of 10 mmol palm olein and 1 mmol glucose.

Table 4. Moisture content of reactants and lipase for glucose palm oleate green synthesis.

Reactants	CPL	Glucose	Palm Olein
Moisture content (% w/w)	5.98 ± 0.18	1.80 ± 0.01	0.58 ± 0.01

3.3. Optimization of Reaction Condition for the Green Synthesis of Glucose Palm Oleate

The reaction was performed in glass bottles with caps. The reaction mixture at the initial stage and at 72 h of reaction is demonstrated in Figure 1A and Figure 1B, respectively. After the synthesized glucose palm oleate was separated and dried, this natural surfactant appeared as present in Figure 1C. The product was stable under ambient conditions during 6 months of storage. The appearance, color, HLB value, and surface tension were consistent when compared to the initial properties. However, longer time stability still needs investigation.

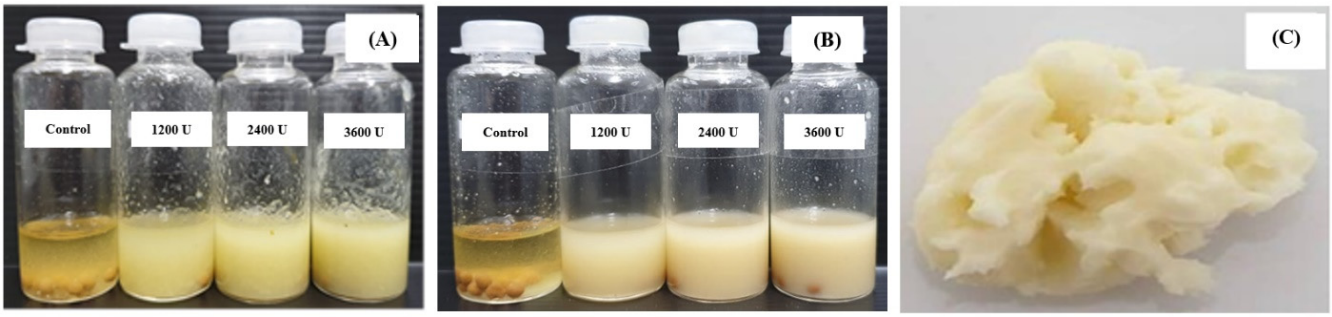

Figure 1. Appearance of glucose palm oleate green synthesis mixture; (**A**) before reaction started, (**B**) reaction mixture at 72 h and (**C**) the glucose palm oleate after drying.

The response values as glucose palm oleate amounts derived from the 17 experimental designs of BBD are revealed in Table 5. The amount of glucose palm oleate in mmol per gram of ester product was calculated by comparing its UPLC peak area with the standard amounts of glucose palmate and glucose oleate. The conversion was obtained by comparing the amount of glucose palm oleate product to the initially used glucose quantity of 1 mmol. The RMS is an appropriate method for studying the effect of process variables and is extensively used for a variety of processes [27].

Table 5. Box–Behnken design with independent variables and response values.

Run	CPL Amount (Unit)	Temp. (°C)	Time (h)	Glucose Palm Oleate	
				mmol/g	% Conversion
1	2400	45	48	0.2756 ± 0.0010	61.73 ± 0.22
2	1200	40	48	0.2038 ± 0.0003	45.65 ± 0.07
3	3600	40	48	0.2622 ± 0.0007	58.73 ± 0.16
4	3600	45	60	0.2261 ± 0.0007	50.65 ± 0.16
5	1200	45	36	0.2119 ± 0.0005	47.47 ± 0.11
6	2400	40	36	0.2037 ± 0.0011	45.63 ± 0.25
7	2400	45	48	0.2888 ± 0.0013	64.69 ± 0.29
8	2400	45	48	0.2714 ± 0.0014	60.79 ± 0.31
9	1200	45	60	0.2072 ± 0.0006	46.41 ± 0.13
10	1200	50	48	0.3504 ± 0.0019	78.49 ± 0.43
11	2400	50	36	0.3118 ± 0.0011	69.84 ± 0.25
12	2400	40	60	0.2073 ± 0.0007	46.44 ± 0.16
13	3600	45	36	0.2557 ± 0.0006	57.28 ± 0.13
14	3600	50	48	0.2270 ± 0.0005	50.85 ± 0.11
15	2400	45	48	0.2801 ± 0.0008	62.74 ± 0.18
16	2400	50	60	0.2498 ± 0.0002	55.96 ± 0.04
17	2400	45	48	0.2729 ± 0.0008	61.13 ± 0.18

As shown in Table 6, the designed model with a p-value of 0.0039 and an R^2 of 0.91 indicated the significance of the model. Among the three variables, temperature exhibited a significant effect on the response with a p-value of 0.0017, while the CPL amount and time of reaction did not affect ester synthesis. Linear term of temperature, interaction term of the CPL amount, and temperature and quadratic term of time were determinant factors ($p < 0.05$).

According to the RSM results in Figure 2, it was confirmed that temperature greatly affected the glucose palm oleate synthesis. This factor responded to the increase in the synthesized products in a linear manner. The increment of temperature increased the kinetic energy of the system resulting in raising the enzymatic reaction rate. This suggests that the transesterification rate is controlled by the reaction steps, rather than the mass transport phenomena of the diffusion of the substrates in the interior of the catalyst [28].

From the RSM result, it was suggested that the highest glucose palm oleate amount of 0.3542 mmol/g could be predicted from the optimum operating conditions of 50 °C, 45 h reaction time, and 1400 U of CPL amount.

Table 6. Analysis of variance (ANOVA) for the selected quadratic model of response glucose palm oleate.

Source	Sum of Squares	Df [a]	Mean Square	*F*-Value	*p*-Value [b]
Model	0.0259	9	0.0029	8.07	0.0039
A: CPL amount (Unit)	6.61×10^{-7}	1	6.61×10^{-7}	0.0019	0.9669
B: Temperature (°C)	0.0086	1	0.0086	24.03	0.0017
C: Time (h)	0.0011	1	0.0011	3.01	0.1264
AB	0.0083	1	0.0083	23.14	0.0019
AC	0.0002	1	0.0002	0.4341	0.5310
BC	0.0011	1	0.0011	3.01	0.1262
A^2	0.0013	1	0.0013	3.58	0.1005
B^2	1.08×10^{-6}	1	1.08×10^{-6}	0.003	0.9576
C^2	0.0052	1	0.0052	14.54	0.0066
Residual	0.0025	7	0.0004	-	-
Lack of fit	0.0023	3	0.0008	15.66	0.0112
Pure error	0.0002	4	0	-	-
Cor Total	0.0284	16	-	-	-

R^2 = 0.91; R^2_{adj} = 0.80; CV = 7.46%; adequate precision = 10.80. [a] Degree of freedom. [b] *p*-value < 0.05 were considered to be significant.

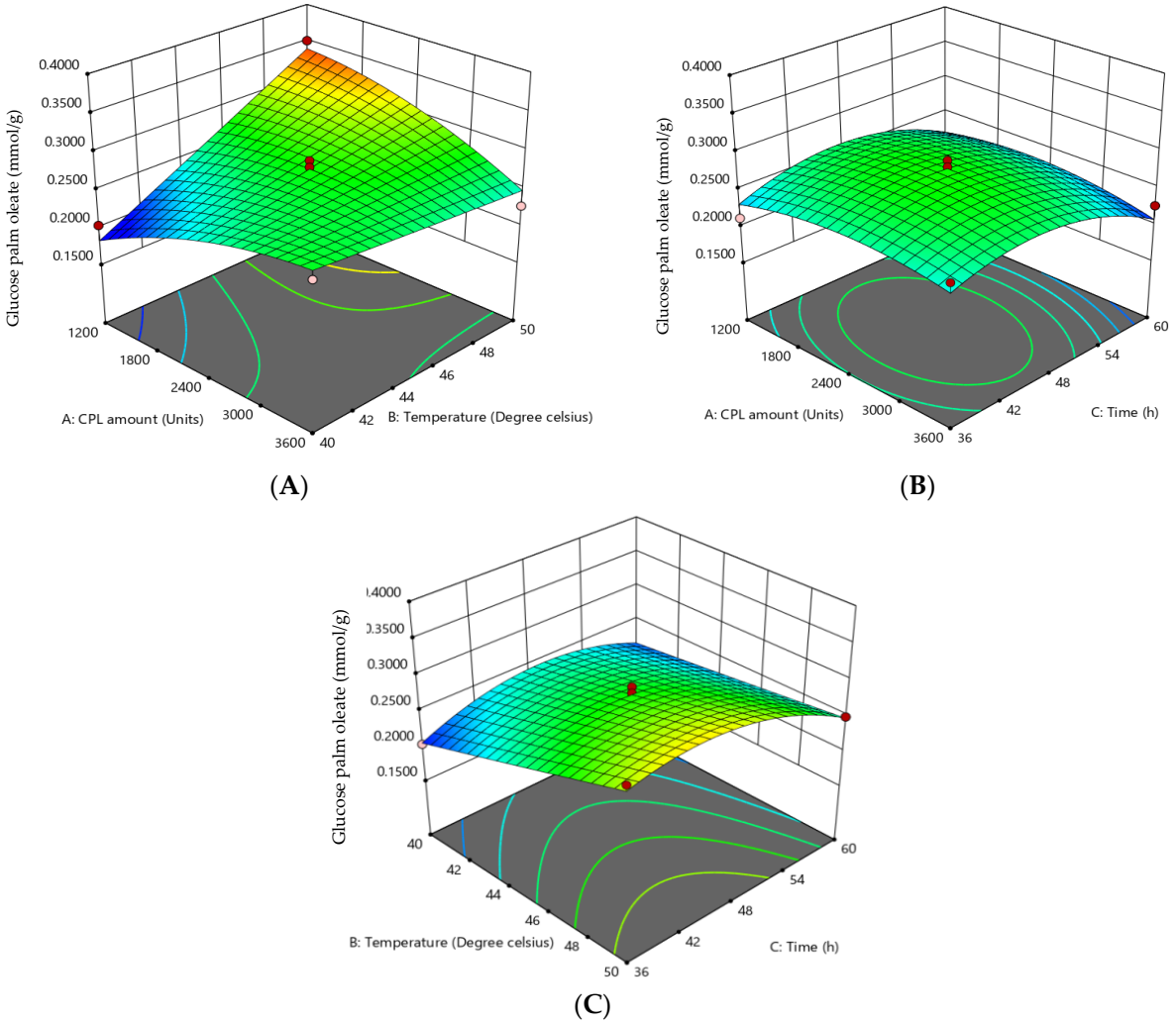

Figure 2. Graphical interpretation of the model describing the dependence of glucose palm oleate content on (**A**) CPL amount and temperature, (**B**) CPL amount and reaction time, and (**C**) temperature and reaction time. The RSM revealed the optimum condition for green synthesis of glucose palm oleate of 50 °C, 45 h reaction time, and 1400 U of CPL.

3.4. UPLC Analysis of Glucose Palm Oleate

The overlay chromatogram in Figure 3 shows the glucose palm oleate ester synthesized from glucose and palm olein. The chromatogram of the control mixture (composed of palm olein and glucose without CPL) and the glucose substrate did not show glucose fatty acid ester peaks. This proved that the transesterification could not occur without the lipase enzyme and the reaction was able to perform by using palm olein as both a reactant and green reaction medium instead of organic solvents. The amount of glucose palm oleate product was calculated and compared with the references. Glucose palmitate and glucose oleate which were used as sugar ester references were eluted at 9.08 and 9.25 min, respectively. The glucose palmitate standard possessed a smaller chain length than glucose oleate; it was consequently eluted before that of glucose oleate. Their calibration curve formulas were y = 122.92x and y = 473.94, respectively.

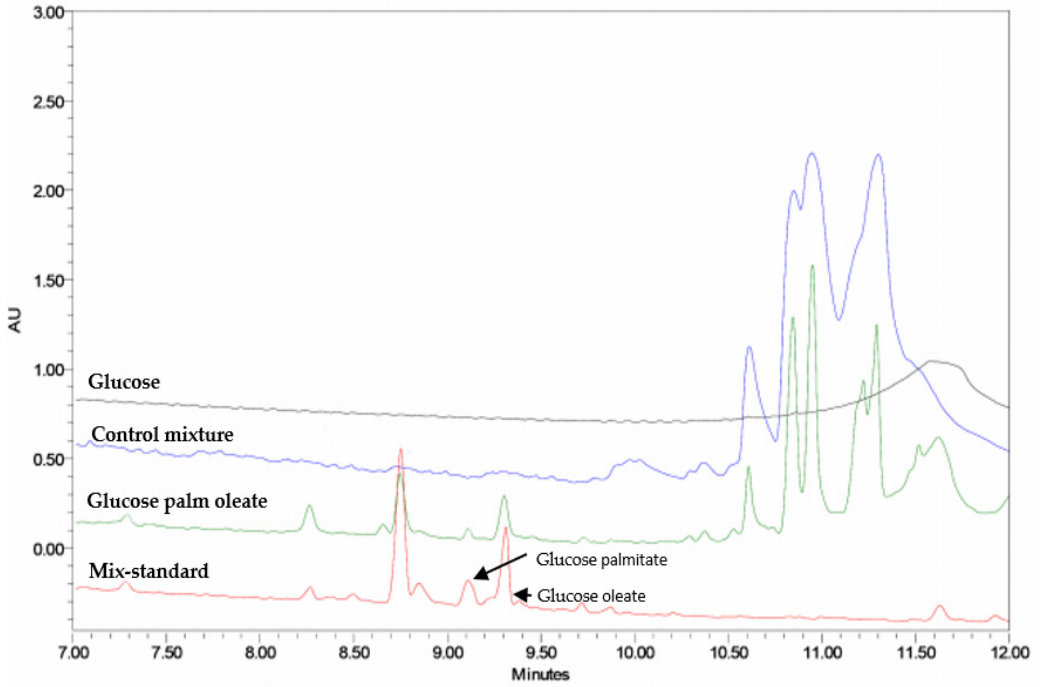

Figure 3. UPLC chromatographic separation of glucose palm oleate.

3.5. FTIR Analysis of Glucose Palm Oleate

Structural confirmation by FTIR analysis of glucose palm oleate is present in Figure 4. The results confirmed the conjugation between palm olein and glucose via transesterification, resulting in glucose palm oleate as the final product. The palm olein spectrum elucidated a triglyceride structure, as it presented C=O stretching at 1750 cm^{-1}, C-O-C stretching at 1172 cm^{-1}, and O-C-H stretching at 1380 cm^{-1} indicating an ester group between glycerol and alkyl chain of fatty acid [29]. The vibration of the glycerol group at 1172 and 1380 cm^{-1} should be absent in the sugar fatty acid ester [30].

The glucose spectrum was similar to a previous report [31]. It presented a strong broad peak of O-H stretching (3300 cm^{-1}) of the hydroxyl group and showed asymmetric stretching of C-H, symmetric stretching of C-H, and C-H bending signals at 2950, 2880, and 1465 cm^{-1}, respectively [30]. There were C-O and C-C vibrations at 1053 representing the ring structure of glucose [30].

The glucose palm oleate spectrum was evidenced to be the ester product of transesterification from those two substrates. Its spectrum combined those of palm olein and glucose. The spectrum exhibited OH-stretching of the hydroxyl group (3300 cm^{-1}) from glucose, with methyl signals (C-H stretching at 2934–2858 cm^{-1}) from palm olein. In addition, the C=O signal of the glyceride ester group from palm olein at 1750 cm^{-1} disappeared [32], while the C=O vibration in the glucose fatty acid ester at 1635 cm^{-1} occurred [32,33].

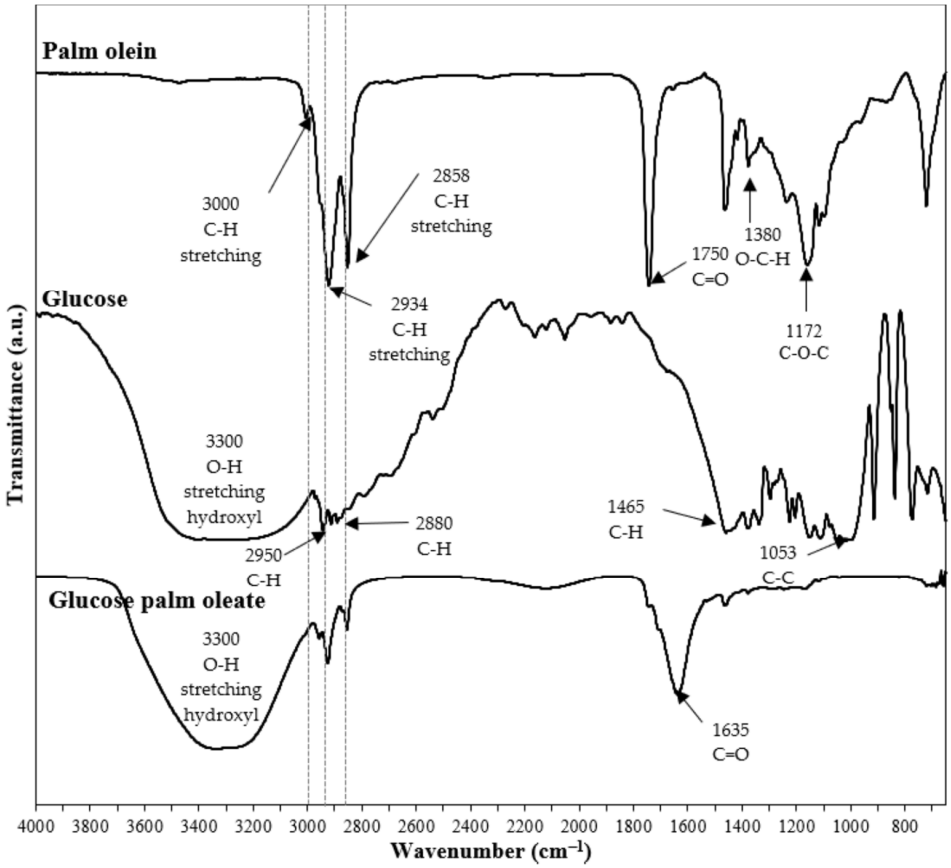

Figure 4. FTIR spectrums of palm olein, glucose, and glucose palm oleate product.

3.6. Thin Layer Chromatography (TLC) of Glucose Palm Oleate

TLC analysis was one evidence verifying the occurrence of glucose palm oleate. It was also employed to evaluate the sucrose monoester synthesis catalyzed by lipase in a non-aqueous biphasic medium [34]. As shown in Figure 5, it can be seen that glucose palm oleate shared the mobility of both palm olein and glucose. As the mobile system was low polarity, palm olein and glucose palm oleate moved faster than glucose due to the like-dissolve-like principle. Glucose, which is a strong nucleophile, reacted with *p*-anisaldehyde producing a dark blue color. This dark spot also appeared in lane 3 of glucose palm oleate, verifying the presence of a glucose moiety in the ester product.

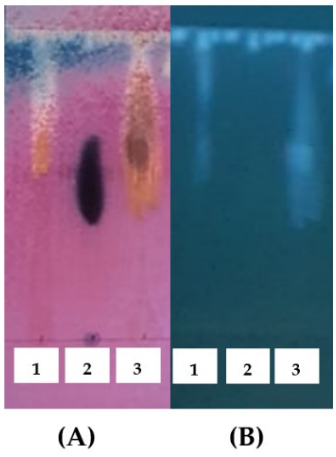

Figure 5. TLC analysis of glucose palm oleate synthesis from glucose and palm olein catalyzed by CPL. Lane 1; palm olein, lane 2; glucose, and lane 3; glucose palm oleate product. The TLC observation under (**A**) visible light and (**B**) UV light.

3.7. Determination of Hydrophilic-Lipophilic Balance Value (HLB)

Glucose palm oleate is considered a natural non-ionic surfactant in which the glucose moiety is the hydrophilic portion, while the hydrophobic portion belongs to fatty acids composition in palm olein. The balance of these groups is expressed as the HLB value. Non-ionic surfactants have been extensively used as emulsifiers in emulsion preparation for various kinds of cosmetic products such as facial creams, body lotions, deodorants, and hair conditioners. For the emulsification process, an emulsifier was selected for the formulation in which its HLB value should be close to the required HLB value of the mixed oil phase used [22,35]. This HLB matching resulted in a stable emulsion.

The HLB value of glucose palm oleate was determined by using the creaming index (CI) method [11]. The emulsion was prepared at different required HLB values ranging from 1.0 to 14.0 which could be reached by a combination of oil phase mixture between the already known HLB oleic acid (HLB = 1) and castor oil (HLB = 14) [36]. The result showed that formulas with required HLB from 5.0–7.0 provided the lowest % CI values of 47.85 ± 1.86%, 37.46 ± 1.69%, and 44.62 ± 1.13%, respectively (Table 7). Therefore, these three formulas were chosen to expand their required HLB. As shown in Table 8, the formula with required HLB 6.2 provides the lowest % CI which is 35.40 ± 3.21%. Hence, the suitable HLB of the non-ionic surfactant glucose palm oleate was approximately 6.2. This value was suggested to be suitable for use in W/O emulsions [37].

Table 7. Required HLB values 1.0–14.0 of emulsion and % CI.

Required HLB of Emulsion	% CI
1.0	68.95 ± 0.35
2.0	53.16 ± 0.06
3.0	55.19 ± 1.54
4.0	48.18 ± 1.20
5.0	47.85 ± 1.86
6.0	37.46 ± 1.69
7.0	44.62 ± 1.13
8.0	56.09 ± 1.04
9.0	64.78 ± 1.67
10.0	65.27 ± 1.54
11.0	70.29 ± 1.71
12.0	72.45 ± 2.56
13.0	74.91 ± 1.54
14.0	79.71 ± 0.00

Table 8. Required HLB values 5.0–7.0 of emulsion and % CI.

Required HLB of Emulsion	% CI
5.0	42.72 ± 2.66
5.2	44.72 ± 1.27
5.4	44.72 ± 0.76
5.6	40.09 ± 1.20
5.8	41.55 ± 2.21
6.0	39.93 ± 0.89
6.2	35.40 ± 3.21
6.4	43.33 ± 0.22
6.6	45.37 ± 2.15
6.8	58.56 ± 5.27
7.0	55.71 ± 1.08

3.8. Microscopic Observation of Emulsions

Microscopic observation of those HLB-determined emulsions was carried out and photographed by using an optical microscope to show the particular structure of emulsion systems. Figure 6 reveals the blue water-soluble color existing in the droplet, while the red oil-soluble color surrounding the continuous phase. This indicated the appearance of the W/O emulsion. The emulsion of required HLB 6.2 had an average smallest droplet size. It also appeared as the most dense with a uniform size distribution. The small size of the inner phase indicated a stable emulsion. Furthermore, an emulsion

with a relatively uniform size distribution is more stable than one with a larger size distribution but the same average particle size [38].

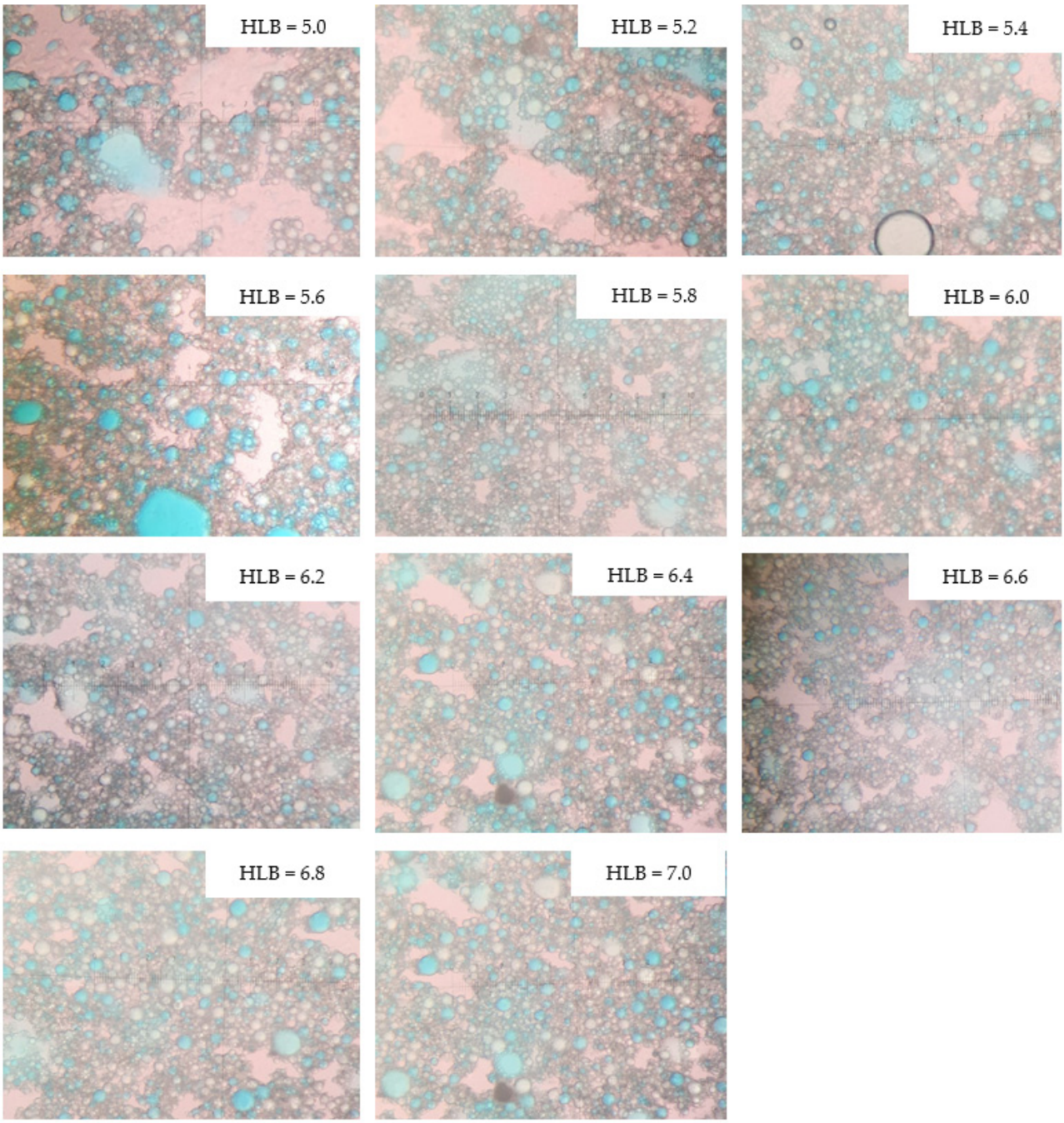

Figure 6. Optical microscopic image of emulsion droplet at different required HLB values ranging from 5.0–7.0 (40× magnification).

3.9. Surface Tension

Surface tension plays an important role in the effectiveness of surfactant material. It has been measured as a function of surfactant concentration [34]. The glucose palm oleate solutions exhibited a pattern in which surface tension decreased as its concentration increased. The critical micelle concentration (CMC) is the intersection of the two linear domains in the surface tension versus concentration of surfactant [39]. Once they reached the CMC point, surface tension became quite constant. The result in Figure 7 demonstrated a CMC value of glucose palm oleate of 3.16×10^{-5} M. From previous studies, CMC values of sugar ester were reported in a variety of results. The

6-substituted products of glucose, sucrose, raffinose, and stachyose ranged from 4.5×10^{-4} to 2.3×10^{-3} M [40]. The sugar ester from sucrose and maltose with mono palmitate were 5.4×10^{-6} to 1.10×10^{-5} M [41]. The sugar head group is apparently a minor factor, and the hydrocarbon tail size is mainly affected that increasing the fatty acid chain length related to lowering the CMC [42].

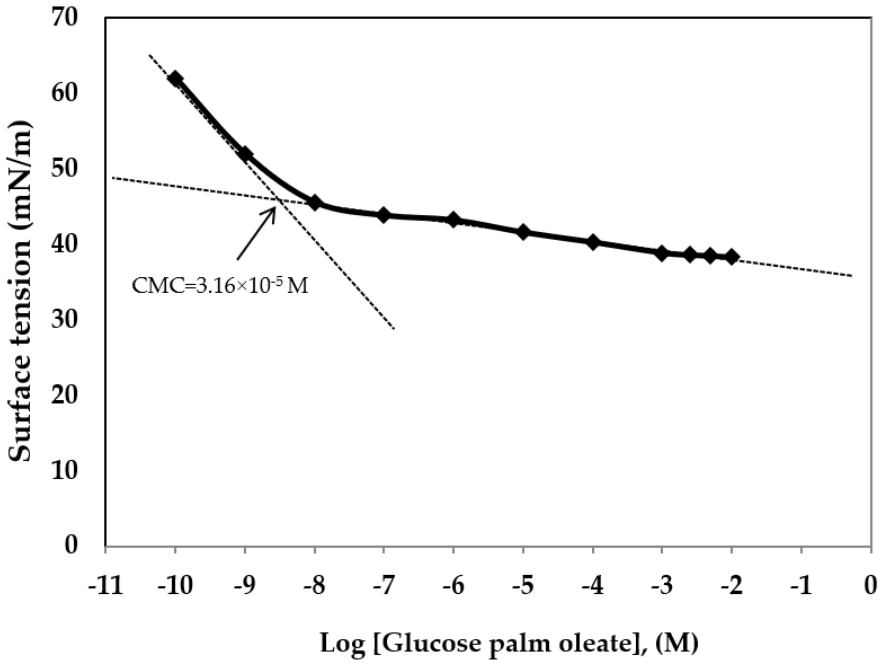

Figure 7. Mean surface tension measurements of glucose palm oleate solution with the corresponding log concentration and indicating CMC point.

4. Conclusions

This study has shown that glucose palm oleate was successfully synthesized by CPL catalysis under a solvent-free system which is an environmentally friendly method and is considered a green method. This study is the first to accomplish the use of palm olein as both a reactant and reaction medium to synthesize glucose fatty acid esters. The RSM suggested the highest glucose palm oleate amount of 0.3542 mmol/g which could be predicted from the optimum reaction conditions of 50 °C, 45 h reaction time, and 1400 U of CPL. FTIR and TLC analyses obviously showed that glucose palm oleate was successfully synthesized from glucose and palm olein. The glucose palm oleate possessed an HLB value of approximately 6.20, which was suitable for preparing a W/O emulsion which was confirmed by microscopic observation. The CMC of this prepared natural surfactant was shown to be 3.16×10^{-5} M.

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