The Influence of Accelerated Stability Test to Physical Properties of Varied-stirring Speed Chitosan-based Nanoemulsion

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Abstract. Preparation of nanoemulsion has been conducted with using squalene as oil phase, chitosan as water phase, then Tween 80 and Span 85 as surfactans. There was an accelerated stability test for the nanoemulsion that had been varied with of stirring speed of homogenization process of 10,000 rpm, 15,000 rpm and 20,000 rpm. These nanoemulsions were placed in an extreme temperature condition of 4 and 40^{o}C for fourteen days. According to this accelerated stability test, there were some changes in physical properties like viscosity, pH, density, and stability percentage after centrifugation. There were some decreasing in viscosity, density and stability percentage of nanoemulsion after finished their extreme temperature conditions but their pH remained constant. For the infra-red spectra, there was a slight change in the intensity, which could be noticed as the result of chemical reaction during accelerated stability test.

Keywords: Nanoemulsion; chitosan; squalene; accelerated stability; stirring speed

Introduction

Chitosan is a natural nontoxic biopolymer produced by the deacetylation of chitin, a major component of the shells of crustaceans such as crab, shrimp, and crawfish [1]. Chitosan is a biodegradable, biocompatible, and bioadhesive polysaccharide [2]. It has been shown that chitosan is non-toxic and soft tissue compatible in a range of toxicity tests [3]. Chitosan has received considerable attention for its commercial applications in the biomedical, pharmaceutical, food, and chemical industries [4-7]. Chitosan was selected for nanoparticles because of its unique character which recognized mucoadhesivity and ability to enhance the penetration of large molecules across mucosal surface and exhibit more superior activities than chitosan [1,8]. Chitosan nanoparticles are obtained by the process of ionotropic gelation based on the intraction between the negative groups of sodium tripolyphosphate (TPP) and the positively charged amino groups of chitosan [1,9-11]. For the las two decades, nanochitosan has been extensively developed and explored for

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pharmaceutical applications [12]. Nanochitosan also can be performed in emulsion form as a part of the emulsion.

An emulsion is formed by homogenizing the two phases together, which are oil and aqueous phase, with the presence of one or more emulsifiers. This usually leads to the formation of droplets coated by an interfacial membrane that consists of a single layer of surface active molecules [13]. Emulsions are studied intensely because of the potential use in many industrial applications such as in pharmaceuticals, food technology, paints, agrochemicals, etc. Moreover, emulsions are formed through creation and stabilization of the interface of one immiscible fluid into another (oil and water) [14]. The addition of chitosan in emulsion system influence the particle diameter which will increase and make it unstable when the concentration exceeded a certain level [13].

In this experiments, we focused on the influence of stirring speed to the physical properties of emulsion system that consist of chitosan as its water phase. There was an accelated stability testing include in the process to know the changes of physical properties due to the different stirring speed.

Experimental

Materials

Squalene oil at purity of 95% was purchased from PT. Ecendo Perkasa which specializes in raw material for cosmetic. Analytical grade of chitosan and citric acid monohydrate ($C_6H_8O_7.H_2O$) used in this research were purchased from E. Merck KGaA Germany. Sodium citrate tribasic dihydrate for molecular biology, Tween 80 (Polisorbate 80), and Span 85 (Sorbitan Trioleat) were purchased from Sigma Aldrich USA. Then aquadest was obtained from a Milli-Q water purification system. All of the materials mentioned above employed directly.

Preparation of nanochitosan

0.2 g powdered chitosan was diluted in 0.2% acetic acid and then was mixed for 24 hours. Then 10 M natrium hydroxide was added as many as six drops untill the pH reached 4.6-4.8. Next, the mixture was added by 0.5% sodium tri polyphosphate with the ratio 3:1 (chitosan:Na-TPP). The mixture was homogenized for 15 min with the same stirring speed for the whole process [9-11].

Preparation of emulsion

Emulsion process was begun by making water phase first that was buffer citrate solution with pH 6.5 that was dissolved in chitosan nanoparticles solution. Buffer solution was

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made by mixing citric acid monohydrate and sodium citrate tribasic dihydrate which was stirred for 15 min used a magnetic stirrer. Then buffer solution was added to nanochitosan solution. Surfactant Tween 80 was added to the obtained water phase while Span 85 was added to Squalene as the oil phase of the emulsion system. The addition of surfactants was done gradually while stirred with a magnetic stirrer for 15 min. After each phase mixed completely, the next process is homogenization with Yellow Line DI 25 Digital Homogenizer. There were stirring speed variations in the homogenization process. Homogenizations were conducted at the stirring speed of 10,000, 15,000 and 20,000 rpm for 30 min by adding the oil phase to the water phase gradually.

Characterization of emulsion

pH, Specific Gravity, and Viscosity

pH measurement [15] was done by using a pH meter with 20 times dilution in the tested samples. Electrode was calibrated with buffer solution standard pH 7.2. While the specific gravity was measured using Pycnometer. For viscosity was measured using Ostwald viscosity.

Centrifugation Test

Centrifugation test [18] was done immediately after emulsion was obtained. Emulsion was incorporated into centrifuge tubes to be centrifuged later at 25^{o}C and speed of 3500 rpm for 20 min using a Centrifuge IEC Centra CI2. This testing showed the percentage of the stability of the prepared emulsions (eq. 1).

$$\text{Emulsion stability (\%)} = \frac{\text{height of emulsion separation}}{\text{total height of emulsion}} \text{ x } 100\% \qquad (1)$$

Accelerated Stability Test

This test was conducted to determine the changes in physical properties of emulsions that have been made. The test was conducted for 14 days by using the two extreme temperatures of 40^{o}C and 4^{o}C [15,16]. The physical properties tested were the same as prior to this test.

Fourier Transform Infra Red (FTIR) Test

All of the emulsion were tested by FTIR in order to know the changes in functional groups absorption in each of them both before and after the accelerated stability test. Moreover, the influence of stirring speed also would be observed. It was used FTIR Shimadzu IR-Prestige 21 with KBr as its pellets. KBr pellets were used as a background to which serves to reduce interference that may cause noise in the spectrum formed when the analysis takes place.

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Results and Discussion

Emulsion Characterizations

These emulsions characterization include the test result of pH, specific gravity, viscosity, and stability through centrifugation both before and after the accelerated stability test. Those physical parameters were significant for emulsion system such as pH which showed the acidity level of emulsion in which would determine the feasibility to be used in human body. Furthermore, stability test that had been done would determine the expired date of emulsion products.

Table 1. Emulsion characterizations.

Testing	Emulsion A (10.000 rpm)		Emulsion B (15.000 rpm)		Emulsion C (20.000 rpm)	
	Before	After	Before	After	Before	After
pH	6.02	6.54	6.16	6.14	6.08	6.23
Specific Gravity (g/ml)	0.8540	0.8113	0.8575	0.6860	0.8660	0.7631
Viscosity (cP)	2638	1927	2475	523	1783	439
Centrifugation (% stability)	90	35	70	50	60	45

pH, Specific Gravity, and Viscosity Measurements

Three types of obtained emulsion have a pH that close to neutral which was around 6 both before and after the accelerated stability test. This was due to the presence of the nonionic surfactant span 85 and tween 80 as well as the buffer solution and nanochitosan from the water phase to maintain the pH of the emulsion remains stable. The pH value close to the pH value of the skin so that it can be used to emulsion dermal applications [15]. In addition, it also indicated that despite having different stirring speed during the process of manufacture, conditions of acidity emulsion would not change after the accelerated stability test.

Emulsion density measurement was done with Pycnometer. There was change in physical properties for the emulsion system before and after the accelerated stability test. Generally, those three emulsions experienced declining value of density. Initially those three emulsions have a specific gravity around 0.8 g/ml. Whereas after the accelerated stability test, the values obtained decreased to about 0.6-0.7 g/ml. This indicated that the density characteristics were influenced by accelerated stability test conditions. This was

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because the extreme temperature changed the molecules density in emulsion system. The molecule density was lowering due to that extreme temperature. Furthermore, the phenomenon of the declining also influenced by the varied stirring speeds. This was due to the greater the stirring speed, the greater the decline along with the research by Anisa et.al [18].

Viscosity measurement was carried out using Ostwald viscosity. As well as the specific gravity, viscosity on those emulsion also impaired after against accelerated stability test. These conditions were different from Anisa et.al's experiment where the faster the stirring speed, the lower the viscosity that would create little average droplet diameter [18].

Centrifugation Test

Centrifugation test is a kind of test conducted in emulsion system [19]. Centrifugation is a process to give pressures on emulsion. Stirring speed of 3500 rpm for 20 min could be said equivalent to the gravity for ±1 year [15]. The percentage of stability was obtained from the height of emulsion separation. Generally it was stated that the lower the emulsion separation, the higher the percentage of stability. From three types of emulsions, the highest levels of stability was 90% from emulsion with a stirring speed of 10,000 rpm and was followed by 70% from the emulsion with a stirring speed of 15,000 rpm and the last was 60% from the emulsion with a stirring speed of 20,000 rpm. After the accelerated stability test, those three emulsions experienced declining in their stability. The largest decrease occurred in the emulsion with the lowest stirring speed. This was in accordance to some research that the lower the stirring speed the greater the decrease in stability [19-21]. This resulted that centrifugation test gave less pressure to emulsion samples. The homogeneous emulsion would only experience little separation of conducted centrifugation test.

FTIR Testing

Based on the results, there could be seen the influence of stirring speed and accelerated stabilization test. Those three types of emulsion produced relatively similar spectra even with different stirring speed. This was because the same amount of material composition it contained. Therefore, the number and the size of spectra were relatively same. The measurement of those emulsions where the accelerated stability test was conducted showed differences in FTIR spectra. Emulsion that resulted from stirring speed of 10.000 and 15.000 rpm had relatively same intensities (Fig.1). However, that did not happen to the type of emulsion with stirring speed of 20.000 rpm (Fig. 1). This was because of the differences in the intensity of emulsion before and after the testing. The changes in

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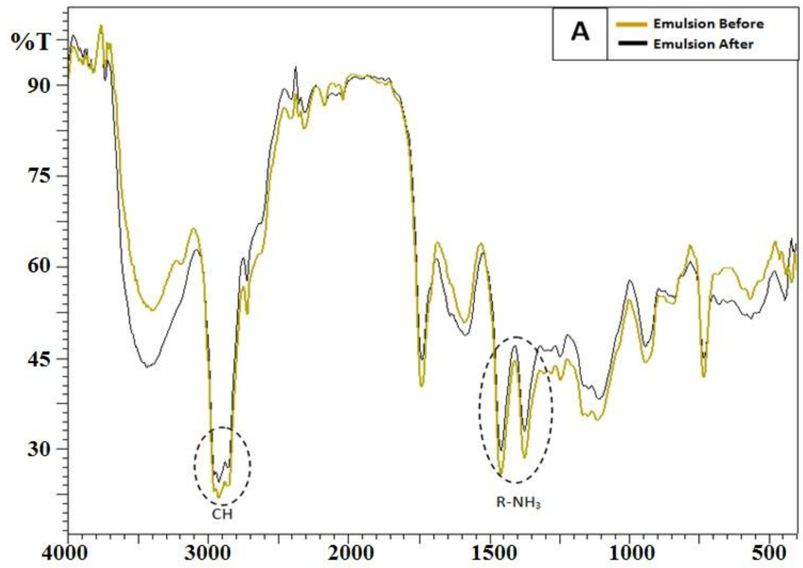
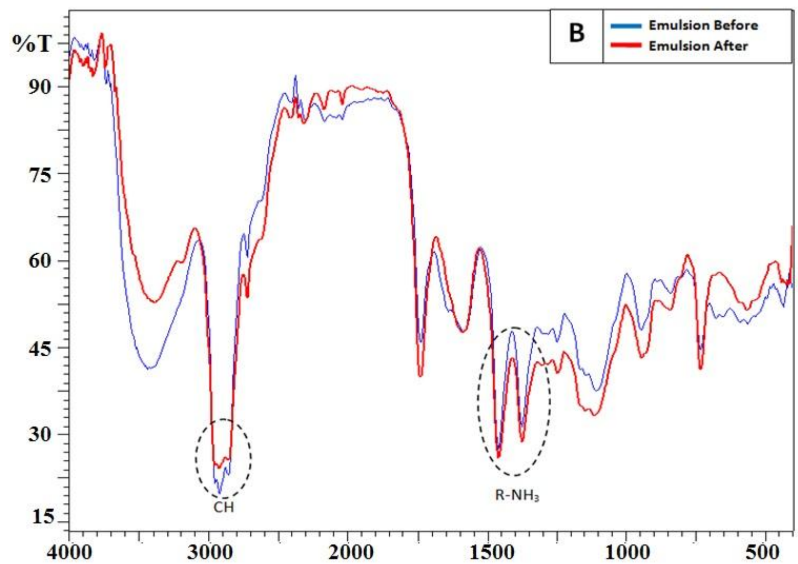
physical properties such as the molecule density due to the stirring speed made the intensity became different. This changes made the after stability test-emulsions had different absorbance with the significant changes in the wavelength of 2000-3000 nm. At this wavelength there were stretching vibration of CH bond and non symmetrical bending vibration of CH_2 bond of the alkyl group (-(CH2) xCH3) from Squalene [22]. In addition, it is also happening at a wavelength of 1250-1500 nm, namely the existence of clusters (R-NH_3) [23]. In this third emulsion, there were changes in the oil or water phase so that resulted in the intensity it produced.





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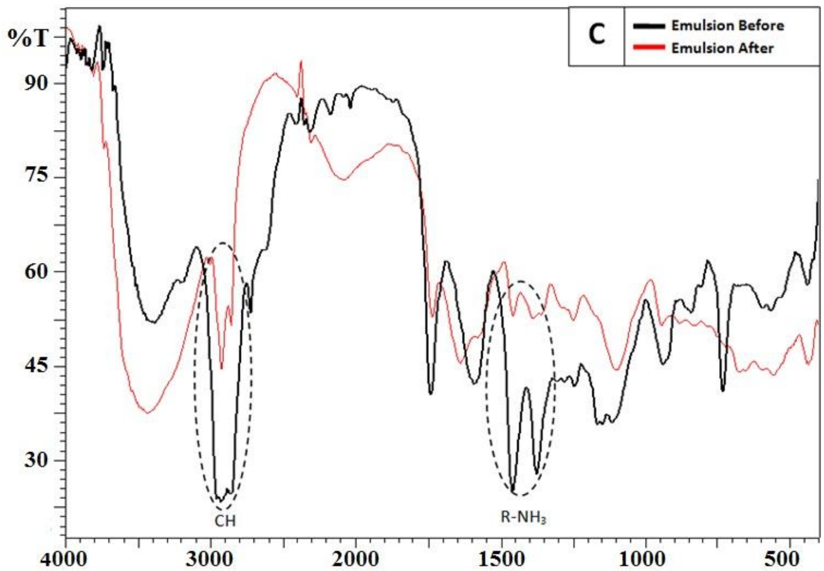

Figure 1. FTIR of emulsion in stirring speed of (A) 10.000 (B) 15.000 (C) 20.000 rpm.

Conclusion

In summary, the physical properties of emulsions which were based on chitosan nanoparticles were influenced by the stirring speed. Those various stirring speed gave different effects such as in viscosity which the highest speed resulted in the lowest value and in percentage stability which show the least stabile in the highest speed. This condition also occurred after the accelerated stability test was conducted. The highest speed resulted in the lowest value of viscosity and the stability got lower in range of 15-55%. Therefore, it could be concluded that the stirring speed and accelerated stability test itself played significant roles in the changes of physical properties of emulsions system.

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