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Effect of Accelerated Stability Test on Characteristics of Emulsion Systems with Chitosan as a Stabilizer

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Abstract

Chitosan has been used as a stabilizer in the preparation of emulsion systems with Squalene as an oil phase, then Tween 80 and Span 85 as a surfactant. Water phase, oil phase and chitosan as a stabilizer stirred with a homogenizer speed of 15,000 rpm for 30 minutes. The results of accelerated stability test with extreme temperatures of 4°C and 40°C showed significant changes in viscosity, density and degree of stability. However, the degree of acidity was relatively still same in which closer to normal pH (6.3-6.4). The particle size analysis showed the size around 3 micrometers. This emulsion system can be applied as the media of active compound delivery, for example is the emulsion system of topical and oral preparation to overcome cellulite problem.

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Keywords: chitosan; emulsion; stabilizer

1. Introduction

An emulsion is formed by homogenizing two phases together, which are oil and aqueous phase, with the presence of one or more emulsifiers [1]. Stability is one of important thing in emulsion systems because they are thermodynamically unstable systems and tend to breakdown over time. This is due to one or several numbers of different physicochemical mechanisms such as creaming, flocculation, coalescence, phase inversion and Ostwald ripening [2]. Emulsions are studied intensely because of the potential use in many industrial applications such as in pharmaceuticals, food technology, paints, agrochemicals etc. In term of pharmaceuticals, there are many examples of emulsion system application such as the topical and oral preparation to overcome cellulite, diabetes, cardiovascular etc. The active compound from herbal extract was a part of emulsion system which included in water phase before the mixing process. As results, there are many producers use this system to be applied in their end-product. Moreover, emulsions are formed through creation and stabilization of the interface of one immiscible fluid into another (oil and water) [3]. The mixing of these fluid that form emulsion will influence the characteristic of its

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stability. Therefore, there will be some stabilizer to prevent the emulsion from some degradation. The stabilizer will facilitate the formation of emulsion and improve the stability by reducing the oil–water interfacial tensions and by forming a protective layer around the droplets to prevent them from aggregating [4]. Therefore chitosan as a polysaccharide are often used as a stabilizer because it is not only to create the desirable textural attributes but also to stabilize the emulsion droplets against gravitational separation [5]. Besides, the use of chitosan in emulsion system will give value-added benefit to the emulsion system itself. The presence of chitosan would improve the creaming stability of O/W emulsion because it would increase the viscosity of a continuous phase,which slows down the diffusion of droplets and flocs. Therefore the formulation that used chitosan as a stabilizer has raised a significant interest in the literature [6].

Over the last decade, chitosan as a deacetylated form of chitin and one of the most abundant naturally occurring biopolymer biopolymers has attracted attention in many industrial fields, such as paper technology, food, water-filtration, agricultural, pharmaceutical and biomedical industries. The size of the chitosan particles required varies depending on the particular industrial field [7]. Due to its biodegradable, biocompatible, mucoadhesive and non-toxic properties [8], this renewable polysaccharide has been widely used in numerous drug delivery systems [9-12]. As a delivery system, chitosan nanoparticles have a special feature which they can adhere to the mucosal surface and transiently open the tight junction between epithelial cells. Some reports have indicated that chitosan can increase membrane permeability, both in vitro [13] and in vivo [14]. Chitosan also enhanced the stability of O/W emulsions by increasing the matrix viscosity and forming a dense polyelectrolytic brush on the water side of oil-water interface [15].

Stability of emulsion can only be seen after the product storage in their shelf life. However this procedure takes quite long time so that it will not work to the development of products that have only short time. Therefore, they need accelerated stability testing. Accelerated stability testing aims to predict how long the product can last the extreme pressure and temperature in order to know the stability of the emulsion product. In this testing, product sample will be placed in particular condition that planned to speed up the changes that commonly may occur in normal condition [16]. Therefore, the objective of this experiment was to better understand the effect of accelerated stability testing against the characteristic of emulsion system that used chitosan as stabilizer.

2. Materials and Methods

2.1. Materials

Squalene oil with purity of 95% was supplied from PT. Ecendo Perkasa which specializes in raw material for cosmetic. Chitosan and citric acid monohydrate ($C_6H_8O_7.H_2O$) with analytical grade were provided from E. Merck KGaA Germany. Tween 80 (Polisorbate 80), Span 85 (SorbitanTrioleat), and sodium citrate tribasic dihydrate for molecular biology were used as supplied from Sigma Aldrich USA. The aquadest used in this research was obtained from a Milli-Q water purification system.

2.2. Preparation of nanochitosan

As many as 0.2 g powdered chitosan was diluted in 0.2% acetic acid and mixed for 24 hours. After that six drops of 10 M sodium hydroxide was added until the pH reached 4.6-4.8. The obtained mixture was added by 0.5% sodium tri polyphosphate with the ratio of 3:1 for chitosan and Na-TPP. The last step was homogenization of the mixture for 15 minutes with the stirring speed of 500 rpm [17-19].

2.3. Preparation of emulsion

The emulsion was obtained by making the water phase first that was buffer citrate solution with pH 6.5 which was already dissolved in chitosan nanoparticles solution. This buffer solution was a mixture of citric acid monohydrate and sodium citrate tribasic dihydrate which was mixed for 15 minutes using a magnetic stirrer. The following step was adding the buffer solution to nanochitosan solution. Both of surfactants were added to different

phase, Tween 80 for 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 minutes. Once each phase mixed completely, homogenization process with Yellow Line DI 25 Digital Homogenizer was started. Homogenization was conducted at the stirring speed of 15,000 rpm for 30 minutes by adding the oil phase into the water phase step by step.

3. Characterization of Emulsion

3.1. Accelerated stability test

This test was conducted to determine the changes in physical properties of emulsions system that has been made. The emulsion was stored in extreme temperature in 4oC as the lowest for 24 hours and the next 24 hours in 40°C as the highest [20]. This procedure was done until 14 days. The following steps were characterizing the emulsion.

3.2. Physicochemical characteristic test

pH measurement [21] 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. By using Pycnometer, the density of emulsion system was measured. Then by using viscometer Brookfield, the emulsion's viscosity was measured. The speed of number 4 spindle was set in 50 rpm consecutively.

3.3. Centrifugation test

Centrifugation test [21] was done by incorporating the emulsion into centrifugation tubes to be centrifuged at 25°C and speed of 3500 rpm for 20 minutes. This testing was done by using a Centrifuge IEC Centra CI2 and the following showed the percentage of the obtain emulsion's stability (Eq. 1).

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

3.4. Fourier Transform Infra Red (FTIR) test

The FTIR test was done in emulsion system to see the changes in functional groups absorption in each of them, both before and after the accelerated stability test. Furthermore, the effect of chitosan as stabilizer would also be observed. This test used FTIR Shimadzu IR-Prestige 21 with KBr as the pellets. KBr pellets were used as a background to reduce interference that may cause noise in the formed spectrum when the sample was analyzed.

3.5. Particle Size Analyzer (PSA) test

The PSA test was conducted in order to measure the particle size of emulsion system. This characterization used equipment of Beckman Coulter Delsanano particle analyzer measured in room temperature with water as the solvent.

4. Result and Discussion

Emulsion system that used chitosan as the stabilizer was characterized including pH, density, viscosity, and stability through centrifugation. These characterizations were also conducted after the emulsion system experienced the accelerated stability test.

Table 1. Physical characteristic of emulsion

Testing	Before test	After test
pH	6,33	6,43
Density (g/ml)	0,858	0,644
Viscosity (cP)	663	155
Centrifugation test (% stability)	60	35

pH measurement was measured by using pH meter and it showed that this emulsion has acidity level near to neutral condition which was about 6.3-6.4; both before and after the accelerated test. The presence of chitosan as stabilizer helped maintaining the level of acidity degree in neutral condition even after the test. This was because chitosan gave the favorable interaction to the emulsion with the pH condition higher than 5 [22]. This emulsion will give some advantages when it comes to application process. This kind of emulsion will help in active compound delivery or dermal application such as the lotion or cream or butter with some treatments [23].

Density was measured by means of pycnometer and generally there was change in emulsion system both before and after accelerated stability test. Initially, the density was 0,858 mg/ml but after the test it changed to 0,644 mg/ml. This declining could be caused by the changes in molecule density of emulsion system. It also can be indicated that the storage at extreme temperature may change the physical structure of emulsion system. This may be due to the fact that higher temperature can increase the degradation of chitosan's effect as the stabilizer [24].

Brookfield viscosity was carried out to measure the emulsion's viscosity. As well as the density, viscosity of the emulsion also decreases after against accelerated stability test. The declining was about four times lower and it may because of the higher the temperature, the lower the emulsion viscosity [24]. Therefore, there may be some degradation of chitosan as a stabilizer that make it not strong enough to maintain the physical condition of the emulsion system.

The aim of centrifugation test in emulsion system is to give some pressures in that system. Stirring of 3500 rpm for 20 minutes could be said equivalent to gravity for ±1 year [21]. Stability percentage was obtained from the separation level of emulsion system. The lower the separation does, the higher the stability percentage will be. As stated before, this test played a role in influencing physical characteristic of emulsion system due to the given pressure. Therefore, the stability fell near to the half of initial stability level. This was due to the given pressure resulted in molecules distribution changes so that the separation level got increase. The higher the pressure, the bigger the separation level would be.

From the emulsion spectra (Fig. 1), it can be seen that there were some characteristic peaks of several compounds it contained. These particular infrared spectra of emulsion system components both before and after the accelerated stability test were summarized in Table 2. Based on Table 2 and Figure 1, it can be seen that the specific infrared spectra in the emulsion was affected by its components. The bands at wavelength range of 2922-2924 and 1460 cm-1 were attributed to the stretching vibration of CH and asymmetric bending vibration of CH_2 from the alkyl groups ($-(CH_2)xCH_3$) of Squalene [17]. Specific infrared spectra at wavelengths 1109-1114, 1741 and 3414-3429 cm^{-1} were assigned to the absorption peaks of Tween 80 and Span 85 as the surfactant. These surfactants have C=O groups in the second ester and the presence of OH groups [18-19]. While the specific infrared spectra at 1579-1593cm^{-1} came from the presence of chitosan and cross ties with sodium tripolyphosphate.

Based on the data in figure and table, it can be seen that, the FTIR spectra of emulsion both before and after the accelerated stability test were similar which no new band appeared and no peak disappeared. This indicates that the compounds of the water phase, oil phase, surfactant, and chitosan espesially as stabilize; as a constituent of the

emulsion was not affected by changes in temperature difference that high enough. Therefore, the results of FTIR suggested that there was no obvious modification of emulsion system's chemical structure.

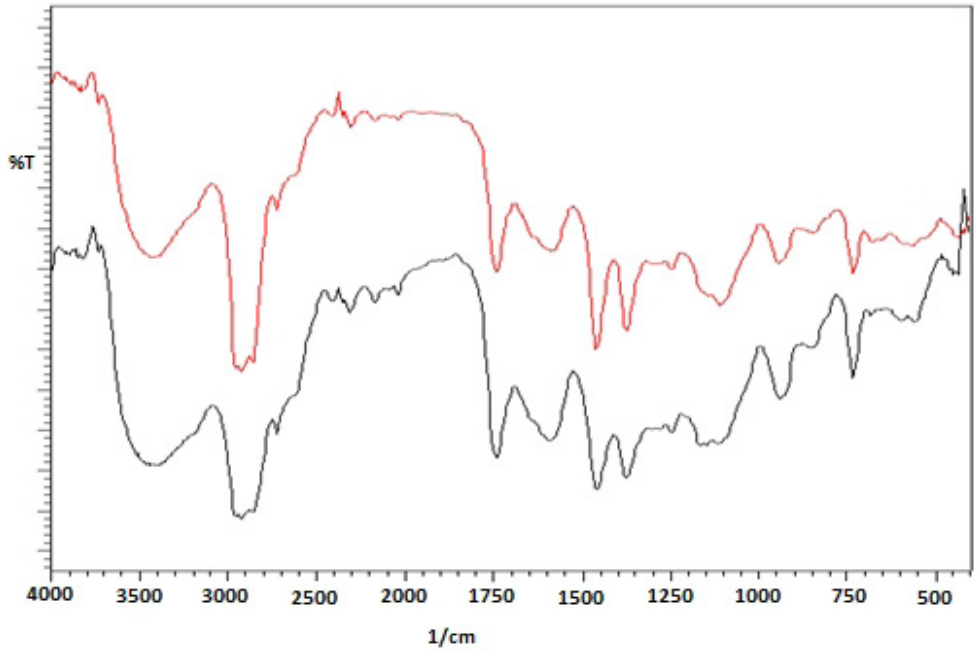

Fig.2.Spectra of emulsion system before ( )andafter () accelerated stability test

Tabel 2. Spectra of emulsion system

Sample	Specific Infra RedSprectra (cm^{-1})						
	υC-O	υC-H_3	υC-H_2	υN-H_2	υC=O	υC-H	υN-H / υO-H
Before	1114	1375	1460	1593	1741	2924	3429
After	1109	1373	1460	1579	1741	2922	3414

In emulsion system, particle size need to be observed in order to know the size it will be categorized. Based on the measurement, it showed particle size of 3 micrometer and polydispersity index of 1. This indicated that this emulsion system had bigger size and categorized as microemulsion. This is inline with centrifugation test result which is the stability dropped into half of initial value. This was because the bigger the size, the unstable the emulsion system will be. The polydispersity index also showed the homogenity level of particle distribution which in the emulsion system it was still in low level.

4. Summary

In summary, this work investigated the emulsion system characteristic after the accelerated stability test with the help of chitosan as a stabilizer. Based on the results, the test influenced the physical characteristic of emulsion system such as density, viscosity, and percentage of stability. Those characteristic got lower after the test while the chemical characteristic got steady. The level of acidity degree and the kind of functional group relatively remained the same. Therefore, it can be concluded that the accelerated stability test played significant roles in the changes of physical properties of emulsions system.

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