Preparation and Characterization of Nano-cream from Squalene in olive oil and Virgin Coconut Oil by Nanoemulsions method

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Abstract

Preparations and characterizations of nano-cream from squalene in olive oil and virgin coconut oil were performed by nanoemulsions method. The emulsion prepared by the Phase Inversion Temperature (PIT) method, using squalene in olive oil or virgin coconut oil, as the oil phase in different ratios, were characterized for their droplet sizes by dynamic light scattering. The ratio between squalene and coconut oil of 0.5: 3.6 provided the smallest droplet size. The emulsion in that conditions were made for the internal phase to be smaller droplet size became nanoemulsions. To enhanced the qualities of the cream in terms of the more quickly absorbed into the skin. Comparison the results of the droplet size between how to make nanoemulsion by using high-energy "sonication" and low-energy "homogeneous dispersing" method at the same time. The results showed the homogeneous dispersing at 45 minutes, a speed of 400 rpm could give the smallest average size in the range of nanoemulsions. The emulsion is transparent white, stable, and did not delaminate even over time. The best conditions were determined to find the important factors which made the cream having small droplet sizes. The statistical analysis method - Design of Experiments (DOE) were applied in the research and Minitab was used to process data. Analysis of variance showed that speed and temperature are significant factors affecting the droplet size of the nano-cream in the significant level of 0.05.

Keywords: Nanoemulsion, Squalene, Virgin coconut oil, Design of experiments.

1. Introduction

Cosmetics are one of the most important industries worldwide. The components of cosmetics are not simple; they usually contain a high number of ingredients and the preparations are time-consuming and tedious [1, 2]. Nanotechnology is a key technology leading to product innovations. Nanotechnology in cosmetics has attracted considerable attention in recent years as potential vehicles for the controlled delivery of cosmetics and personal care products [3]. Nano-emulsions are oil-in-water (o/w) or water-in-oil (w/o) transparent or translucent colloidal dispersions, usually in the 20–500 nm size range [4]. The advantage of nanoemulsions over the ordinary emulsion, is a long term kinetic stability due to their very small droplet sizes [5], which result in a large reduction in the gravitational force. Thus, Brownian motion suffices to and have high kinetic thermodynamically stability. This means that no creaming, sedimentation and flocculation occurs during storage. Weak flocculation is prevented, which enables delivery system to remain dispersed with no separation [6].

Nanoemulsions can be prepared by using a high-energy emulsification method (e.g., high-pressure homogenization, sonication) or by a low-energy emulsification method (using

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physicochemical properties of the components) [7]. In 1906, squalene was discovered from a shark liver extract. Squalene is unsaturated hydrocarbon intermediate of cholesterol metabolism. It is an isoprenoid compound with six isoprene units and has the chemical formula - $C_{30}H_{50}$. Another source of squalene is in plants and human. Olive oil can give the highest amount of squalene among all edible oils. Squalene can protect the skin from ultraviolet radiation, improve the moisture balance, and the elasticity of the skin [8]. Virgin coconut oil contains high levels of vitamin B and antioxidant-rich-components. Coconut oil also helps body to produce rising collagen to slow skin aging.

Design of Experiments (DOE), the statistical analysis method, was applied to design experiments by using Minitab program to find the important factors which made the sample to have small droplet size. The aim of this study was to prepare and characterize nano-cream from squalene in olive oil and virgin coconut oil by nanoemulsions method, and to find the appropriate ratios and the important factors this effected to the droplet size of nano-cream.

2. Experimental Details

The cosmetic grade of squalene in olive oil, propylene glycol, Tween® 80 and dissodium EDTA were kindly provided from Chanchao longevity Co.,Ltd (Thailand). Virgin coconut oil (cosmetic grade) was purchased from Boonchai Chemical (Thailand). The preservative nonyl phenol ethoxylate surfactant was purchased from Chemipan Corporation Co., Ltd (Thailand). Span® 80 (AR grade) was purchased from Fluka (Thailand).

2.1. Preparation of nanoemulsion

First, Phase Inversion Temperature (PIT) method was chosen to prepare an emulsion using squalene in olive oil or virgin coconut oil as the oil phase in different ratios as the followings: 4.1:0.0, 3.5:0.6, 2.7:1.4, 2.5:1.6, 1.3:2.8, 1.1:3.0, 0.9:3.2, 0.7:3.4, 0.5:3.6, 0.3:3.8, 0.1:4.0 and 0.0:4.1. The water and oil phases were heated separately at 70°C for 10 minutes, the water phase was added into the oil phase (squalene in olive oil, virgin coconut oil, propylene glycol, span®80, tween®80 and disodium EDTA). The mixture was then cooled to 25°C. The droplet size was measured. The conditions which could give the smallest droplet size, we brought to make the internal phase to be smaller droplet size became to nanoemulsions. Comparison the results of the droplet size between using high-energy "Sonication" where the high frequency was applied in different times of 10, 15, 30, 45 and 60 minutes., and took pulse to solution every 2 seconds pause 1 second and set power at 70 percent by sonometer and low-enery "Homogeneous Dispersing" method, stirred at 400 rpm at the same time by Analog homogenizer. The method, which could give the smallest droplet size, was applied in the step of design of experiment (DOE) to determine the important factors that effected to the droplet size.

2.2. Design of Experiment

Design of Experiment (DOE) was applied in the research and processing data using Minitab program. Fractional Factorial Design (2^k-1) was chosen to be an experiment design to find the important factors that made the cream had small droplet size from the first step. Defined the factors and treatments which predicted to effect to the droplet size of nano-cream. Randomization and replication were made for reliable data analysis.

2.3. Determination of nanoemulsions droplet size

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The mean droplet size of the nanoemulsions was determined by Particle Analyzer (Delsa™Nano C). All of conditions were measured at room temperature 25 °C.

2.4. Stability study

The preliminary stability of the nanoemulsions preparation was evaluated at 24 hours by centrifuge test at 3500 rpm for 30 minutes using centrifugation. Stability was assessed by macroscopic emulsion observation. The purposes of these tests were to select stable nanoemulsion droplet size and stable physicochemical properties. The selected nanoemulsions were prepared in triplicate, and the samples were stored at low temperature (4±2°C), room temperature (25±2°C) and high temperature (40±2°C). Tests were performed at 24 hours, 7, 15 and 30 days after preparation. The analysis measurements were droplet size and pH value.

3. Results and Discussion

3.1. Preparation of nanoemulsions

Table 1 showed the comparison the droplet radius (nm) and zeta potential (mv) from the different oil phase ratios. The ratio between squalene and coconut oil 0.5: 3.6 provided the smallest droplet size from 12 different formulations. In the first step, the emulsion that was made from the conditions for the internal phase to be smaller droplet size relied on the sonication method for 15 minutes. The results showed the mean droplet radius of 201.15±0.5 nm and 202.73 ± 1.4 nm, respectively. It was found that as the ratios in oil phase changed, the average of droplet size also changed. The ratios of virgin coconut oil showed that they affected the transparence or translucence of the nano-cream and the more virgin coconut oil, the higher viscosity. Zeta potential in was at -14.41±0.7 and -9.93±0.5 mv, respectively.

Table 1. Comparison of the best two recipes, show radius of droplets (nm) and Zeta-potential (mV)

Squalene in olive oil (%)	Virgin Coconut Oil (%)	Droplet radius (nm) ± S.D.	Zeta potential (mV) ± S.D.
0.5	3.6	201.15±0.5	-14.41±0.7
0.3	3.8	202.73±1.4	-9.93±0.5

Table 2. Study the effect of time to droplet size and the pH values between sonication and homogeneous dispersing methods

Time (min)	Sonication method		Homogeneous Dispersing method	
	Droplet radius (nm) ± S.D.	pH values ± S.D.	Droplet radius (nm) ± S.D.	pH values ± S.D.
10	333.40 ± 1.5	6.44 ±0.01	216.63 ± 2.3	6.61 ±0.02
15	285.33 ± 1.4	6.77 ±0.02	467.07 ± 1.3	6.65 ±0.03
30	252.63 ± 1.1	6.75 ±0.05	194.27 ± 2.1	6.68 ±0.03
45	328.80 ± 1.4	6.33 ±0.01	165.93 ± 1.5	7.10 ±0.03
60	239.33 ± 2.0	6.68 ±0.04	308.47 ± 1.7	6.67 ±0.01
90	300.13 ± 2.1	6.54 ±0.01	224.03 ±2.7	6.62 ±0.05

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From the first step, the ratio between squalene and coconut oil 0.5: 3.60 provided the smallest droplet size. The emulsion in the conditions was made for the internal phase to be smaller droplet size and became to nanoemulsions. The results of the droplet size between using sonication and homogeneous dispersing methods at 10, 15, 30, 45, 60 and 90 minutes were compared to search for the cream formulation that can be absorbed quickly on the skin. The results showed that the homogeneous dispersing at 45 minutes could give the average smallest size. This finding led to the study of the important factors which effected the droplet size of nano-cream. The samples in different conditions showed the pH values average in the range of 6-7 that were mild acid to neutral which are appropriate on human skin (Table 2).

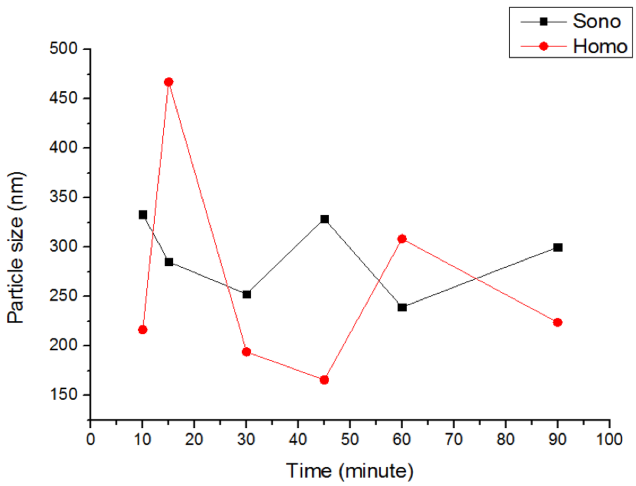

Fig. 1. Droplet size between sonication and homogeneous dispersing method

3.2 Analysis data from Minitab program

The ratio between squalene and coconut oil of 0.5: 3.6 using homogeneous dispersing at 45 minutes was further investigate to find the important factors which made the cream to have a small droplet size. Designed the experiments was fractional factorial design (2^k-1) by Minitab program. The 5 factors including time (A), speed (B), temperature (C), container (D) and length of probe (E) were investigate for their effects on the droplet size of nano-cream. And each of factors had two treatments.

Considered the p-value from Table 3, almost all factors were less than 0.05. That mean were almost all factors were significant. To confirm the significant factors, normal plot of the standardized effects was investigated.

Normal plot the standardized effects in Figure 2 showed the effect values. The effects were not closed to the linear that were significant effect. Opposed to the effects were closed the linear or near zero that were not significant effects. The important factors that affected the droplet size of nano-cream were speed (B), temperature (C), and interaction between speed and temperature (BC) in the significant level 0.05.

Factorial plot from Figure.3 showed the main effects for nano-cream found at 25°C and speed 400 rpm by homogeneous dispersing could give the smallest droplet size.

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Table 3. Analysis of variance from Minitab program

Term	Effect	Coef	T	P
Constant		1107.1	77.34	0.000
Time	-138.3	-69.2	-4.83	0.000
Speed	-436.4	-218.2	-15.24	0.000
Temp.	611.5	305.8	21.36	0.000
Container	99.5	49.7	3.47	0.001
Probe	-76.7	-38.4	-2.68	0.012
Time*Speed	-156.0	-78.0	-5.45	0.000
Time*Temp.	94.6	47.3	3.30	0.002
Time*Cont.	59.5	29.8	2.08	0.046
Time*Probe	-18.0	-9.0	-0.63	0.534
Speed*Temp	288.4	144.2	10.07	0.000
Speed*Cont.	-93.6	-46.8	-3.27	0.003
Speed*Probe	-51.0	-25.5	-1.78	0.084
Temp.*Cont.	98.9	49.4	3.45	0.002
Temp.*Probe	132.8	66.4	4.64	0.000
Cont.*Probe	-89.5	-44.7	-3.13	0.004

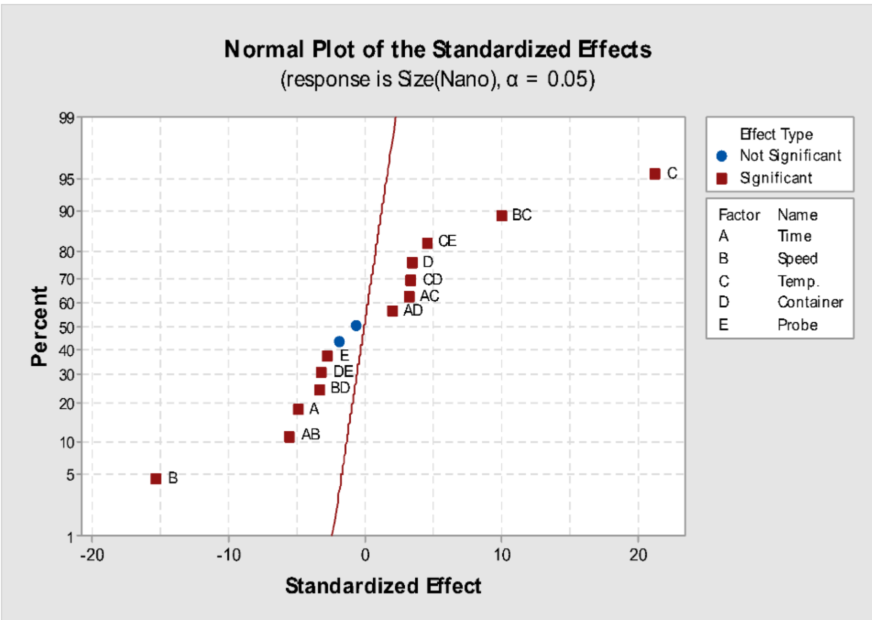

Fig. 2. Normal Plot of the Standardized Effects

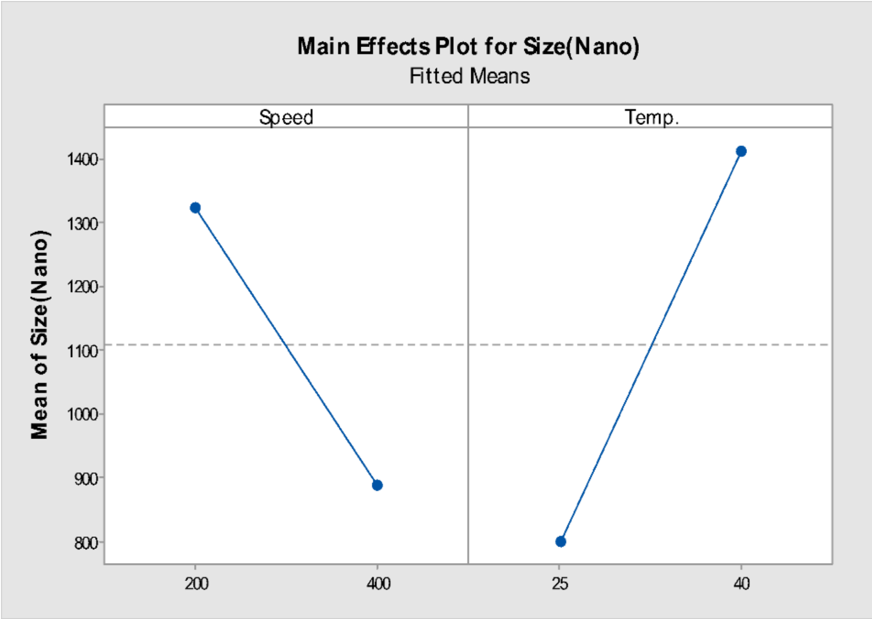

Fig. 3. Factorial Plot for study the main effects

3.3 Stability study

The stability of the nanoemulsion preparation was evaluated at 24 hours by centrifuge test at 3500 rpm, for 30 minutes. Stability was assessed by macroscopic emulsion observations. The nanoemulsions were not creaming, and have good stabilities. The selected nanoemulsion was prepared in triplicate and the samples were stored at low temperature (4±2°C), room temperature (25±2°C) and high temperature (40±2°C). Tests were performed at 24 hours, 7, 15 and 30 days after preparation. The analysis measurements were droplet size and pH value. Monitoring the pH value is important for determining the emulsion's stability because pH changes indicate the occurrence of chemical reactions that can compromise the quality of the final product. Emulsions produced with vegetable oils may experience a decrease in pH due to the hydrolysis of fatty acid esters into free fatty acid degrading products [9]. The nanoemulsions had stable average pH values in the range of 6-7 that were mild acid to neutral, and it is also appropriate on human skin. Measured the droplet size by Particle Analyzer before and after stability test has changed or not. However, found the temperatures at 4 and 25° C at various times the average particle size trended to the prior tests. However, upon a storage at 40 ° C, for 30 days, it was found that the average droplet size trended to slightly increase. This may lead to aggregation and deformation of nanoemulsion. The sample was not stable within 48 hours and then stored the samples at room temperature.

4. Conclusion

The ratio between squalene and virgin coconut oil of 0.5: 3.6 provided the smallest droplet size using homogeneous dispersing method which controlled the factors during the sample centrifuged found that 45 minutes, at 25 ° C, speed 400 rpm, volume size 250 ml) and position of probe in the middle of substances, could give the smallest droplet size.

Stability test found thermodynamically and kinetically stable system. Further studies are required to improve the quality of products for applications in industry to evaluate their irritating potential and moisturizing activity. The ability to penetrate the skin either in vitro (in vitro skin permeation studies) or in the skin of human volunteers (in vivo skin permeation studies) satisfaction survey of the real users will improve the formulations and in terms of marketing products.

Acknowledgements

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