Research Article

Development of Nanoemulsions Containing Essential Oils for Gel Formulations

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Abstract

The formulations of nanoemulsions containing lime-peel essential oils (LPO) and LPO-encapsulated nanoemulsion gels (nanoemulgels) for a cosmetic product were investigated. The IC_{50} value for LPO essential oils, which was determined using the 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) method to determine the antioxidant activity of the oils, is 21.7 ± 0.5 mg/ml. To facilitate the preparation of the nanoemulsion formula and ensure that the essential oils were in the form of oil-in-water (O/W) nanoemulsions, the most suitable surfactant, and cosurfactant utilized were Brij IC20 and glycerine, respectively. When LPO (concentration of 3% by weight) and the surfactant and cosurfactant (total concentration of 20% by weight) were mixed at a weight ratio of 3:1, the resulting LPO nanoemulsion contained fine particles with a uniform particle size distribution, low zeta potential, and good physical stability. All results exhibited that nanoemulsions stabilized by a mixture of Brij IC20 and glycerine are suitable as delivery vehicles for LPO. Nanoemulgel was also manufactured utilizing the nanoemulsion. The optimal gelling agent for the nanoemulgel was determined to be Carbopol 940 with a concentration of 0.3 % by weight. The nanoemulgel was physically stable with a pH of 5.3 (near the skin pH) and an adequate viscosity of 1290 cP.

Keywords: Co-surfactant, Essential oils, Hydrogels, Nanoemulgel, Nanoemulsions, Physical stability, Surfactant

1 Introduction

Nowadays, the popularity of herbal products has increased significantly because many consumers are interested in using natural extracts for health enhancement as well as natural medicine. As a result, extracts from essential oils derived from the extraction of different parts of a plant such as leaves, flowers, fruits, peels, or stems, are widely used. Lime-peel essential oil was reported to have high antimicrobial activity such as antifungal [1], and antibacterial activities [2], antioxidant activities and hypolipidaemic effects [3].

In particular, the consumption of cosmetics, herbal medicine products, and natural ingredients is likely to grow rapidly. However, the utilization of essential oils is limited due to their volatility and water insolubility. This is due to the fact that essential oils are hydrophobic and consist of a mixture of varying lipophilic aroma compounds including lipophilic terpenoids, phenylpropanoids and short-chain aliphatic hydrocarbon derivatives, making them immiscible with water [4].

Therefore, nanoencapsulation technology is often applied to increase the efficacy and maintain the quality of essential oils. Nanoemulsions represent one of the nanoencapsulation technologies commonly used for cosmetic and drug delivery systems. As a colloidal dispersion with kinetic stability, a nanoemulsion consists of oil, water, and surfactants. The typical droplet size of nanoemulsions is usually in the range of 20–200 nm. Currently, nanoemulsions have gained wide interest in pharmaceutical research as drug delivery systems. The interest stems from the ability of nanoemulsions to increase the apparent aqueous solubility and control the release of hydrophobic molecules, the reasonable production cost, and the ease of scale-up for production and high stability.

Nanoemulsions more or less require the input of external energy to transform the immiscible oil and water phases into colloidal dispersions with thermodynamic stability. Generally, the low-energy techniques to produce nanoemulsions include phase inversion temperature (PIT), phase inversion composition (PIC), and self-emulsifying methods. Alternatively, nanoemulsions can be prepared by high-energy emulsifying devices, including microfluidizers, high-pressure homogenizers, sonicators, and ultrasound generators. Generally, smaller nanoemulsion droplets are formed when the system undergoes a phase inversion in response to a change in either composition or temperature [5]. Naturally, the low-energy methods are preferable. Therefore, the aim of this study was to formulate and produce stable LPO nanoemulsions via the phase inversion method. This method was selected because it is an effective low-energy method to encapsulate sensitive compounds in cosmetic and pharmaceutical products [6]. It was also challenged to investigate the effect of the nature and type of non-ionic emulsifiers and cosurfactants on the emulsion properties of the LPO formulation. The physical (such as particle size, polydispersity index, turbidity, viscosity), morphological and antioxidant properties of the LPO nanoemulsions were further investigated. In addition, nanoemulsion technology can be applied together with nanoemulgel technology in order to reduce the degradation of essential oils and increase the efficiency of storage as well. The nanoemulgel is a system in which nanoemulsions are mixed with hydrogels, thereby leading to higher stability and more attractive appearance as cosmeceutical products.

2 Materials and Methods

2.1 *Chemicals*

Brij C20 (Ceteth-20), Brij IC20 (Isoceteth-20), Tween 20, Tween 60, and Tween 80 were purchased from Croda (Thailand). Brij CS25 (Ceteareth-25), Brij L23 (Laureth-23), Brij S100 (Steareth-100), Brij S2 (Steareth-2), and Brij S721 (Steareth-21) were gifts from Croda (Thailand). Cetomacrogol 1000 (Ceteareth-20), glycerine, propylene glycol, butylene glycol, Carbopol 940, Carbopol ultrez 20, Carbopol ETD2020 and Sodium CMC were purchased from S. Tong Chemicals (Thailand). Brij O10 (Oleth-10), Cremophor EL (Polyoxyl 35 Hydrogenated Castor Oi), tocopherol acetate, and 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (USA). Dehyton K (Cocamidopropyl betaine (30% wt/wt) in water) and Texapon N28 L (sodium laureth sulfate (28% wt/wt) in water) were supplied by BASF (Thailand). Emal AD-25 (ammonium lauryl sulfate (25% wt/wt) in water) was purchased from Honghuat (Thailand). Isoprene glycol was bought from Bonanzatech (Thailand). Softisan GC8 (glyceryl caprylate) and Imwitor GO 90 (glyceryl oleate) were gifts from IOI Oleochemical (Germany). Glyceryl monostearate, Sepimax zen, and Microcare PHC were purchased from Chemico Inter corporation (Thailand). Hyaluronic acid was purchased from Namsiang Group (Thailand). Absolute ethanol was supplied by Fisher Scientific (UK). Deionized (DI) water was used throughout this investigation.

2.2 *Antioxidant activity*

DPPH radical is reduced by antioxidant molecules, resulting in a change in color from a purple solution to a colorless solution. Therefore, the DPPH radical scavenging activities of LPO were evaluated by the measurement of the faded color using the method described by Prommaban *et al.* [7]. A serial dilution of lime-peel essential oils (LPO) with absolute ethanol was prepared. Tocopherol acetate was used as the standard to prepare a calibration curve. The ability to scavenge the stable DPPH free radicals was tested by mixing a portion of the sample solution with an equal volume of DPPH. The mixture was then allowed to react in an amber bottle for 30 min at room temperature. Reduction of the DPPH radicals was measured at 517 nm by a microplate reader (Synergy H1M, Biotek Instruments, Cambridge, USA). The concentration of the samples causing 50% inhibition

(IC_{50}) of DPPH radicals was compared with the standard tocopherol acetate. All determinations were carried out in triplicates.

2.3 *Formulation of nanoemulsions*

2.3.1 Selection of surfactants

The first step of this work was set up to select the suitable type of surfactants. Sixteen types of surfactants were investigated for nanoemulsion formulation. They comprised the three types of nonionic, amphoteric, and anionic surfactants. The investigated nonionic surfactants were Brij C20, Brij CS25, Brij IC20, Brij L23, Brij O10, Brij S100, Brij S2, Brij S721, Cetomacrogol 1000, Cremophor EL, Tween 20, Tween 60, and Tween 80. The amphoteric surfactant was Dehyton K. The anionic surfactants were Emal AD-25 and Texapon N28 L. Using a fixed surfactant concentration of 20% wt/wt, the LPO nanoemulsions were prepared by the phase inversion temperature (PIT) method. The water and surfactant-containing oil phases were heated up to 75 °C while continuously stirring at 600 rpm (Model MR Hei Standard, Heidolph, Germany) until the mixture cooled to 25 °C. When a clear nanoemulsion sample was obtained, the oil content was gradually increased stepwise by 1% wt/wt until the appearance of the sample turned cloudy. The boundary of each nanoemulsion existence was determined using triplicate samples to ensure an accuracy of less than ± 1% wt/wt for each of the tested surfactants.

2.3.2 Selection of cosurfactants

The investigation of the selection of surfactants revealed Brij IC20 to be the most suitable surfactant. Next, it was combined with one of the seven types of cosurfactants, namely, glycerine, propylene glycol, butylene glycol, isoprene glycol, Softisan GC8, Imwitor GO 90, and glyceryl monostearate. The employed fixed concentration of the surfactant-cosurfactant mixture (S_{mix}) and their weight ratio were 25% wt/wt and 3:1, respectively. As the previous study, nanoemulsions containing triacetin as oil with various ratios (S_{mix}) of cremphor EL to PEG 400 (surfactant and cosurfactant), the maximum nanoemulsion region was obtained with the S_{mix} 3:1 and the minimum was obtained with the S_{mix} 1:1 [8]. To illustrate the example of measurement for the preparation of nanoemulsion, nanoemulsion containing 2 %w/w LPO, 20 %w/w Brij IC20 : glycerine (weight ratio 3:1) and 78 %w/w water was weighed 2 g of LPO, 15 g of Brij IC20, 5 g of glycerine and 78 g of water to prepare 100 g of sample. The order of materials weighed was oil, surfactant/cosurfactant and water, respectively. The method of LPO nanoemulsion preparation and the selection of the most suitable cosurfactant was the same as the surfactant selection process.

2.3.3 Preparation of nanoemulsion by phase inversion composition method (PIC)

Based on the results of the surfactant and cosurfactant selection, Brij IC20 and glycerine at a fixed surfactant and cosurfactant mixture (S_{mix}) concentration of 20% wt/wt and weight ratio of 3:1, respectively, should constitute the oil phase of the LPO nanoemulsions. At first, the oil phase comprising LPO, Brij IC20, and glycerine was thoroughly mixed at 25 °C, 600 rpm (Model MR Hei Standard, Heidolph, Germany). After that, water was gradually added slowly while stirring until a homogeneous LPO nanoemulsion was obtained.

2.3.4 Characterization of nanoemulsions

- Droplet size analysis

The droplet size, polydispersity and zeta potential of nanoemulsions were measured simultaneously using a Zetasizer (Model Zetasizer Nano ZS, Malvern, United Kingdom) at 25 °C. All samples were stored in the concentrated form and then freshly diluted to surfactant and cosurfactant at the concentration of 1 %w/w immediately before the measurements in order to avoid the effect of multiple scattering, likely to occur at high surfactant concentrations.

- Ternary phase diagram

The phase inversion composition (PIC) method was used to prepare a ternary-phase diagram by varying the concentrations of LPO, surfactant, and cosurfactant. Samples were routinely visually inspected for stability immediately after preparation, after one day, one week, one month, and three months. By investigating only inside the region of nanoemulsions with a clear appearance, samples showing turbidity, gelation, or phase separation were not further studied.

- Phase inversion temperature and cloud point

The phase inversion temperature of the clear nanoemulsions and the cloud point of the corresponding micelle solution was determined by inserting a thermometer inside a sample bottle placed in a water bath on top of a hot plate. The samples were gradually heated slowly until they became turbid and thereafter left to cool to room temperature while constantly stirring. The temperatures at which the onset of turbidity (upon heating) and the appearance of clarity (upon cooling) occurred were recorded as the phase inversion temperature and the cloud point, respectively.

- Type of nanoemulsions

The o/w (oil in water) type of nanoemulsions was confirmed by diluting the nanoemulsions with water from a range of surfactant (s_{mix}) concentrations of 20% wt/wt down to 2% wt/wt. Then the diluted samples were kept at room temperature and inspected for stability immediately after dilution as well as after one day, one week, and one month [9]. If no phase separation occurred, then the o/w type was confirmed.

2.3.5 Stability of nanoemulsions

The samples of selected nanoemulsions were prepared and stored at 4 °C, 25 °C, and 40 °C, respectively, for one month, two months, and three months after preparation as well as alternating heating-cooling cycles (samples stored and collected at 4 °C alternating with 40 °C for 3 cycles). The analytical measurements were droplet size, polydispersity, and zeta potential.

2.4 *Formulation of nanoemulgels*

2.4.1 Preparation of hydrogels

Five types of gelling agents, each at two different concentrations, were investigated for nanoemulgel formulation, namely, Carbopol 940, Carbopol ultrez 20, Carbopol ETD2020, Sepimax zen, and Sodium CMC. The first four represented synthetic polymers while sodium CMC was a natural polymer. The hydrogels were prepared at 0.1 and 0.3% wt/wt concentration of gelling agents by dissolving the gelling agents in water using a high-speed homogenizer at 5,000 rpm (Model SilentCrusher M, Heidolph, Germany) and room temperature until complete dissolution. The pH was neutralized by the addition of triethanolamine (TEA). The formed hydrogels were kept for 24 h and observed for homogeneity, pH, transparency, viscosity, and spreadability. The compositions of the different formulations are listed in Table 1.

2.4.2 Preparation of nanoemulgels (NEG)

The above five types of gelling agents at 0.3 %wt/wt concentration were next used to prepare nanoemulgels. The nanoemulgels were prepared by mixing the investigated hydrogel with the selected nanoemulsions. The hydrogel was prepared by dissolving the gelling agent, preservative, moisturizer, humectant, and Tween 20 in water using a high-speed homogenizer at 5,000 rpm and room temperature until complete dissolution. The selected nanoemulsion was mixed with each hydrogel while constantly stirring to obtain the nanoemulgel, which was neutralized by the addition of triethanolamine (TEA). The compositions of the different formulations are listed in Table 2.

2.4.3 Characterization of nanoemulgels

Physical characteristics of nanoemulgels. The physical characteristics of the prepared nanoemulgels were checked at room temperature for color, odor, and phase separation. The viscosity of the prepared nanoemulgels was determined by using a Brookfield Viscometer (Model DV2T, AMETEK Brookfield, United States of America) with a spindle number of 62 at room temperature and the spindle speed was set at 20 rpm. The pH of the prepared nanoemulgels was determined by using a digital pH meter (Model SevenCompact S210-Uni, METTLER TOLEDO, Switzerland) at room temperature.

Table 1: Compositions of all 10 hydrogel formulations.

Formulation	**Concentration (%wt/wt)**					
	DI Water	**Carbopol 940**	**Carbopol Ultrez 20**	**Carbopol ETD2020**	**Sepimax Zen**	**Sodium CMC**
F1	99.9	0.1	-	-	-	-
F2	99.7	0.3	-	-	-	-
F3	99.9	-	0.1	-	-	-
F4	99.7	-	0.3	-	-	-
F5	99.9	-	-	0.1	-	-
F6	99.7	-	-	0.3	-	-
F7	99.9	-	-	-	0.1	-
F8	99.7	-	-	-	0.3	-
F9	99.9	-	-	-	-	0.1
F10	99.7	-	-	-	-	0.3

Table 2: The compositions of 5 nanoemulgel formulations.

Part	Materials	Concentration (%wt/wt) in				
		NEG1	NEG2	NEG3	NEG4	NEG5
Solid	Carbopol 940	0.3	-	-	-	-
	Carbopol ultrez 20	-	0.3	-	-	-
	Carbopol ETD2020	-	-	0.3	-	-
	Sepimax zen	-	-	-	0.3	-
	Sodium CMC	-	-	-	-	0.3
	Hyaruronic acid (Humectant)	0.2	0.2	0.2	0.2	0.2
Liquid	Selected nanoemulsion (20% Brij IC20: glycerine (3:1) 3% LPO, H_2O)	72.34	72.34	72.34	72.34	72.34
	DI water	24.86	24.86	24.86	24.86	24.86
	Butylene glycol (Moisturizer)	0.5	0.5	0.5	0.5	0.5
	Tween 20	1.2	1.2	1.2	1.2	1.2
	Microcare PHC (Preservative)	0.6	0.6	0.6	0.6	0.6
pH regulator	Triethanolamine	q.s.	q.s.	q.s.	q.s.	q.s.

2.4.3 Stability of nanoemulgels

Samples of the selected nanoemulgels were prepared and stored at 25 °C, 40 °C, and 4 °C, respectively, for two weeks after preparation as well as alternating heating-cooling cycles (samples collected at 40 °C alternating with 4 °C for 3 cycles). The analytical measurements were physical characteristics, pH and viscosity.

3 Results and Discussion

3.1 *Antioxidant activity*

The scavenging of oxidants causing cellular damage and accompanying aging was assessed by DPPH assays based on a single electron transferring mechanism to terminate the chain reaction of free radicals. The concentrations of both LPO and tocopherol acetate causing 50% inhibition (IC_{50}) of DPPH radicals are listed in Table 3. According to the results, the IC_{50} of essential oils from lime peel (LPO) exhibited a significantly lower IC_{50} than that of tocopherol acetate. This illustrates that lime-peel essential oils have promising antioxidant activity worth utilizing as an active ingredient in cosmetic products. It has been reported that the antioxidant activities of citrus essential oil were considerably high due to the high amounts of monoterpenes and oxygenated composition and may be distinguished depending on their chemical composition. The presence of limonene, a major constituent of the oils, as well as other monoterpene compounds including -terpineol and perillyl alcohol, may contribute to the free radical scavenging capabilities of citrus essential oils [10].

Table 3: Biological activities of lime-peel essential oils (LPO) and tocopherol acetate.

Samples	DPPH (IC_{50}, mg/mL)
LPO	21.7 ± 0.5
Tocopherol acetate	45.7 ± 0.7

3.2 *Formulation of nanoemulsions*

3.2.1 Selection of surfactants

A large number of surfactants may cause skin irritation. Therefore, the proper selection of surfactants is necessary. It is important to determine the required surfactant concentration properly and use a minimum concentration in the formulation [11]. Figure 1 shows the maximum amount of LPO that can be dissolved without causing turbidity or phase separation in the presence of different surfactants. It was found that both Tween 20 and Brij IC20 (20%wt/wt) could solubilize the maximum amount of LPO, i.e., 3%wt/wt, but the viscosity of the sample stabilized by Tween 20 was higher than the sample using Brij IC20. The higher viscosity might transform to gel in the stability study under various conditions of temperature after 3 months. Therefore, the proper selection of surfactant was Brij IC20. After the selection of Brij IC20 as the surfactant, Figure 2 shows the maximum amount of LPO that can be dissolved without causing turbidity by different amounts of Brij IC20. It was found that the sample using 25%wt/wt of Brij IC20 solubilized the maximum amount of LPO, i.e. 4% wt/wt. As the previous study by Malcolmson *et al.*, the areas of nanoemulsion existence exhibited that the three-component oil/surfactant/water systems alter oil-in-water nanoemulsion formation in a complex way depending on the relative size and structure of the surfactant hydrophobe, the number of attached ethylene oxide units and the nature of the oil phase to be incorporated [12].

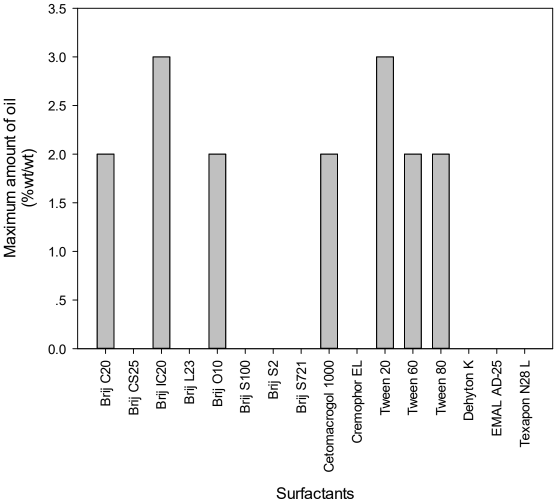

Figure 1: Upper limit of the amount of lime-peel oils (LPO) (%wt/wt) incorporated into oil-in-water nanoemulsions stabilized by 20% of each surfactant which remained stable for 1 month.

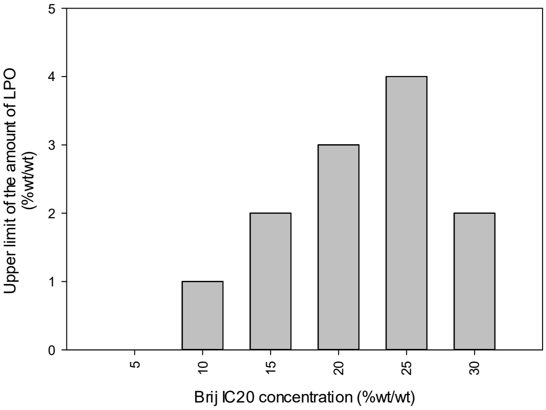

Figure 2: Upper limit of the amount of lime peel oils (LPO) (%wt/wt) incorporated into nonionic nanoemulsions containing different concentrations of Brij IC20 surfactant at room temperature.

3.2.2 Selection of cosurfactants

Typical cosurfactants are n-alcohols and alkyl carboxylic acids. Cosurfactants are miscible with surfactants and form mixed micelles. Because their head groups are small, they also increase the surface activity and decrease the interfacial tension of the micelles [11]. The investigations clearly distinguished the ability of various cosurfactants to improve the nanoemulsification of the selected surfactant, Brij IC20. Figure 3 shows the maximum amount of LPO that can be incorporated in the nanoemulsions in the presence of Brij IC20 surfactant and a different cosurfactant without causing turbidity. It was found that the sample using the glycerine as cosurfactant solubilized the maximum amount of oil, i.e., 5 %wt/wt. Based on the result, glycerine was selected as the most suitable cosurfactant for the nanoemulsion formulation.

After the selection of Brij IC20 and glycerine as the surfactant and cosurfactant, respectively. Figure 4 shows the maximum amount of oil that can be dissolved without causing turbidity at different concentrations of Brij IC20-glycerine at a weight ratio of 3:1 and room temperature. It was found that samples using 25 wt/wt of Brij IC20-glycerine concentration solubilized the maximum amount of LPO, i.e., 4% wt/wt. However, the high amount of surfactant-cosurfactant may cause skin irritation, therefore, it was decided that the proper Brij IC20-glycerine concentration should be 20% wt/wt, which solubilized 3% wt/wt of LPO.

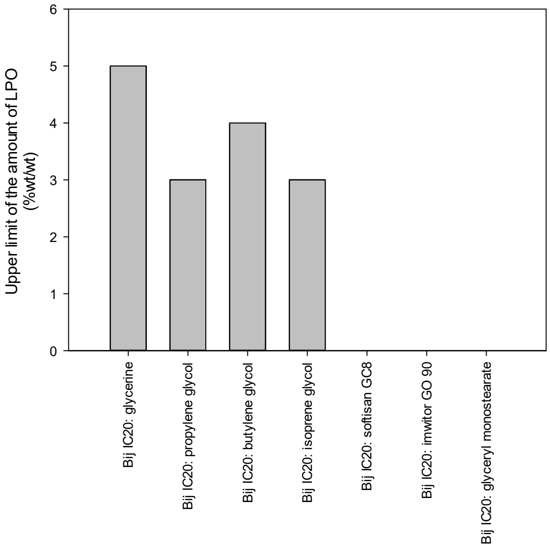

Figure 3: Upper limit of the amount of lime peel oils (LPO) (%wt/wt) incorporated into nonionic nanoemulsions containing 25% wt/wt of the mixture of surfactant and a different cosurfactant with a weight ratio of 3:1 at room temperature.

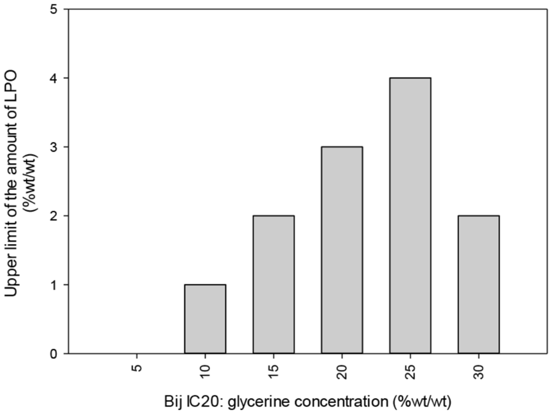

Figure 4: Maximum amount of lime-peel oils (LPO) (% wt/wt) incorporated into nonionic nanoemulsions containing different Brij IC20-glycerine concentrations at a weight ratio of 3:1.

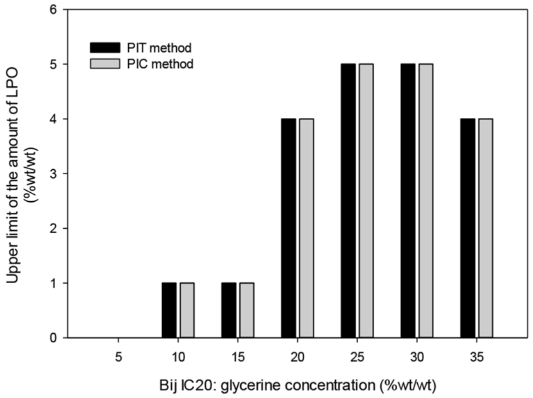

Figure 5: Maximum amount of lime-peel oils (LPO) (% wt/wt) incorporated into nonionic nanoemulsions containing different Brij IC20-glycerine concentrations at a weight ratio of 3:1 and prepared by two different methods.

3.2.3 Effect of preparation method on nanoemulsion formation

The objective here was to compare and choose between the Phase Inversion Composition (PIC) and Phase Inversion Temperature (PIT) methods. The PIC method involves the gradual progressive addition of one of the components either water or oil to a mixture of the other two components, namely, either oil/surfactant or water/surfactant, respectively [13]. The PIT method involves the heating of the water and surfactant-containing oil phases up to 75 °C before cooling the mixture to 25 °C. Therefore, the PIC method is advantageous over the PIT method when handling components with temperature-stability problems, or systems containing surfactants that are not of the polyoxyethylene type [14]. Based on the results of previous experiments, Brij IC20 was used as a surfactant and glycerine as a cosurfactant. They were mixed (S_{mix}) in the proportion of 3:1. Figure 5 shows that all of the samples prepared by the PIC method could dissolve LPO to a maximum equal to the PIT method. For this reason, the PIC method was selected for the preparation of nanoemulsions for further study.

3.2.4 Characterization of nanoemulsions

Based on the results of previous experiments, various samples of nanoemulsions were prepared by the PIC method using Brij IC20 and glycerine as surfactant and cosurfactant, respectively, with S_{mix} in proportion 3:1 and at 20% wt/wt concentration.

- Droplet size analysis

Table 4 shows that the particle sizes of the micelle and nanoemulsion samples at LPO concentrations of 0, 1, 2, and 4 percent by weight were 11.8 ± 0.5, 11.5 ± 0.8, 12.9 ± 1.3, and 14.9 ± 1.3 nm, respectively. Note that, when the oil concentration increased, the particle size of nanoemulsions tended to increase as well with sizes much smaller than 50 nm, and the resulting nanoemulsions were transparent [15]–[17]. This result is in agreement with the previous study that the addition of the smaller molecular volume oils (namely ethyl butyrate, ethyl caprylate, and tributyrin) into the Brij O10 micelles caused relatively small changes in size [18]. In theory, the polydispersity index can vary from 0–1, which indicates the degree of uniformity of the particle size distribution. When close to 0, it indicates an essentially uniform particle size distribution [19].

The corresponding zeta potentials of the micelle and nanoemulsion formulations were found to be 0.6 ± 0.6, 0.0 ± 0.5, 0.2 ± 0.2, and 0.2 ± 0.9 mV, respectively. Generally, the zeta potential of micelle and nanoemulsion prepared by a non-charged surfactant is very low but a breakup of the structures can create higher zeta potentials. Therefore, it can be concluded that all nanoemulsions do not have any structural breakup and remain stable [5].

- Ternary phase diagram

The region of nanoemulsion existence was determined by varying the concentration of either a single surfactant or a mixture of surfactant and cosurfactant, and then plotting the results, on a weight basis, on a partial ternary phase diagram in order to understand the phase behavior of the nanoemulsion formulations.

Table 4: Physicochemical characteristics of nanoemulsions that were analyzed right after preparation.

Sample	**Particle Size (nm)**	**Polydispersity Index (PdI)**	**Zeta Potential (mV)**
20% Brij IC20 : glycerine (3:1) 0% LPO, H_2O (2% dilution)	11.8 ± 0.5	0.3 ± 0.1	0.6 ± 0.6
20% Brij IC20 : glycerine (3:1) 1% LPO, H_2O (2% dilution)	11.5 ± 0.8	0.2 ± 0.0	0.0 ± 0.5
20% Brij IC20 : glycerine (3:1) 2% LPO, H_2O (2% dilution)	12.9 ± 1.3	0.2 ± 0.1	0.2 ± 0.2
20% Brij IC20 : glycerine (3:1) 4% LPO, H_2O (2% dilution)	14.9 ± 1.3	0.1 ± 0.1	0.2 ± 0.9

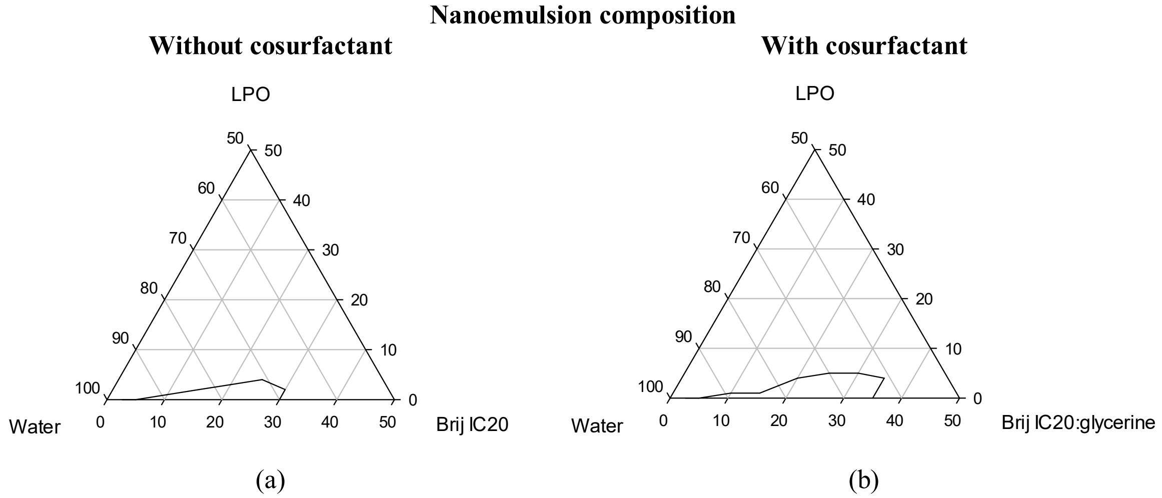

Figure 6: Partial ternary phase diagrams for the LPO-in-water nanoemulsions prepared using either (a) a single surfactant (Brij IC20) or (b) a mixture of surfactant and cosurfactant (Brij IC20 and glycerine) in aqueous solution after 1-month storage at room temperature.

Based on the results of Figures 2 and 4, it is found as shown in Table 4 that the use of only Brij IC20 as surfactant yielded a smaller region of nanoemulsion existence than the case of adding glycerine as cosurfactant. This indicates that the addition of cosurfactant could help to increase the storage capacity of nanoemulsions [20], [21] as seen from the enlarged region of nanoemulsions. In addition, the partial ternary phase diagrams shown in Figure 6 confirmed that the prepared nanoemulsions were oil-in-water nanoemulsions [9].

- Phase inversion temperature and cloud point

Table 5 shows that the cloud point of the surfactant solution (without oil) changing from clear to turbid was 78.8 ± 1.2 °C. Similarly, the phase inversion temperatures causing the samples of nanoemulsions at the LPO concentration of 1, 2, and 4 percent by weight to change from clear to turbid were 64.8 ± 6.4, 28.0 ± 5.0, and 30.4 ± 4.9 °C, respectively, which gave insight into the temperature that will cause phase separation of the nanoemulsion. It also indicates the appropriate operating temperature. This suggests that the temperature for the use or storage of these nanoemulsions should not exceed the phase inversion temperature that causes phase separation upon cooling because it may cause the nanoemulsion to return to two phases and result in turbidity [5]. Therefore, the nanoemulsion samples at every oil concentration should be used or stored at temperatures below 28 °C.

Table 5: Phase inversion temperature of nanoemulsions and cloud point of the surfactant solution.

Oil Concentration (%wt/wt)	**Phase Inversion Temperature or Cloud Point (°C)**
0	78.8 ± 1.2
1	64.8 ± 6.4
2	28.0 ± 5.0
4	30.4 ± 4.9

- Type of nanoemulsions

The type of nanoemulsions was investigated experimentally by the dilution method using the samples of nanoemulsions prepared by the phase inversion composition (PIC) method. At the total concentrations of surfactants and cosurfactants in a three-to-one ratio equalling 10, 15, 20, 25, 30 and 35 percent by weight, it was found that all nanoemulsions were well dispersed in water. After collecting and storing the diluted samples for 1 day, 1 week, and 1 month, respectively, all samples did not have phase separation. Therefore, it can be concluded that all prepared nanoemulsions were of the oil-in-water (O/W) type

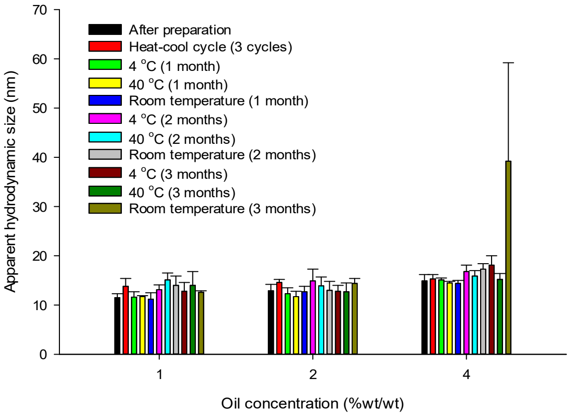

Figure 7: The variation in the apparent hydrodynamic size of micelles and nanoemulsions containing 20 %w/w Brij IC 20:glycerine (3:1) with different lime-peel oil (LPO) contents, temperatures, and durations of the stability test.

6.2.5 *Stability of nanoemulsions*

Table 6 shows the storage of the nanoemulsion samples in 4 conditions:

- Room temperature (stored for 1 month, 2 months and 3 months)
- Heat-cool cycle (alternating between 4°C and 40 °C for 3 cycles)
- 4 °C (stored for 1 month, 2 months, and 3 months)
- 40 °C (stored for 1 month, 2 months, and 3 months)

It was found that the particle size of the nanoemulsion samples at the oil concentration of 1 and 2 percent by weight showed only a slight increase in particle sizes, thus indicating that all of the samples were physically stable (Figure 7). However, in the case of nanoemulsion samples with an oil concentration of 4 percent by weight, it was found that at room temperature (25 °C), there was a tendency for the size of the particles to increase significantly after 3 months, resulting in slightly turbid nanoemulsions samples [9]. Therefore, it can be concluded that the nanoemulsions with an LPO concentration equal to 3 percent by weight (at the total concentration of surfactant and cosurfactant equal to 20 percent by weight) can avoid the loss of physical stability of the nanoemulsions.

Table 6: The stability and particle size of nanoemulsions

Sample	Measurement	Particle Size (nm)
20% Brij IC20: glycerine (3:1) 1% LPO, H_2O (2% dilution)	Right after preparation	11.5 ± 0.8
	Heat-cool cycle (3 cycles)	13.8 ± 1.6
	4 °C (1 month)	11.6 ± 1.1
	40 °C (1 month)	11.7 ± 0.2
	Room temperature (1 month)	11.2 ± 1.3
	4 °C (2 months)	13.1 ± 1.0
	40 °C (2 months)	15.1 ± 1.4
	Room temperature (2 months)	14.0 ± 1.9
	4 °C (3 months)	12.8 ± 1.8
	40 °C (3 months)	14.0 ± 2.8
	Room temperature (3 months)	12.6 ± 0.3
20% Brij IC20: glycerine (3:1) 2% LPO, H_2O (2% dilution)	Right after preparation	12.9 ± 1.3
	Heat-cool cycle (3 cycles)	14.6 ± 0.6
	4 °C (1 month)	12.3 ± 1.2
	40 °C (1 month)	11.7 ± 1.1
	Room temperature (1 month)	12.7 ± 1.1
	4 °C (2 months)	14.9 ± 2.4
	40 °C (2 months)	13.9 ± 1.8
	Room temperature (2 months)	13.0 ± 1.8
	4 °C (3 months)	12.8 ± 1.2
	40 °C (3 months)	12.7 ± 1.8
	Room temperature (3 months)	14.4 ± 1.0

Table 6: *(Continued)*

Sample	Measurement	Particle Size (nm)
20% Brij IC20: glycerine (3:1) 4% LPO, H_2O (2% dilution)	Right after preparation	14.9 ± 1.3
	Heat-cool cycle (3 cycles)	15.3 ± 0.9
	4 °C (1 month)	15.1 ± 0.4
	40 °C (1 month)	14.5 ± 0.3
	Room temperature (1 month)	14.4 ± 0.6
	4 °C (2 months)	16.8 ± 1.3
	40 °C (2 months)	15.9 ± 1.1
	Room temperature (2 months)	17.3 ± 1.1
	4 °C (3 months)	18.1 ± 1.9
	40 °C (3 months)	15.2 ± 1.2
	Room temperature (3 months)	39.2 ± 20.0

3.3 *Formulation of nanoemulgels*

3.3.1 Selection of gelling agent and optimization of its concentration

Table 7 reveals that the suitable type and amount of gelling agents in hydrogel preparation were Carbopol 940, Carbopol ultrez 20, Carbopol ETD2020, Sepimax zen, and Sodium CMC, all with a concentration of the gelling agent equaling 0.3 percent by weight. The mentioned gels were satisfactory with an appropriate viscosity, and a pH similar to the human skin, therefore posing no harm to the skin. As for the lower concentration of the gelling agents, the viscosity became too low and unsuitable for use in preparing hydrogels. Table 8 shows nanoemulgels prepared with Carbopol 940, Carbopol ultrez 20, Carbopol ETD2020, Sepimax zen, and Sodium CMC, respectively, at the concentration of 0.3. percent by weight. It reveals that the suitable gelling agents in the preparation of nanoemulgel were Carbopol 940 (NEG1) and Carbopol ultrez 20 (NEG2) because the gels had a good appearance with an appropriate viscosity and a pH similar to that of the skin.

Table 7: Physical characteristics of hydrogels with different gelling agents at 2 concentrations.

Sample	Gelling Agent	Amount of Gelling Agent (%wt/wt)	Appearance	pH	Transparency	Viscosity	Texture
F1	Carbopol 940	0.1	Homogeneous	5.3	Clear	Low	Slightly viscous
F2	Carbopol 940	0.3	Homogeneous	5.2	Clear	High	Good
F3	Carbopol ultrez 20	0.1	Homogeneous	5.5	Clear	Low	Slightly viscous
F4	Carbopol ultrez 20	0.3	Homogeneous	5.1	Clear	High	Good
F5	Carbopol ETD2020	0.1	Homogeneous	5.3	Clear	Low	Slightly viscous
F6	Carbopol ETD2020	0.3	Homogeneous	5.3	Clear	High	Good
F7	Sepimax zen	0.1	Homogeneous	5.4	Clear	Low	Slightly viscous
F8	Sepimax zen	0.3	Homogeneous	5.3	Clear	moderate	Medium viscosity
F9	Sodium CMC	0.1	Homogeneous	5.5	Clear	Low	Fluid
F10	Sodium CMC	0.3	Homogeneous	5.5	Clear	moderate	Medium viscosity

Table 8: Physical characteristics of nanoemulgel formulations with different gelling agents.

Sample	Gelling Agent	Amount of Gelling Agent (%wt/wt)	Appearance	pH	Transparency	Viscosity	Texture
NEG1	Carbopol 940	0.3	Homogeneous	5.3	Slightly opaque	High	Good
NEG2	Carbopol ultrez 20	0.3	Homogeneous	5.4	Clear	High	Good
NEG3	Carbopol ETD2020	0.3	Homogeneous	5.2	Clear	High	Medium viscosity
NEG4	Sepimax zen	0.3	Homogeneous	5.4	Clear	Moderate	Fluid
NEG5	Sodium CMC	0.3	Homogeneous	5.3	Clear	Low	Fluid

Table 9: Physical characteristics of nanoemulgels, NEG1 and NEG2.

Sample	Physical Stability			pH	Viscosity (cP)
	Phase Separation	Color	Odor		
NEG1	None	None	None	5.3	1296
NEG2	None	None	None	5.4	1296

Table 10: Stability and characteristics of nanoemulgels, NEG1 and NEG2.

Sample	Measurement	Physical Stability			pH	Viscosity (cP)
		Phase Separation	Color	Odor		
NEG1	Right after preparation	None	None	None	5.3	1296
	Heat-cool cycle (3 cycles)	None	None	None	5.2	1170
	4 °C (2 week)	None	None	None	5.3	1370
	40 °C (2 week)	None	None	None	5.1	798
	Room temperature (2 week)	None	None	None	5.2	1088
NEG2	Right after preparation	None	None	None	5.4	1296
	Heat-cool cycle (3 cycles)	None	None	None	5.4	1074
	4 °C (2 week)	None	None	None	5.5	1241
	40 °C (2 week)	None	None	None	5.3	840
	Room temperature (2 week)	None	None	None	5.4	1044

3.3.2 Characterization of nanoemulgels

Nanoemulgel samples were prepared using Carbopol 940 (NEG1) and Carbopol ultrez 20 (NEG2) gelling agents, respectively, at 0.3% by weight (Table 9). Obviously, both samples were physically stable and had the same viscosity, while NEG1 and NEG2 had pH values of 5.3 and 5.4, respectively.

3.3.3 Stability of nanoemulgels

Table 10 compares the physical stability and characteristics of the nanoemulgel samples NEG1 and NEG2 right after preparation and storage in various conditions, such as alternating high and low temperatures for 3cycles and under constant-temperature storage at 4, 40 and 25 °C for a period of 2 weeks. All samples were found without phase separation, discoloration, and foreign odors. Therefore, it can be concluded that the various storage temperature conditions did not affect the physical stability of the samples NEG1 and NEG2.

It was observed that the NEG1 samples (using the Carbopol 940 for sample preparation) at alternating high and low temperatures, constant 40, and 25 °C tended to show a decrease in the pH. Similarly, the NEG2 samples (using the Carbopol ultrez 20 for sample preparation) at a constant temperature of 40 °C tended to reduce the pH but tended to increase the pH at a constant 4 °C.

In terms of the viscosity, it was observed that the NEG1 samples at alternating high and low temperatures, constant 40 and 25 °C showed a tendency to reduce the viscosity compared to that obtained right after sample preparation. The viscosity of NEG1 samples dramatically decreased in the case of constant 40 °C. However, the viscosity of the NEG1 samples tended to increase in the case of constant 4 °C. As for NEG2 samples, it was found that, at alternating high and low temperatures, constant 40 and 25 °C, the viscosity of the samples tended to decrease compared to that obtained right after sample preparation. In conclusion, the NEG1 samples had an increased viscosity after storage at low temperatures and a reduced viscosity after storage at high temperatures. In contrast, NEG2 samples had a lower viscosity after storage at all temperature conditions.

4 Conclusions

Judging from the above experimental results, it may be concluded that the addition of the cosurfactant, glycerine, to Brij IC20 NE led to an increase in the area of NE existence prepared by phase inversion composition with low energy. O/W nanoemulsions prepared using Brij IC20:Glycerine (S_{mix} 3:1) and LPO contained fine particles with a uniform particle size distribution, low zeta potential with good physical stability. Moreover, nanoemulgel NEG1 (containing 3% wt/wt of LPO and total 20% wt/wt of Brij IC20 and glycerine at 3:1 S_{mix} ratio and 0.3% wt/wt of Carbopol 940) is the optimum formulation because it displayed excellent physical stability, and had an appropriate viscosity of about 1296 cP and a pH in the range of 5.3–5.5, which is similar to the skin (pH 5.3).

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Author Contributions

N.S., P.N. and O.L.: conceived and planned the experiments and made final evaluations of the results; N.S., P.D., P.B. and P.N.: conducted the experiments and wrote an original draft; O.L. and W.T.: supervising, reviewing, editing, funding acquisition and project administration. All authors have read and agreed as regards the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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