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Formulation and stability of whitening VCO-in-water nano-cream

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ABSTRACT

Virgin coconut oil (VCO)-in-water, nano-emulsion in the form of cream stabilized by Emulium Kappa® as an emulsifier, was prepared by using the Emulsion Inversion Point method. A nano-emulsion with droplet size <300 nm was then obtained. VCO has recently become a more popular new material in the cosmetic industries. Emulium Kappa® is an ionic emulsifier that contains sodium stearoyl lactylate, the active whitening ingredient was Kojic Dipalmitate. Ostwald ripening is the main destabilizing factor for the nano-emulsion. This decline can be reduced by adding non-soluble oil, namely squalene, to the virgin coconut oil. We tested VCO:squalene in the ratios of 10:0, 9.8:0.2, 9.6:0.4, 9.4:0.6, 9.2:0.8, 9:1 and 8:2 and discovered that squalene's higher molecular weight (above critical molecular weight) resulted in low polarity and insolubility in the continuous phase. The continuous partitioning between the droplets results in the decline of Ostwald ripening. Furthermore, flocculation may occur due to the instability of nano-emulsion, especially for the preparations with little or no squalene at all. The stability of the nano-emulsion was evaluated by the electrophoretic properties of the emulsion droplets. The zeta potential values for the emulsion increased as the percentage of squalene oil increased.

1. Introduction

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 (Porras et al., 2004; Usn et al., 2004), formed by the dispersion of one liquid phase into the second liquid phase to form a droplet (Fernandez et al., 2004; Solans et al., 2005; Maestro et al., 2006; Anton et al., 2007). The interest on studies of this type of emulsion began in the early 19th century, but it exploded recently due to cosmetic and pharmaceutical applications of novel systems generating nano-particles (Anton et al., 2007). Nano-emulsions also are known as miniemulsions (Fernandez et al., 2004; Tadros et al., 2004; Solans et al., 2005; Liu et al., 2006; Maestro et al., 2006; Anton et al., 2007), fine disperse emulsions (Liu et al., 2006), submicron emulsions (Fernandez et al., 2004; Solans et al., 2005; Liu et al., 2006; Maestro et al., 2006), ultrafine emulsions (Fernandez et al., 2004; Solans et al., 2005; Porras et al., 2008), translucent emulsions (Fernandez et al., 2004; Ee et al., 2008), emulsoides (Maestro et al., 2006), and unstable microemulsions (Maestro et al., 2006). Nano-emulsions have a long term kinetic stability due to their very small droplet sizes (Tadros et al., 2004; Solans et al., 2005; Liu et al., 2006; Maestro et al., 2006) which result in a large reduction in the gravitational force. Thus, Brownian motion suffices to overcome gravity (Betz et al., 2005; Solans et al., 2005; Maestro et al., 2006) and prevent sedimentation and creaming during storage. Nano-emulsions are attractive systems for many industrial applications (Wang et al., 2007; Gutierrez et al., 2008) due to their purity, simplicity (Sonneville-Aubrun et al., 2004), the ability to sterilize them through filtration, and the increased bioavailability of drugs solubilized in them (Wang et al., 2007; Kotyla et al., 2008). These properties make them efficient trans-dermal delivery systems for the active ingredients or fragrances incorporated in many personal care products (Tadros et al., 2004). Nano-emulsions can be prepared by using a high-energy dispersion method and condensation, or by a low-energy method (Fernandez et al., 2004; Solans et al., 2005; Liu et al., 2006; Maestro et al., 2006; Anton et al., 2007; Wang et al., 2007; Ee et al., 2008). The dispersion, or high-energy method, requires a large input of mechanical energy (Fernandez et al., 2004; Solans et al., 2005; Anton et al., 2007; Wang et al., 2007) during the process of emulsification and condensation. The condensation, or low-energy method, takes advantage of the physico-chemical properties of the system in which changes in curvature and phase transition occur during the emulsification process (Tadros et al., 2004; Solans et al., 2005; Liu et al., 2006; Maestro et al., 2006; Anton et al., 2007; Wang et al., 2007). Changes in the spontaneous curvature of the surfactant during emulsification are key factors in the formulation of a nano-emulsion (Fernandez et al., 2004; Liu et al., 2006). Three low-energy methods may be used in order to formulate a nano-emulsion: (i) Phase Inversion Temperature (PIT) method (Solans et al., 2005; Wang et al., 2007). (ii) The Emulsion Inversion Point (EIP) method (Maestro et al., 2006) in which the degree of

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ionization of the surfactant changes the curvature transition and is altered by changing the relative amount of water or oil (Fernandez et al., 2004; Tadros et al., 2004; Liu et al., 2006; Wang et al., 2007). (iii) Self emulsification (Bouchemal et al., 2004).

Although, nano-emulsions are considered as stable systems, there are still two major sources of instability: one is the nature of the oil phase and the other is the presence of polymers that thicken or cause the nano-emulsion to be converted into a gel (Capek, 2004; Liu et al., 2006). Ostwald ripening or molecular diffusion is probably the most serious instability problem (Tadros et al., 2004; Solans et al., 2005; Anton et al., 2007), and is governed by inter-droplet oil diffusion and by differences in the solubility of small and large droplets. Ostwald ripening theory suggests that stable systems will result if a second insoluble or very poorly soluble oil phase (e.g. squalene oil) is added to the system (Tadros et al., 2004). This addition causes significant partitioning between different droplets and results in an equilibrium due to the differences in the droplet sizes and chemical resources. Besides, an increased surfactant content at the interface also reduces Laplace pressure (Fernandez et al., 2004).

In 1906, squalene was discovered from a shark liver extract (Senthilkumar et al., 2006). Squalene is unsaturated hydrocarbon intermediate of cholesterol metabolism. It is an isoprenoid compound with six isoprene units and has chemical formula which is $C_{30}H_{50}$ (Bhattacharjee and Singhal, 2003; Senthilkumar et al., 2006). Another source of squalene is olive oil (Bondioli et al., 1993; Nenadis and Tsimidou, 2002) and human beings. In human beings, squalene may protect the skin from ultraviolet radiation and against several carcinogens (Senthilkumar et al., 2006).

This research has started from the condensation method with an ionic system. The objective of this research is to discover the formulation of nano-emulsion in the form of cream containing a whitening active ingredient and then to evaluate its stability. The hypothesis of this research is that the phase inversion method yield emulsion droplets that are in nanometer size that provides a possibility to obtain a stable cream with squalene. The significance of this research is to establish that a solid emulsifier with Emulsion Inversion Point method has been used to prepare nano-cream. In addition, this paper aims to make the emulsion as part of the electrical circuit through a modified system.

2. Materials and methods

2.1. Materials

Emulium Kappa® (EK), consisting of (candelilla/jojoba/rice bran polyglyceryl-3-esters, glyceryl stearate, stearoyl alcohol, and sodium stearoyl lactylate) was purchased from Gattfossé, France. EK is a solid emulsifier. Propylene glycol (PG) was purchased from Sigma. After combining with propylene glycol at the ratio of 9:1, EK has a Hydrophylic-Lipophilic Balance (HLB) value of 10.35 which produces o/w emulsion (Mollet and Grubenmann, 2001a; Block, 1996; Eccleston, 2006). However; the HLB value represents surfactant/cosurfactant molecules alone without taking into account its interaction with oil and water in the overall emulsion system (Izquierdo et al., 2005). Virgin coconut oil (VCO) was purchased from Adirondack Co., Ltd (Selangor, Malaysia) and was filtered through 0.45 μm methyl cellulose acetate filter paper before use. Squalene oil was purchased from Fluka. Kojic dipalmitate (KDP) (the whitening agent) was purchased from Beijing Brilliance Biochemical Co., Ltd. (Hou Modern, China). In all experiments, the emulsifier and oil fractions were kept consistent so that they were the only variables directly involved in the emulsion phase inversion and the ternary system: aqueous phase, surfactant, and oil.

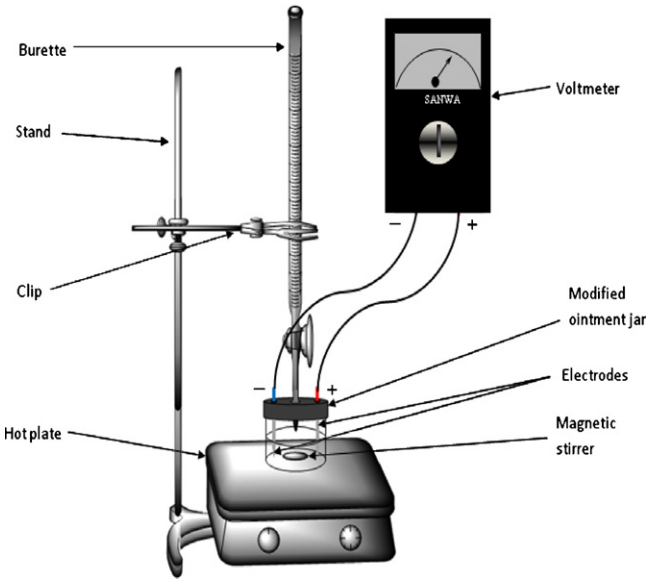

Fig. 1. System used to prepare nano-emulsion by condensation method.

2.2. Method

2.2.1. Nano-emulsion preparation

Our work was based on the formulation of Liu et al. (2006). Emulsions were prepared from a mixture of VCO and surfactant by slowly adding water and mixing it by using a magnetic whisk. Two electrodes of a volt meter (SANWA, Tokyo, Japan) were placed inside the emulsion to determine the point of the inversion (Fig. 1). Water was added at 1.0 mL min^{-1} by using a 50 mL burette. The temperature was kept consistent at $65 \pm 2^{\circ}$. The fraction of the emulsifier and the oil phase were also kept consistent 14% (w/w):12.8% (w/w). The oil phase is a mixture of two oils (VCO and Squalene) with different ratios. The VCO to Squalene ratios used were 10:0, 9.8:0.2, 9.6:0.4, 9.4:0.6, 9.2:0.8, 9:1 and 8:2 (Block, 1996; Mollet and Grubenmann, 2001a; Leal-Calderon, 2007). The HLB value of the system was 10.35.

We designed a new system in order to prepare nano-emulsions. The emulsion is part of an electrical circuit. Two holes were made in the cover of an ointment jar which could resist temperatures up to 70 °C. Electrode bars that reached the bottom of the ointment jar were installed in these holes, and then sealed with silicon. In the middle of the cover, a third hole was made to allow the entry of water droplets from the burette. In this experiment, water evaporation and loss had to be controlled carefully due to higher operating temperature (up to 70 °C). The higher temperature was needed to solubilize the emulsifier and the kojic dipalmitate. The volume of water is the key factor in the transition step (between w/o and o/w), so the amount of water lost through evaporation should be minimized.

2.2.2. Determination of droplet size

Oil droplet size (as dynamic laser light scattering) and its distribution was measured using ZetaSizer apparatus (Malvern, UK). The Helium–neon laser, 4 mW, was operated at 633 nm, with scatter angle fixed at 173°, and at a temperature of 25 °C. The Poly Dispersity Index (PDI) was discovered to be the relative error between curve fit and experimental values. A PDI < 0.1 was considered to be a good quality, while the values of 1 were considered to be poor quality samples. All of the measurements were performed under standard conditions of dilution and temperature. All the measurements were performed in triplicate.

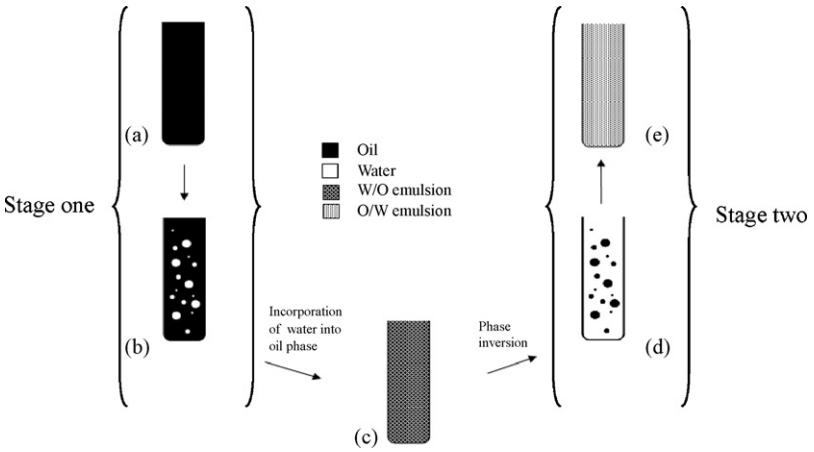

Fig. 2. The stages of nano-emulsion formation of EK/PG/VCO/squalene system by Emulsion Inversion Point method.

2.2.3. Electrophoretic properties

The difference in the potential between the surface of the tightly bounded layer and the electroneutral layer of the solution is the Zeta-potential (Mollet and Grubenmann, 2001a,b; Kim, 2004; Minko, 2006). This value has practical application in determining the stability patterns and it reflects the degree of repulsion between the adjacent, similarly charged, and dispersed particles. If the zeta potential is less than a critical value, which varies in the system, then the repulsive forces are less than the attractive forces and the particles assemble together. This phenomenon is known as flocculation (Minko, 2006). The droplet zeta potential was measured by using Zeta Potential Analyzer (ZEECOM, TOUOZUMI, Japan) and zeecom Ver2.07m as the software. The migration voltage was 80 V and the emulsion temperature was 25 °C. The dimension of the cell used in the measurement was 0.75 mm in thickness and 10 mm in width. The inter-electrode gap was 9 mm. All the measurements were performed in triplicate.

3. Results

3.1. Pre-experimental condition

Each emulsion was prepared by using the same method and condition. Emulium Kappa®–Propylene glycol with kojic dipalmitate was dissolved in the VCO–Squalene mixture at 65 °C by stirring it with a magnetic whisk at 250 rpm for 10 min. Water could be added only after solubilization of the mixture in order to form a clear transparent solution.

3.2. Nano-emulsion

During the addition of water, the emulsion passed through two stages. The first stage include the incorporation of water-in-oil (w/o). During this stage, the emulsion become irregular in appearance with a large fraction that could be observed by eye. Conductivity during the first stage fluctuated at the beginning since the water fraction could conduct electricity; but after 2–3 min of the addition of the water, the conductivity was zero because the water had completely incorporated into the oil phase and the emulsion appeared homogeneous. The second stage of emulsification was the incorporation of oil-in-water (inversion of w/o to o/w). This stage also began with the presence of a large, distinct water fraction and an apparently non-homogeneous emulsion. At the beginning of stage two, i.e. the point of transition, the conductivity change from 0 to 100%. The emulsion was homogenized by mixing it with a magnetic whisk until it turned completely homogeneous (Fig. 2). After homogenization, the emulsion was cooled to room temperature. The resulting emulsion was viscous (viscous cream) which was due to the proportion of surfactant and the lower proportion of water used. After cooling to room temperature, sufficient water was added to make it 100% (w/w) and the water and emulsion were mixed by using a hand spatula until homogenization process was complete.

As noted above, the conditions under which Emulium Kappa was dissolved in the oil phase should be consistent. The surfactant was 14% (w/w) and the oil phase was 12.8% (w/w). In order to study the effect of squalene oil on stability, the ratio of surfactant to oil was kept consistent (1.4:1.2). Seven ratios of VCO:squalene (as mentioned in Section 2.2.1) were evaluated. The quantity of water added for the inversion of formulations was almost equal to 12 mL.

3.3. Particle size

The resulting average droplet diameters of nano-emulsions with different squalene concentrations listed in Table 1 are given in Fig. 3. It was discovered that as the sequalene concentration increased, the Ostwald ripening however decreased and this in turn decreased the droplet growth. The droplet growth actually depends on the VCO:squalene ratio. The changes in droplet size as a function of storage time for the nano-emulsions with different VCO:squalene ratios are given in Fig. 3. Droplet size increased very slowly and the growth rate decreased with the increasing squalene ratios, i.e. the stability of the emulsion improved with the addition of squalene. The primary droplet size was not affected by the squalene ratios as shown in Fig. 4. The addition of up to 20% squalene to the oil phase systematically had reduced the rate of droplet growth from 14.94 to 0.97 nm day^{-1} as shown in Fig. 5. If squalene alone had been used as

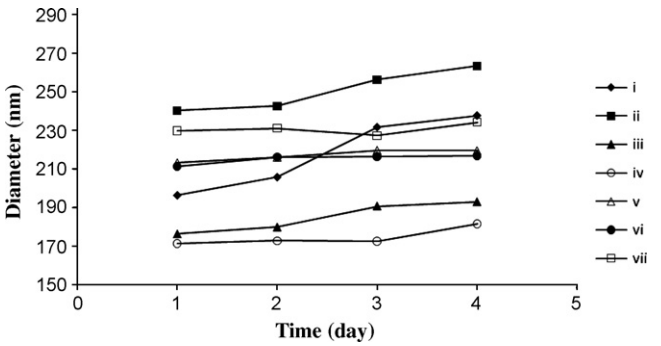

Fig. 3. Radius of droplet as a function of time for formulations with 14% (w/w) surfactant and 12.8% (w/w) oil with different squalene proportions.

Table 1
The composition of formulations, radius of droplets (nm), Zeta-potential and Ostwald ripening.

Formulations	VCO (%)	Squalene oil (%)	Droplet radius (nm) ± S.D.*	Zeta potential (mV) ± S.D.**	Ostwald ripening (nm day^{-1})
i	12.80	0.00	196.4 ± 2.6	−65.1 ± 1.3	14.94
ii	11.84	0.24	240.2 ± 1.8	−71.2 ± 1.4	8.36
iii	11.60	0.48	176.3 ± 1.6	−78.6 ± 1.8	6.07
iv	11.36	0.72	171.3 ± 2.2	−84.3 ± 0.5	3.04
v	11.10	0.98	213.3 ± 3.3	−90.2 ± 0.6	2.21
vi	10.88	1.20	211.3 ± 0.6	−94.9 ± 0.6	1.70
vii	9.66	2.42	229.6 ± 3.5	−101.8 ± 0.2	0.97

Note: The other component of the formulation were: Kojic dipalmitate 2 g, EK:PG (9:1) 14 g, Propyl paraben 0.01 g, and Methyl paraben 0.2 g, and they were kept consistent for all of the formulations.

* Droplet diameters were measured for freshly prepared nano-emulsions and they were given ± standared deviation (S.D.).

** Zetapotentials were measured for freshly prepared nano-emulsions and they were given ± standard deviation (S.D.).

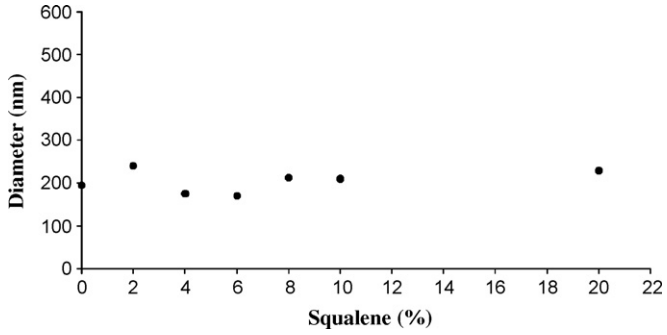

Fig. 4. Radius of droplet as a function of squalene concentration for formulations with 14% (w/w) surfactant and 12.8% (w/w) oil.

the oil phase, the system would be very unstable and the creaming would begin within 1–2 h. Thus the surfactant is discovered to be not suitable for the emulsification of squalene (Tadros et al., 2004).

3.4. Zeta-potential

The resulting zeta-potentials of nano-emulsions with different squalene concentrations listed in Table 1 are given in Fig. 6. The droplets of the nano-emulsions were negatively charged. The zeta potential was −65.1 mV for formulation (i) and increased dramatically to −101.8 mV for formulation (iv) as shown in Fig. 6. The stability of the emulsion could be increased by expanding the surface charge, as the repulsive forces between the droplets help prevent flocculation (Liu et al., 2006). In the system studied, it was revealed that the surface charges of the droplets increased as the squalene percentage increased (Table 1). The negative charges on the surface of VCO droplets covered with EK were the result of the adsorption of OH^- ions on the o/w interface through hydrogen bounding.

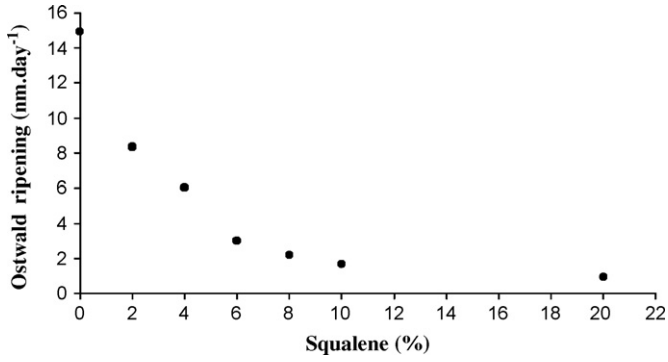

Fig. 5. Ostwald ripening as a function of squalene concentration for formulation with 14% (w/w) surfactant and 12.8% (w/w) oil.

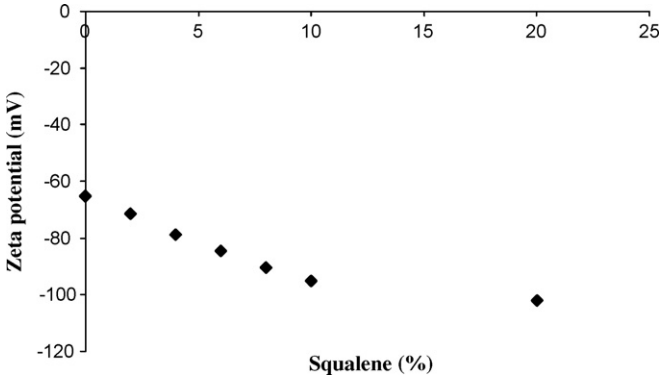

Fig. 6. Zeta-potential as a function of squalene concentration for formulation with 14% (w/w) surfactant and 12.8% (w/w) oil.

4. Discussion

Nano-emulsions in the form of cream were prepared using the Emulsion Inversion Point method. In order to explain the mechanism that happened during the process of this Emulsion Inversion Point method, the steps of ultrafine-droplet formation in this low energy method is described as follows. When the water was added into the oil phase, the system would form the w/o emulsion, but with the increasing water volume, water droplets started to assemble and merge together to form bicontinous or lamellar structures (Fernandez et al., 2004). These structures would decompose into small oil droplets with the increasing water volume (i.e. after EIP). These decompositions occurred due to the fact that the interfacial tension at the inversion point was at the minimum. Basically, because the study had used a higher surfactant concentration (14%), this solubilized all the oil near the EIP that produced monomodal emulsion with ultrafine-sized droplet. On the other hand, this higher surfactant concentration, however, would enhance the stability of the emulsion by increasing the viscosity of the external phase. During the emulsification process, the volume of water was considered a critical factor in the process of inversion and at the same time, the rate of water flow was also considered an important factor because the experiment was run under higher temperature (65 °C) and this would increase the evaporation rate of water leading to the decrease water volume. For this reason, a modified system was used to decrease the rate of evaporation. The electrical circuit was used to determine exactly the point of inversion as shown in Fig. 1. The stages of nano-emulsion formation of EK/PG/VCO/Squalene system by Emulsion Inversion Point method was shown in Fig. 2. The radius of droplets as a function of time for formulations with 14% (w/w) surfactant and 12.8% (w/w) oil with different Squalene proportions was shown in Fig. 3.

The primary droplet diameter centered around 171.3–240.2 nm was not affected by the squalene ratio as shown in Fig. 4. On

the other hand, it appeared that the higher the squalene the concentration was, the smaller the Ostwald ripening that could occur. The Ostwald ripening decreased dramatically from 14.94 to 0.97 nm day^{-1} when the squalene concentration was increased from 2 to 20%, i.e., the growth rate was decreased approximately 15th times as shown in Fig. 5 and this finding corresponded with that as reported before (Fernandez et al., 2004; Tadros et al., 2004). Since the objective of the study was to identify the most stable nano-emulsions, it was observed that the differences between the Ostwald ripening of the formulation vi and vii was very small (1.7–0.97 nm day^{-1}) but the differences in the squalene concentration was the double (10–20%), therefore vi would be considered best than vii from the economical point of view. The zeta potential for the formulation shown in Table 1 shows that the charge increased from −65.1 to −101.8 mV with the increased squalene ratio as shown in Fig. 6. Thus the repulsion forces between the droplets would be increased; this would lead to the enhancement of the stability of the nano-emulsions. The droplet charge was negative and this was due to the adsorption of hydroxyl ions on the non-polar VCO droplet by the hydrogen bound. These results correspond with the work reported before (Liu et al., 2006).

The addition of squalene in our study was compatible with the Ostwald ripening theory which suggests that stable systems would produce results if a second insoluble or very poorly soluble oil phase was added to the system. This finding correspond with those of other reports (Tadros et al., 2004). This addition caused significant partitioning between different droplets and results in an equilibrium due to the differences in droplet size and chemical potential. If one component were to have zero solubility in the continuous phase, then the size distribution will not deviate from the initial one, i.e., the growth rate is zero (Tadros et al., 2004).

5. Conclusions

Nano-emulsion whitening cream made from VCO and EK could be obtained by using the Emulsion Inversion Point method. Ostwald ripening (the main instability mechanism), could be reduced by adding insoluble oil (squalene) to the system. This phenomenon may be explained by the equilibrium that was established between the differences of chemical potential of different droplet size and the differences of chemical potential of the two oils. The results of the zeta potential and the particle sizes well correlated with each other.

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