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Formation of Stable Palm Kernel Oil Esters Nanoemulsion System Containing Hydrocortisone

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A physical and chemically stable palm kernel oil esters emulsion system with nanosized droplet was developed as a delivery system for hydrocortisone (hydrophobic drug). A simple low energy emulsification method was used in forming the nanoemulsions. The influence of added solvents (isopropanol and ethanol) on particle size and stability of oil in water nanoemulsion was investigated. Formation of nanoemulsion with solvent, increase the solubility of hydrocortisone in the oil phase and thus make the nanoemulsion more stable. Reducing the solvent to lipoid ratio showed no significant difference in the mean particle size. However, after solvent removal particle size increased over time. As for zeta potential value, all nanoemulsions exhibited values below -30 mV which indicated good stability. The DSC thermograms for stable nanoemulsions proved that hydrocortisone is in a non-crystalline state, suggesting that hydrocortisone is homogenized well in the nanoemulsion system. These results showed that nanoemulsion with solvent appear to be a promising transdermal delivery vehicle for hydrocortisone.

Keywords: Oil in water nanoemulsion, Transdermal, Solvent, Binary surfactant, Isopropanol, Ethanol, Hydrocortisone.

INTRODUCTION

Emulsions are formed when two immiscible liquids are mixed in the presence of a surfactant. Nanoemulsions are a class of emulsion with the droplet size ranging from 20 to 500 nm[1]. Nanoemulsions are also referred as miniemulsion[2], ultrafine emulsion[3] and submicron emulsion[4]. Due to their particle size, nanoemulsions appear to be transparent or translucent. However, nanoemulsions with particle size of more than 100 nm appear to have a milky white appearance. This is due to the refraction of light by scattering of small droplets[5].

The low surface tension with particle having high surface area makes nanoemulsions as a promising delivery system in pharmaceutical, cosmetic[6], agriculture[7] and chemical[8] industries. The small particle size of nanoemulsions makes them less prone to ostwald ripening, coalescence and flocculation which limit applications in various industries[9]. However, despite these advantages, the main problem when working with nanoemulsions is the stability.

Suitable components such as oil and surfactants are vital in the formation of nanoemulsion. The components selected should be pharmaceutically acceptable for transdermal application and shows no sign of irritancy and sensitivity towards skin. Besides that, selected components should also fall into the generally regarded as safe (GRAS) category.

Shakeel *et al.*[10] reported that in selecting surfactants for o/w nano-emulsion the required hydrophilic lipophilic balance (HLB) value should at least be greater than 10. In our study, Lipoid S75 and tween 20 were used as the surfactant to help emulsify the prepared nanoemulsion. Tween 20 and lipoid S75 have HLB values of 16.7 and 7.0, respectively. From Griffin's formulae (1), hydrophilic lipophilic balance numbers of mixed binary surfactant system obtained is 11.84. Tween 20 is a non-ionic surfactant while Lipoid S72 is an amphoteric surfactant. The choline group carries a positive charge while the phosphate group carries a negative charge.

$$HLB_{mix} = \frac{(wt\ \%\ of\ surfactant\ A)}{100} \times (HLB_A - HLB_B) + HLB_B \quad (1)$$

The main obstacle in formulating hydrophobic and partially lipophobic drug[11] is the solubility. Higher solubility of drug in oil would help the drug to maintain in the solubilized form. Laugel *et al.*[11] conducted a test on the solubility of hydrocortisone and found the partition coefficient to be 15.38. Therefore they concluded that hydrocortisone preferably remained in the oil phase. Since hydrocortisone has a high log P value, solvents are added in order to increase the solubility of hydrocortisone. There has always been a debate that solvents cause irritancy to skin, hence only class 3 (solvents with low toxic potential) is used in this work.

Steroidal drugs are widely used in transdermal application to treat inflammatory diseases. Many of these drugs have poor absorption and thus bioavailability due to the skin barrier function. Over the years, works have been carried out to maximize the potency of steroid drugs while minimizing the side effects[12]. Therefore by incorporating the hydrophobic steroidal drug into nanoemulsion, offers an opportunity for better bioavailability and reduced side effects. This is because nanoemulsions have the ability to control drug release as well as drug targeting to where the inflammatory reactions occur. Besides that, with its small particle size, penetration through the layers of skin is easier, thus reducing the side effects in the targeted organ and organs surrounding it.

In this study, formation and characterization of oil in water nanoemulsion loading a steroidal drug for transdermal application was carried out. The oil phase used was palm kernel oil esters. It is known for having excellent wetting behavior but without the greasy feeling when applied on skin[13]. The model steroidal drug was hydrocortisone, which was widely used as an anti-inflammatory agent. Since the log P value of hydrocortisone was low, effect of solvents in the formation of nanoemulsion was studied.

EXPERIMENTAL

Hydrocortisone was purchased from Alfa Aesar (Britain). Lipoid S75 was obtained from Sigma-Aldrich (Munich, Germany) and Tween 20 from Merck (Hohenbrunn, Germany). Palm kernel oil esters (PKOEs) were used as the oil phase while deionized water as the aqueous phase. Both PKOEs and deionized water were prepared in our laboratory. The solvents used were, ethanol (EtOH), isopropanol (IPA) and ethyl acetate (EA) which were obtained from Sigma-Aldrich chemicals. A preservative, phenonip was obtained from Gattefosse (North America, USA).

Ternary phase diagram: The phase behaviour study was carried out using titration method. Water was titrated into a mixture of oil and surfactants (60:40). These mixtures were vortexed to obtain homogenous emulsions and then centrifuged for visual observation. The physical appearance of the prepared emulsions was recorded. A ternary phase diagram was constructed with three axis representing water, oil and surfactant, respectively using software, Chemix version 3.5 phase diagram plotter (UK).

Selection of nanoemulsion composition: From the ternary phase diagram, compositions were selected from the homogenous region. The chosen compositions were characterized and then used for further studies. The chosen compositions contain less than 50 % of surfactants, as emulsion rich in surfactants is believed to cause irritant to the skin[10].

Preparation of oil in water (o/w) nanoemulsion containing hydrocortisone: Both aqueous and oil phases were prepared separately. Lipoid S75 was dissolved in selected solvent at room temperature before the addition of drug (hydrocortisone) and palm kernel oil esters. On the other hand, the aqueous phase mixture containing Tween 20 and deionized water was heated until all Tween 20 was dissolved. At room temperature, the two phases were mixed drop wise and prehomogenised for 4 h. The solvents used in this research are class 3 solvents.

Particle size analysis: The particle size of the nanoemulsion formulation prepared was measured by Nanophox particle size analyser (SympaTec GmbH, Germany) with Photon Cross Correlation Spectrometer (PCCS) using laser light scattering. The samples were loaded onto 1 cm^2 cuvettes and were inserted into a thermostated chamber. Measurements were taken at 25 ºC and light scattering was monitored at a 90º angle. The particle size values were obtained by taking the average of 5 measurements. All samples were measured immediately after dilution with deionized water. The graph of particle size distribution by volume *versus* particle size was chosen and analyzed.

Zeta potential: The surface charge present was measured using zetasizer 4 (Malvern Instruments, Worcestershire, UK) by laser doppler electrophoresis. In order to measure the surface charge the samples were diluted with deionized water. The samples were then injected into a capillary cell for charge measurement. Zeta potential with the values +30 mV and above and -30 mV and below indicates good stability.

pH: The pH values of the nanoemulsion formulations prepared were measured at 25 ºC using a Delta 320 pH meter (Mettler-Toledo, Schwerzenbach, Switzerland).

Refractive index: The refractive index of formulations with and without drug was measured using Refractor 30PX/GS (Mettler Toledo, Schwerzenbach, Switzerland).

Differential scanning calorimetry (DSC): DSC thermograms of raw materials and nanoemulsion samples were obtained using a Mettler instrument (Mettler-Toledo AG, Greifensee, Swiizerland) after 90 days of sample preparation. Samples (12 mg) were placed in an aluminium crucible. The thermal behavior was in the range of 20-350 ºC at a heating rate of 10 ºC per min with flow rate of nitrogen gas at 50 mL per min.

Physical stability studies: Prepared formulations were centrifuged for 0.5 h at 4000 rpm[14] and were observed for phase separation. All the formulations without significant phase separation were stored in three different temperatures; 5 °C (refrigerator), 25 ºC (room temperature) and 45 ºC in an incubator (Shaking Incubator DK-S1020, DAIKI Sciences Co. Ltd, Korea). Formulations were also subjected to heat cool cycle (6 cycles) between temperatures 4 °C and 45 ºC with storage at each temperature not less than 24 h. The samples were observed visually and tested for particle size and zeta potential each week for 3 months.

RESULTS AND DISCUSSION

Fig. 1 shows the ternary phase diagram of water/lipoid: Tween 20 (60:40)/PKOEs. Low surfactant concentration showed high tendency of phase separation. Composition for formulations were chosen from the homogeneous phase as shown in Fig. 1 for further characterization.

Selection of composition from homogenous phase: Table-1 shows a total of 9 formulations which were selected from the homogenous phase. Formulation NEC was chosen for further studies as other prepared formulations showed immediate phase separation. Table-2 shows the percentage of compositions (wt%) of the chosen formulation. The formulations were formulated with different solvents (isopropanol and

TABLE-1
SHORT TERM STABILITY OF FORMULATIONS FROM THE HOMOGENEOUS PHASE

Formulations	NEA	NEB	NEC	NED	NEE	NEF	NEG	NEH	NEI
Short term stability	X	X	✓	X	X	X	X	X	X

TABLE-2
COMPOSITION OF NEC NANOEMULSION FORMULATIONS CONTAINING SOLVENTS

Formulation	Composition (wt %)							
	Water	PKOEs	Lipoid	Tween 20	HC	Phenonip	Solvent (IPA/EtOH)	Oil:surfactant
NEC[IPA:Lipoid (1:1)]	12.25	5	12	8	0.5	0.25	12	1:4
NEC[IPA:Lipoid (0.5:1)]	18.25	5	12	8	0.5	0.25	6	1:4
NEC IPA SE	12.25	5	12	8	0.5	0.25	12	1:4
NEC[EtOH:Lipoid (1:1)]	12.25	5	12	8	0.5	0.25	12	1:4
NEC[EtOH:Lipoid (0.5:1)]	18.25	5	12	8	0.5	0.25	6	1:4
NEC EtOH SE	12.25	5	12	8	0.5	0.25	12	1:4

SE refers to solvent evaporation. 1:1/0.5:1 refers to solvent to lipoid ratio

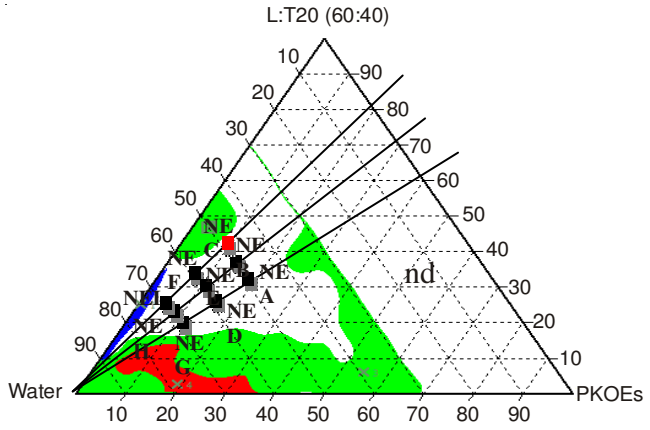

Fig. 1. Phase behavior of Water/Lipoid:Tween20 (60:40)/PKOEs system at 25ºC. White- homogenous; blue- isotropic; red-3 phases;green-2 phases, nd-not determined

ethanol). The formulations have the same oil and water content but different lipoid to solvent ratio.

Ethanol, ethyl acetate and isopropanol were used as the solvent due to their low toxicity towards skin. Besides that, these solvents are also commonly used as an ingredient in pharmaceutical products.

Effect of solvents on particle size and zeta potential: Table-3 summarizes the mean particle size and zeta potential for both formulation with isopropanol NEC(IPA), ethanol NEC(EtOH) and for both formulations after solvent evaporation NEC(IPA)SE and NEC(EtOH)SE. Particle size measurements were not recorded for formulations with ethyl acetate because hydrocortisone was not fully solubilize. As a result, sedimentation of drug occurs after centrifugation of freshly prepared nanoemulsion.

The mean droplet size for NEC(IPA) and NEC(EtOH) were 185.90 nm and 198.0 nm respectively. The zeta potential values obtained were -53.2 mV for NEC(IPA) and -58.6 mV for NEC(EtOH). From the results obtained there were no significant difference in the zeta potential values between the two formulations. Since the use of solvents was less favorable, removing of solvents through solvent evaporation at room temperature technique is taken into consideration. Solvent evaporation process contributes to the decrease in mean particle size for both the

TABLE-3
EFFECT OF SOLVENTS ON THE PARTICLE SIZE AND ZETA POTENTIAL VALUE FOR NEC[IPA:LIPOID (1:1)], NEC [ETOH:LIPOID (1:1)], NEC(IPA)SE AND NEC(ETOH)SE

Formulations	Particle size (nm)	Zeta potential (mV)
NEC [IPA:Lipoid (1:1)]	185.90 ± 2.90	-53.2
NEC[EtOH:Lipoid (1:1)]	198.00 ± 1.70	-58.6
NEC(IPA)SE	160.6 ± 2.44	-54.0
NEC(EtOH)SE	156.7 ± 1.51	-53.5

formulations. On the other hand, zeta potential values remained the same. This indicated that the presence of solvents did not affect the surface charge of the formulation. The particle size and zeta potential value for NEC(IPA)SE and NEC(EtOH)SE are 160.6 nm (-54 mV) and 156.7 nm (-53.5 mV), respectively.

NEC(EtOH) shows a slightly larger particle size compared to NEC(IPA). The formation of nanoemulsion involves the dissolution of oil phase in the organic solvent. Solvents (EtOH and IPA) help promotes this process. As a result during the evaporation process, solvent is removed from the oil phase and causes the particle size to shrink[15].

The high negative surface charge indicates good physical stability of the nanoemulsion formulations as well as electrostatic stability[16]. An emulsion has a higher percentage to remain stable when having zeta potential values above +30 mV or below -30 mV[17]. If all the particles in an emulsion have a high positive or negative zeta potential value they will repel each other, thus reduce the occurrence of coalescence and flocculation[18]. The similar zeta potential values indicated similar interfacial properties of the nanoemulsion particles[19].

Effect of solvent to lipoid ratio on particle size and zeta potential: The mean particle size and surface charge value for formulations with different solvent to lipoid ratio is given in Table-4. Interestingly, NEC[IPA: Lipoid (0.5:1)] and NEC [EtOH: Lipoid (0.5:1)] that comprised slightly less solvent compared to NEC[IPA: Lipoid (1:1)] and NEC [EtOH: Lipoid (1:1)] were observed to have similar particle size. The mean particle size recorded for NEC[IPA: Lipoid (0.5:1)]is 183.40 nm while for NEC[EtOH: Lipoid (0.5:1)] is 198.70 nm. In addition, the zeta potential value obtained were -56.4 mV and -44.7 mV for NEC[IPA: Lipoid (0.5:1)] and NEC EtOH:Lipoid (0.5:1)] respectively[20].

TABLE-4
EFFECT OF SOLVENTS TO LIPOD RATIO ON THE PARTICLE SIZE AND ZETA POTENTIAL VALUE FOR NEC[IPA:LIPOID (1:1)], NEC[EtOH:LIPOID (0.5:1)], NEC[IPA:LIPOID (0.5:1)] AND NEC[EtOH:LIPOID (0.5:1)]

Formulations	Particle size (nm)	Zeta potential (mV)
NEC [IPA:Lipoid (1:1)]	185.90 ± 2.90	-53.2
NEC[EtOH:Lipoid (1:1)]	198.00 ± 1.70	-58.6
NEC [IPA:Lipoid (0.5:1)]	183.4 ± 2.13	-56.4
NEC [EtOH:Lipoid (0.5:1)]	198.7 ± 1.61	--44.7

O'Donnell *et al.*[20] Reported that decreasing the volume of solvent used will result in the increase of particle size. The lower volume of solvent use cause the viscosity of the internal phase of the emulsion to increase. However, when emulsifying the oil phase containing solvent with water, the solvent will diffused out from the oil droplet into the continuous phase. Thus, the decreased in droplet size[15].

Nevertheless, our results showed that there is no significant difference in particle size when the volume of solvent is reduced. This could be due to the volume of solvent in all four formulations do not vary much. Therefore the amount of solvent diffused into the water phase is almost similar. Besides that the surfactant concentration which plays an important role in determining the particle size remains constant in all formulations. Hence, no reduction of particle size reported.

pH test: The pH for all the formulations was found to be approximately pH 5. The pH value showed good compatibility with the pH of skin (pH 5.5). The skin surface is known to be acidic with a pH of 4.2 -5.6 [21]. The acidic nature of the skin is influenced by sweat, hydration and sebum[22].

Refractive index: The refractive index of freshly prepared formulations and formulations that were stored in room temperature for 3 months is as shown in Table-5. This value indicated the isotropic nature of formulations[23]. Therefore formulations with lower solvent to lipoid ratio and after solvent evaporation were not tested for refractive index values. This is because formulations turned opaque after removal of solvent. Results showed that there were no significant changes in the refractive index for fresh formulation and formulation after 3 months of storage at room temperature. This indicated that the nanoemulsions prepared were thermodynamically and chemically stable. In other words, there are no interactions between the nanoemulsion components and the drug[10].

TABLE-5
REFRACTIVE INDEX VALUE OF FRESHLY PREPARED FORMULATIONS AND FORMULATIONS AFTER 3 m OF STORAGE

Formulations	Fresh Samples	Samples after 3 m
NEC[IPA:Lipoid (1:1)]	1.4270	1.4251
NEC[EtOH:Lipoid (1:1)]	1.4232	1.4219

Stability test: Figs. 2 and 3 show the mean droplet size for NEC[IPA: Lipoid (1:1)], NEC[EtOH: Lipoid (1:1)], NEC [IPA: Lipoid (0.5:1)] and NEC[EtOH: Lipoid (0.5:1)] over a period of 3 months. From the figure shown no significant change in particle size were observed. This indicated that the prepared nanoemulsion formulations could resist flocculation, coalescence and ostwald ripening processes[18].

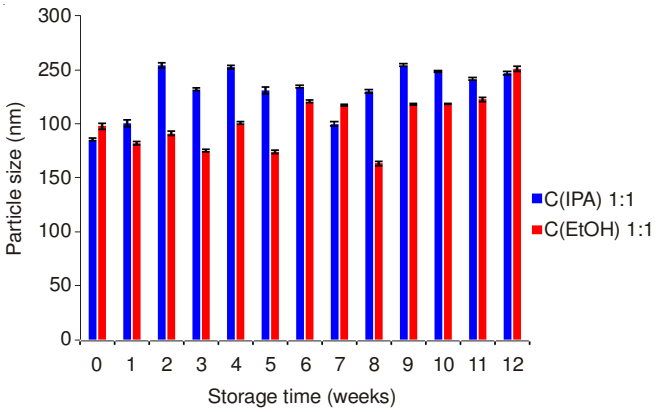

Fig. 2. Mean particle size of hydrocortisone loaded NEC with different solvent over a period of 3 months. (0 week refers to freshly prepared nanoemulsion). NEC[IPA:Lipoid (1:1)]-12.25% water, 5 % palm kernel oil esters (PKOEs), 12% Lipoid S75, 8 % Tween 20, 12 % IPA 0.5 % hydrocortisone and 0.25 % phenonip, NEC[EtOH:Lipoid (1:1)]-12.25 % water, 5 % palm kernel oil esters (PKOEs), 12% Lipoid S75, 8 % Tween 20, 12 % EtOH, 0.5 % hydrocortisone and 0.25 % phenonip

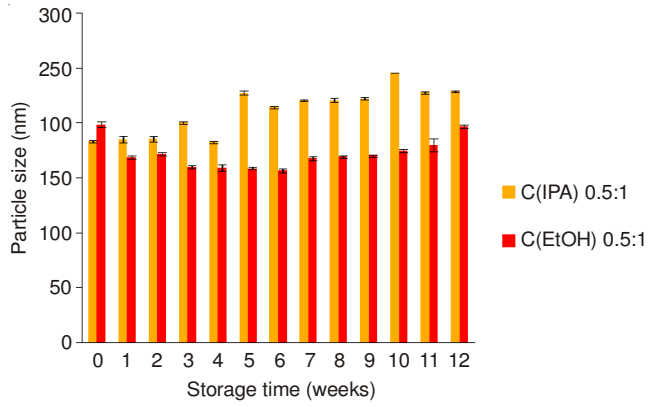

Fig. 3. Mean particle size of hydrocortisone loaded NEC with different solvent to lipoid ratio over a period of 3 months. (0 week refers to freshly prepared nanoemulsion). NEC[IPA:Lipoid (0.5:1)]-18.25 % water, 5 % palm kernel oil esters (PKOEs), 12 % Lipoid S75, 8 % Tween 20, 8 % IPA 0.5% hydrocortisone and 0.25 % phenonip, NEC[EtOH:Lipoid (0.5:1)]-12.25 % water, 5 % palm kernel oil esters (PKOEs), 12 % Lipoid S75, 8 % Tween 20, 6 % EtOH, 0.5 % hydrocortisone and 0.25 % phenonip

Fig. 4 illustrates the mean particle size for NEC(IPA)SE and NEC(EtOH)SE formulations. Both the formulations showed increase in mean particle size over time. The particle size of NEC(IPA)SE and NEC(EtOH)SE grew up to 320.50 nm and 305.80 nm, respectively. Yet, no phase separation was observed. The total free energy and interfacial area of particles could be reduced by the increase in particle size[24]. The mechanism that caused this process are mainly coalescence and ostwald ripening[25]. In order to determine the mechanism that governed this process the results of Fig. 4 were replotted as shown in Fig. 5.

The coalescence graph (Fig. 5a) showed that no linear variation was obtained while the ostwald ripening graph (Fig. 5b) was found to be in linear relationship of mean particle size *versus* time. This indicated that ostwald ripening dominated the instability mechanism for NEC(IPA)SE and NEC(EtOH)SE. In O/W emulsion the Ostwald ripening rate is very much dependent on the solubility of oil in the aqueous

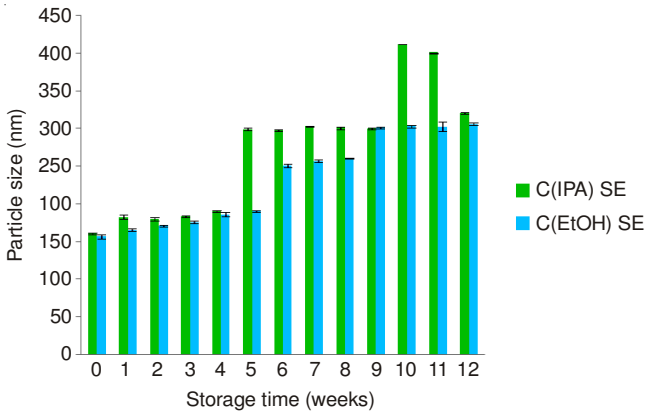

Fig. 4. Mean particle size of hydrocortisone loaded NEC after solvent evaporation over a period of 3 months. (0 week refers to freshly prepared nanoemulsion). NEC(IPA)SE-12.25 % water, 5 % palm kernel oil esters (PKOEs), 12 % Lipoid S75, 8 % Tween 20, 12 % IPA 0.5 % hydrocortisone and 0.25 % phenonip, NEC(EtOH)SE-12.25 % water, 5 % palm kernel oil esters (PKOEs), 12 % Lipoid S75, 8 % Tween 20, 12 % EtOH, 0.5 % hydrocortisone and 0.25 % phenonip

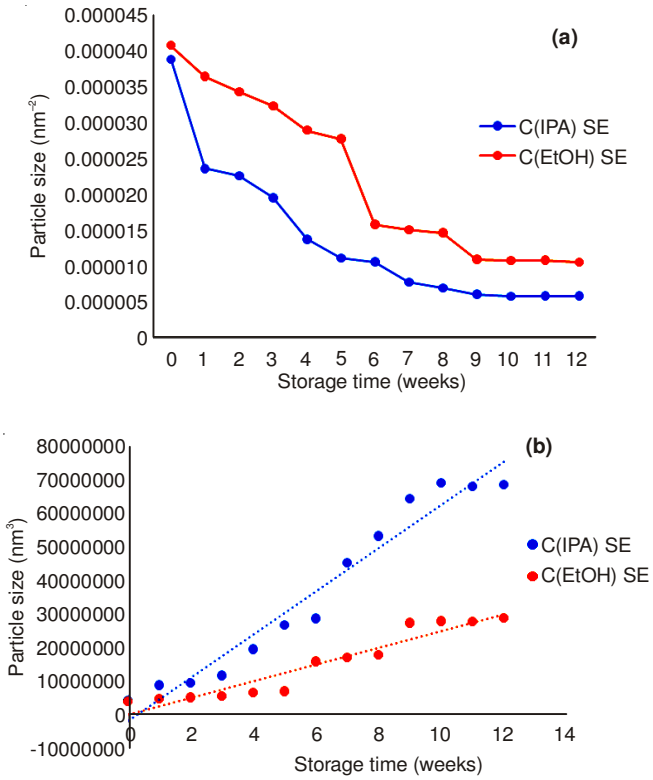

Fig. 5. Effect of time on mean particle sizedistribution of (a) coalescence and (b) Ostwald ripening formed in NEC(IPA)SE-12.25 % water, 5 % palm kernel oil esters (PKOEs), 12 % Lipoid S75, 8 % Tween 20, 12 % IPA 0.5 % hydrocortisone and 0.25 % phenonip and NEC(EtOH)SE-12.25 % water, 5 % palm kernel oil esters (PKOEs), 12 % Lipoid S75, 8 % Tween 20, 12% EtOH, 0.5% hydrocortisone and 0.25 % phenonip

phase. Therefore Ostwald ripening rate can be reduced by the help of solvent which promotes the solubility of oil prior to homogenization. In the case of NEC(IPA)SE and NEC(EtOH) SE, these nanoemulsions underwent solvent evaporation. This could possibly reduce the solubility of oil thus Ostwald ripening takes place.

The zeta potential value however showed no significant difference for all formulations (NEC[IPA:Lipoid (1:1)], NEC[EtOH:Lipoid (1:1)], NEC[IPA:Lipoid (0.5:1)], NEC [EtOH:Lipoid (0.5:1)], NEC(IPA)SE and NEC(EtOH)SE). All zeta potential values recorded were below -30 mV. Part of the negative surface charge measured on the droplets comes from the phosphate group in Lipoid S75. Besides that the absorption of OH^- or H_3O^+ ions from water in the continuous phase is also a contributing factor. Another possibility would be from the anionic impurities such as free fatty acid that is present within the oil[26]. As for the particle size, our results showed that there was a tremendous increase over time for solvent evaporated formulations. This could be due to some destabilization process. No phase separations were observed for all the formulations that underwent heat cool cycle (6 cycles) and which were stored in 45 ºC.

Differential scanning calorimetry: Nanoemulsions which remained stable for 3 months were kept for thermal analysis to investigate the crystalline behavior of the drug (hydrocortisone). Since NEC(IPA)SE and NEC(ETOH)SE exhibits growth of particle size over time, these formulations did not proceed for thermal analysis. Fig. 6a shows the DSC thermograms of hydrocortisone while Fig. 6b shows the thermograms of prepared nanoemulsions where A, B, C, and Drepresents (NEC[IPA:Lipoid (1:1)], NEC[EtOH:Lipoid (1:1)], NEC[IPA:Lipoid (0.5:1)] and NEC[EtOH:Lipoid (0.5:1)], respectively.

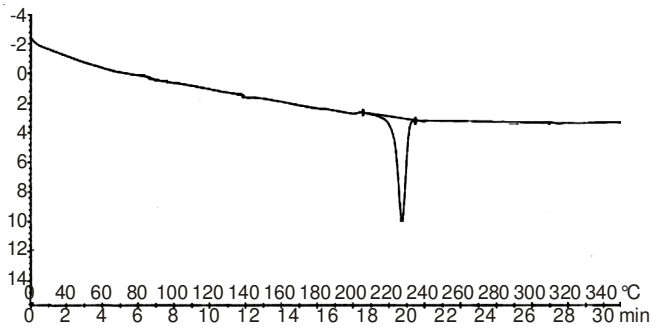

Fig. 6a. Differential scanning calorimetry thermograms of hydrocortisone

The DSC curve of hydrocortisone shows a melting peak at 226ºC resembled the melting point of the drug from the previous work[2] reported to be 220 ºC. In Fig. 6b, no peak of hydrocortisone was observed at this temperature for all the nanoemulsion samples prepared. This could explain that hydrocortisone was dispersed in the nanoemulsions in an amorphous or non-crystalline state[2]. This phenomenon was observed for other drugs in previous studies as reported. Kheradmandnia *et al.*[4] reported that ketoprofen was dispersed in an amorphous state in their SLNs.

The peak visible in the thermograms of nanoemulsions formulations was the vaporization of water at approximately 100 ºC, corresponding to the boiling point of water. The hydrocortisone in all the nanoemulsion formulationswas not present in a crystalline state but was well dispersed in an amorphous form. Nanoemulsions have proved to be a suitable carrier for hydrophobic drugs.

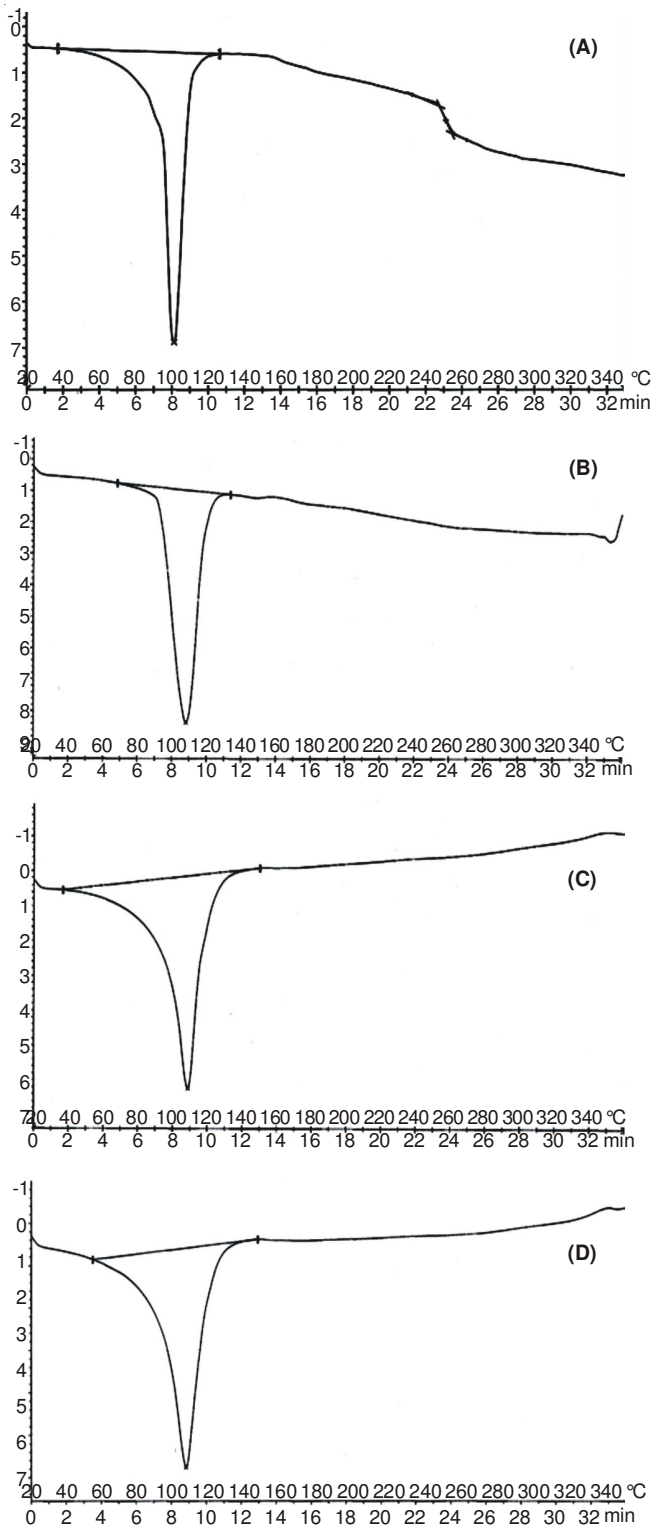

Fig. 6b. Thermograms of prepared nanoemulsions where (A), (B), (C) and (D) represents (NEC[IPA:Lipoid (1:1)], NEC[EtOH:Lipoid (1:1)], NEC[IPA:Lipoid (0.5:1)] and NEC[EtOH:Lipoid (0.5:1)], respectively

Conclusion

In this work, nanoemulsions containing solvent which were stable for 3 months at temperatures 5, 25 and 45 ºC were successfully prepared. The stability of the nanoemulsion formulations was due to the increase of drug solubility by the presence of solvent. Hence, a low toxicity solvent could be used in the nanoemulsions system. The solvents used did not affect the pH of the prepared formulation (pH 4-5). The pH recorded was compatible with the skin's pH. The addition of solvent not only improved the stability but showed no obvious effect on the particle size throughout the storage period. In addition, in the DSC analysis, no hydrocortisone peak was visible, suggesting that the hydrophobic drug was well dispersed in the nanoemulsions. This finding shows that the nanoemulsion was a suitable carrier for hydrocortisone. Due to the positive results from this study, future studies on the drug release and TEM images will be carried out for these formulations for further validation.

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