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Formation of Nanoemulsion Containing Ibuprofen by PIC Method for Topical Delivery☆

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Abstract

This study reports the formation of nanoemulsions from palm-kernel oil esters (PKOE)/Cremophor EL/water systems intended for topical administration of a non-steroidal anti-inflammatory drug, ibuprofen. Nanoemulsions containing 2% ibuprofen, various oil:surfactant ratios (10:90, 20:80 and 30:70) and 80% of water were selected from the ternary system of PKOE/Cremophor EL/water and prepared by the phase inversion composition (PIC) method. The characterization of the nanoemulsions such as droplet size, polydispersity index, zeta potential, stability and the permeation of ibuprofen from nanoemulsions were evaluated.

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The prepared nanoemulsions exhibited good stability without any phase separation, sedimentation or creaming for the period of tested experimental time (6 months). The permeation study of ibuprofen was performed on Franz type-diffusion cells through human abdominal skin. The median values of the fluxes obtained as well as the median of the percentage of permeated amount at 24h were not statistically different. The mean profiles of permeated ibuprofen versus time from PKOE was greater ($p < 0.05$) than those obtained from Miglyol 812.

Keywords: Nanoemulsion; PIC Method; palm kernel oil esters; ibuprofen.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used drugs to reduce pain and inflammation [1]. The administration of NSAIDs through the skin would be preferable to reduce the gastrointestinal side effects and to increase patient compliance, avoiding first-pass metabolism, and maintaining the plasma drug level for a longer period of time [2,3]. Ibuprofen, one of the most used NSAIDs, has been formulated into many topical preparations but it is difficult to maintain effective concentrations, since it possesses poor skin permeation ability. In order to enhance the permeation, various vehicle systems have been explored including the addition of enhancers in the formulation and changing the droplet size of the internal phase in case of emulsions [4].

One of the most promising vehicles for enhancement of ibuprofen permeation is the use of nanoemulsions. Nanoemulsions are transparent or translucent dispersions of oil and water stabilized by an interfacial film of surfactant molecules having a droplet size in the nanometer scale usually less than 200 nm [5]. The small size which implies stability against creaming or sedimentation and a large interfacial area, together with low viscosity are the reasons for the nanoemulsions' increasing use in different applications [6,7]. Many studies have shown that nanoemulsion formulations possess improved transdermal and dermal delivery properties *in vitro* as well as *in vivo* [8,9] in comparison to conventional topical formulations such as conventional emulsions and gels [10].

In the preparation of nanoemulsions, a combination of high-energy and low energy emulsification methods has proven to be an efficient way to obtain nanoemulsions with small and uniform droplets [11,12]. The formation of nanoemulsions by the phase inversion composition (PIC), a low energy emulsification method, has drawn a great deal of attention from scientists in various fields (including pharmaceutical sciences) [13,14]. It has been reported that in order to obtain small droplet-sized (less than 50 nm) nanoemulsions, a liquid crystalline phase should be formed during the emulsification process. The wider this liquid crystalline phase, the easier to reach the equilibrium along the emulsification path, and smaller droplet-sized nanoemulsions are obtained [7].

The oil component selected for nanoemulsion formulation in this work was palm kernel oil ester (PKOE) which shows interesting properties for pharmaceutical applications [15]. Palm oil consists of triglycerides, a combination of glycerol and different fatty acids. The fruit of the palm tree *Elaesis guineenis* is the source of two distinctively different oil types. The outer pulp contains palm oil and the nut in the fruit contains kernels that are the source of palm kernel oil. PKOE produced by the reaction between oleyl alcohol and palm kernel oil through the enzymatic process of transesterification, is rich in oleyl laurate, C30:1 (54.1%) and it consists mostly of relatively shorter chain esters. Therefore, it is appropriate for its incorporation as an oil phase in the formulation of nanoemulsions for topical applications [16].

In this study, nanoemulsions seem to be interesting dosage form for topical delivery of ibuprofen. Therefore, the aim of this work was to formulate kinetically stable nanoemulsions containing ibuprofen and to study the ability of the nanoemulsions deliver ibuprofen topically by *in-vitro* study. Permeation of ibuprofen from nanoemulsions formulated with PKOE was compared with the results obtained from nanoemulsions formulated with Miglyol 812, a medium chain triglyceride.

2. Experimental

2.1 Materials

PKOE was prepared in the laboratory according to the method of Gunawan *et al*. (2004) [17] and purified with methanol (1:1, w/w). Miglyol oil (caprylic/capric triglyceride) was purchased from Fagron (Barcelona, Spain). A non-ionic surfactant, Cremophor EL, was supplied by BASF (Germany). Water was deionized by Milli-Q filtration. Ortho-Phosphoric acid (85%), acetonitrile and potassium dihydrate phosphate were purchased from Merck (Darmstadt, Germany). Ibuprofen, [2-(4-isobutylphenyl)-propionic acid], is an NSAID that, according to the Biopharmaceutics Classification System (BCS), is considered a class II drug, showing high permeability and pH dependent solubility with an acidic pKa and logP (n-octanol/water) values of 4.6 and 3.68, respectively [18]. Ibuprofen was purchased from Eurochem (China).

2.2 Construction of the ternary phase diagram and selection of nanoemulsions composition

A mixture of ibuprofen and PKOE (oil phase) was stirred until it became homogeneous (for about 1 h) using a magnetic stirrer at room temperature. The oil phase was mixed with Cremophor EL at various weight ratios ranging from 0:100 to 100:0. Then, the mixtures were placed into 10 ml screw-cap glass tubes, and were homogenized using a vortex mixer (VM-300, Gemmy Industrial CORP-Taiwan). Water (5 wt.%) was then added and the samples were vortexed for 5 min. The samples were then centrifuged at 4000 rpm for 15 min and observed using polarized light. The steps were repeated with the addition of 10, 20, 30, 40, until 90 wt.% of water. The experiment was repeated without ibuprofen in the oil phase. Ternary phase diagrams were constructed by using the Chemix School v3.50 software (Arne Standnes, Norway).

From the ternary phase diagram constructed, different oil to surfactant ratio (10:90, 20:80 and 30:70) from water/CrEL/PKOE:Ibuprofen system were selected to produce nanoemulsion formulations. Oil to water ratio was kept constant at 20:80, which was based on the best stability of the system with respect to their smallest droplet size compared to 30:70, 40:60 and 50:50 (data not shown). Ibuprofen (2.0 wt %) was dissolved in the oil phase of the nanoemulsion formulations for all formulations.

2.3 Preparation and characterization of Nanoemulsions

Nanoemulsions were prepared by the following methods: A) Stepwise addition of water into the mixture of two components (oil and surfactant). B) Addition of water at once into the mixture of two components (oil and surfactant). C) Addition of oil at once into the mixture of two components (water and surfactant). The samples were homogenized by using a vortex mixer at 25°C. All the samples were kept at 32°C.

The mean droplet size and polydispersity of the nanoemulsions were measured by dynamic light scattering using a Malvern 4700 photon correlation spectrometer (Malvern Instrument, Malvern, UK) with an argon laser (λ=488 nm) at variable intensities. Measurements were performed at a scattering angle of 90° and a constant temperature of 32°C (skin temperature). The DLS data was analyzed by the cumulants method to obtain the *z*-average mean diameter and polydispersity index (PDI). All the samples were diluted with water prior to measurement.

2.4 In-vitro Permeation Studies

Comparative *in vitro* studies were performed with Franz type-diffusion cells. A sample of nanoemulsion (0.3 mL) was added to the donor compartment, which was subsequently occluded. The receptor compartment was filled with PBS (phosphate-buffered saline pH 7.4, sink conditions) and maintained at a constant temperature of 32 ± 0.5ºC. Abdominal human skin samples (0.4 mm thickness) from the same donor were used as a permeation membrane and their barrier integrity was characterized by Transepidermal Water Loss (TEWL) measurements. 0.4

mL samples were withdrawn from the receptor compartment at 2, 4, 6, 16, 18, 20, 22 and 24 h, and replaced with the same volume of PBS at 32ºC.

The ibuprofen content of the samples was analyzed by HPLC (Waters LC-Module I), operated at ambient temperature, consisting of an automatic auto sampler system equipped with a 200 µl loop injection valve, a variable wavelength UV–vis detector and a Spherisorb ODS 2 (150 mm × 4.6 mm, 5 µm). The samples were eluted by the mobile phase consisting of a mixture of acetonitrile/water (55/45 ratio, 1 L), potassium dihydrate phosphate (KH_2PO_4, 2.758 g) and 85% orthophosphoric acid (H_3PO_4, 4.7 mL) which were filtered separately. The flow rate was 1 ml/min, UV detector 254 nm, injection volume was 50 µl and the retention time was 5.66 min. Calibration curves (0.5640–100.84 µg/ml) were constructed from the linear plots of peak area vs. concentration. The calibration was performed by diluting ibuprofen in PBS, pH 7.4.

The permeation rates of ibuprofen at a steady-state (J, $\mu g.cm^{-2}h^{-1}$) was estimated from the slope of the linear part of the plot of cumulative permeated ibuprofen across the skin area vs. time, and lag time (T_L) by the x-intercept. Skin permeability coefficient (Kp), and P_1 ($K \cdot L$) and P_2 (D/L^2) parameters were calculated as follows:

$$Kp = J/C_0 \quad (1)$$

$$P_2 = 1/6 \cdot T_L \quad (2)$$

$$P_1 = Kp/P_2 \quad (3)$$

where Co = donor concentration of ibuprofen; K = partition coefficient of the permeating solute skin/vehicle; D = diffusion coefficient of the solute in the skin and L = effective thickness of the membrane [19,20]. Data was shown as mean ± SD (n = 3). Statistical data was analyzed by non-parametric analysis (Kruskall-Wallis) with a significance level of 0.05.

3. Results and Discussion

3.1 Phase behaviour study

Fig. 1 shows the ternary phase diagrams of water/surfactant/PKOE systems with and without the present of ibuprofen, which prepared according to the method A. Cremophor EL was selected in this study due to the widely usage in topical preparations. The skin irritation evaluation test made by Ammar *et al*. (2009)[21] classified that Cremophor EL as practically non-irritant. In the ternary phase diagrams, isotropic liquid phase, (L_1), a clear and transparent solution, liquid crystalline phase, (L_c), and multilayer region (M), were observed. It should be noted that the L_1 region is increased after addition of ibuprofen.

3.2 Preparation and characterization of nanoemulsions

The compositions of the selected formulations are described in **Table 1**. Previous research indicated that the formation of nanoemulsions by low-energy methods was related to the phase transitions during emulsification involving lamellar liquid crystalline phases [22, 23].

Droplet size and stability of nanoemulsions of the PKOE/Cremophor EL/water system with water content of 80 wt. % were compared to those of the Miglyol 812/Cremophor EL/water system previously reported [24], as appropriate for pharmaceutical applications.

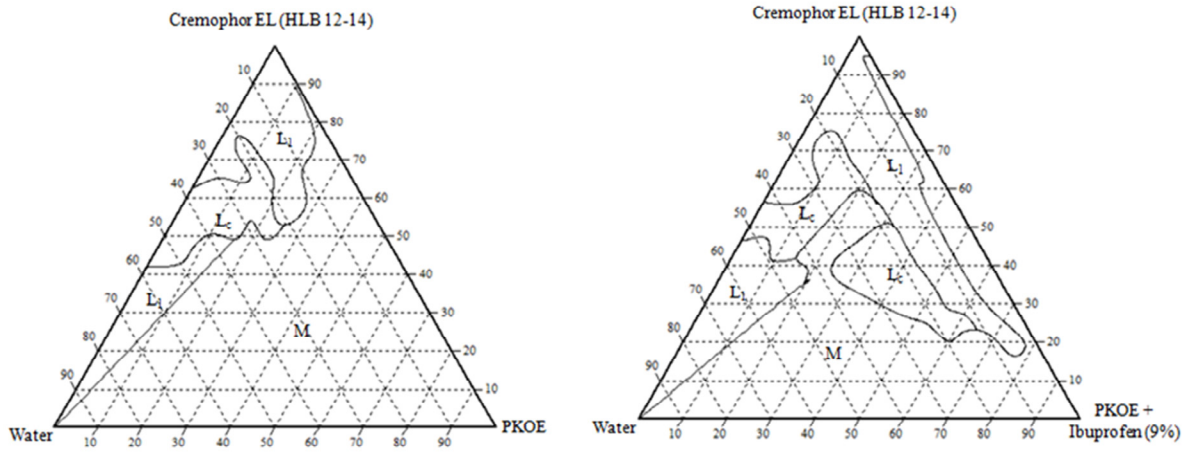

Fig. 1. Ternary phase diagram of the water/Cremophor EL/PKOE systems (a) without, and (b) with ibuprofen at 32 ºC; where L_1, Isotropic liquid region; Lc, Liquid crystalline region; M, Multilayer region.

Table 1. Composition of the selected nanoemulsion containing ibuprofen and their characterizations.

Formulation	O/S ratio	Composition (wt %)[a]				Droplet size	Polydispersity
		Cremophor EL	PKOE	Miglyol 812	Ibuprofen	(nm)	Index[b]
F1	10:90	18	2	-	-	14.7	0.14
F2	20:80	16	4	-	-	23.4	0.285
F3	30:70	14	6	-	-	-	(~1)
F4	10:90	18	-	2	-	13.3	0.0827
F5	20:80	16	-	4	-	18	0.117
F6	30:70	14	-	6	-	24.7	0.141
F7	10:90	16.2	1.8	-	2	32.9	0.49
F8	20:80	14.4	3.6	-	2	17.7	0.223
F9	30:70	12.6	5.4	-	2	27.1	0.268
F10	20:80	14.4	-	3.6	2	16.8	0.147

[a] O:W ratio = 20:80;
[b] A measure of the narrowness of the particle size distribution, with an **index** of less **than 0.2** indicating a monodisperse droplet size

Samples F1-F3 contained PKOE, while samples F4-F6 contained Miglyol 812. Samples F7-F10 contained 2 wt. % of ibuprofen. Particle size was determined by DLS and the effect of dilution was studied with two formulations (F2 and F10). The dilution results showed that droplet size and the distribution were not changed at dilution factors higher than 10. Table 1 shows that the droplet size of nanoemulsions without ibuprofen (F1-F6) increased as the oil/surfactant ratio was increased for PKOE/Cremophor EL/water and Miglyol 812/Cremophor EL/water systems. It was found that polydispersity indexes also increased with the increase in oil:surfactant ratio. The smallest droplet size of 13.3 nm and 14.7 nm were obtained at oil:surfactant ratio of 10:90 for both systems, respectively. The polydispersity index of sample F3 (O/S ratio of 30/70) was too high and therefore, droplet size value could not be determined.

When ibuprofen was added to nanoemulsions of systems, PKOE and Miglyol 812 (Formulations F7-F10), the droplet size and polydispersity index were significantly influenced. At the oil/surfactant ratio of 20:80, the droplet size and polydispersity index decreased from 23.4 nm to 17.7 nm and 0.285 to 0.223, respectively. At a higher oil/surfactant ratio, 30:70, addition of ibuprofen (sample F9), influenced in a decrease in the droplet size and the

polydispersity. The sample changed from a non-measurable sample by DLS and a polydispersity index near to 1 for a sample with a droplet size of 27.1 nm and a polydispersity index of 0.268. The cause of the decrease in size with the incorporation of ibuprofen could be attributed to the amphiphilic behaviour of the drug, where the terminal of the carboxyl group of ibuprofen may interact with the Cremophor EL, resulting in a reduction of the interfacial tension, thus causing a decrease in the droplet size [25]. From the results obtained, sample F8 with the lowest droplet size and polydispersity index was selected for further study.

Fig. 2 shows the droplet diameter as a function of time for samples F2 and F8, without and with ibuprofen, respectively. Stability results indicated that sample F8 (containing ibuprofen) is stable and did not have phase separation for 4 weeks whereas the droplet size in sample F2 (without ibuprofen) increased over time. It has been suggested that the incorporation of ibuprofen at the interface could enhance the elasticity of the surfactant film [26] resulting in the enhancement of the stability of the nanoemulsions [27].

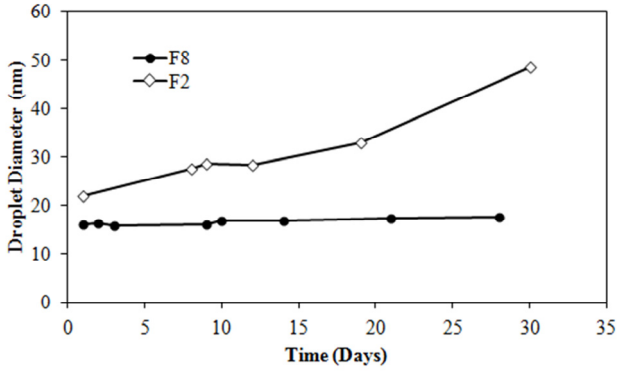

Fig. 2. Droplet diameter of nanoemulsions of the water/Cremophor EL/PKOE system as a function of time for formulation F2 (without ibuprofen) and F8 (with ibuprofen) with 80 wt. % water content, at 32°C.

3.2 In vitro permeation studies

Comparative in-vitro skin permeation studies were performed from nanoemulsions of both systems, PKOE (F8) and Miglyol 812 (F10), all of which have the same amount of ibuprofen (2 wt.%). Table 2 shows the median and the range of the permeation parameters obtained for both formulations. The median values of the fluxes obtained (6.41 and 6.58 $\mu g \cdot cm^{-2} \cdot h^{-1}$ from F8 and F10, respectively) were not statistically different.

Table 2. Median and range values of permeation parameters (n=5-6).

Samples	Flux J ($\mu g \cdot cm^{-2} \cdot h^{-1}$)	Permeated amount at 24h (%)	Lag Time T_L (h)	$K_p \cdot 10^4$ ($cm \cdot h^{-1}$)	$P_1 \cdot 10^3$ (cm)	P_2 (h^{-1})
F8	6.41	5.41	0.3	3.23	0.0684	78.33
	(3.76-8.48)	(3.1-5.21)	(0.3-2.7)	(1.88-4.29)	(0.0012-1.55)	(0.27-186.6)
F10	6.58	5.88	1.91	3.19	1.09	0.29*
	(5.08-9.26)	(4.79-8.05)	(1.15-2.99)	(2.54-4.63)	(0.85-1.32)	(0.19-0.49)

*$p = 0.03$

However, Fig. 3 indicated that the mean profiles of permeated ibuprofen as a function of time of F8 was initially slightly greater than that of F10, and with statistical significance ($p < 0.05$) for the first 6 h of the permeation study.

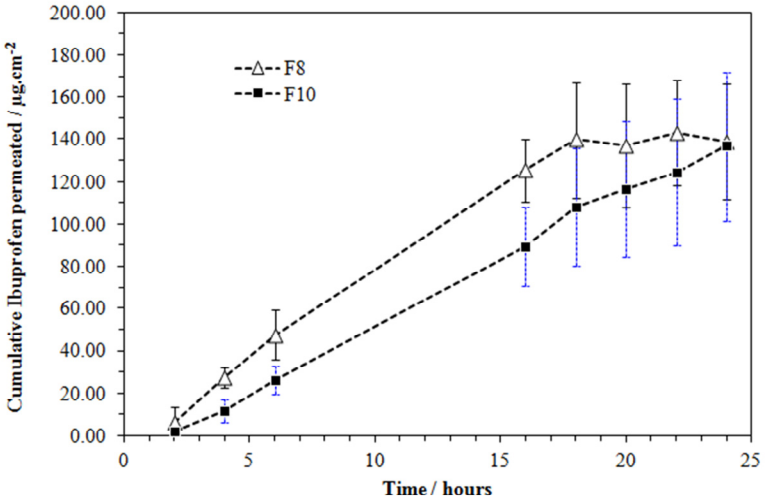

Fig. 3. Permeation profiles of ibuprofen through human abdominal skin from 2% ibuprofen nanoemulsions of different oils. The error bars represent the standard deviation from the mean.

Diffusivities are a function of the molecular structure of ibuprofen as well as the barrier material (skin). In our case, the same drug, membrane and formulation were used and only the type of oil used was different. We could attribute these differences in an increase of the thermodynamic activity of ibuprofen due to a change in the vehicle where ibuprofen would be more soluble, or due to a different interaction of the oils with the intercellular stratum corneum lipids. The use of the vegetable oil PKOE in substitution of Miglyol 812 could be an alternative in the development of nanoemulsions for topical delivery.

4. Conclusion

PKOE nanoemulsions containing 2% ibuprofen were successfully formed by stepwise addition of water to oil/surfactant mixtures. Droplet sizes below 50 nm with polydispersity indexes below 0.2 were obtained. The addition of 2% ibuprofen enhanced the stability of the nanoemulsions system. The permeation flux through human skin of ibuprofen obtained from PKOE and Miglyol were not significantly different. The use of the vegetable oil PKOE could be an alternative to Miglyol 812 in developing nanoemulsions for topical delivery.

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