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THE PALM KERNEL OIL ESTER NANOEMULSION SYSTEM USING PLURONIC F-127 AS A POLYMERIC SURFACTANT VIA A HIGH ENERGY EMULSIFICATION METHOD

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ABSTRACT

The study of formation of nanoemulsions by low energy emulsification method was carried out in water/surfactant/oil system. Palm kernel oil ester (PKOE) was used as the oil phase while Pluronic F-127 was chosen as the surfactant. A ternary phase diagram was constructed for the water/Pluronic F-127/PKOE system by changing the composition concentration at constant temperature. The pre-emulsions were prepared based on the constructed phase diagram via a low energy emulsification and these were characterised with respect to centrifugation force and particle size. Since the particle size formed was large, they were then subjected to high energy emulsification to reduce the particle to nano-size. Formulations with particle size from 93-127 nm were successfully formed. Kinetic stability was assessed by measuring particle size as a function of time and storage condition. Droplet size of the formulations did not show any significant changes with 135 nm after three months. The morphology study was carried out on two formulations using low energy and high energy emulsification. Formulation using high energy emulsification showed an image with smaller and well distributed droplets compared to low energy emulsification.

Keywords: palm kernel oil ester, nanoemulsions, high energy emulsification method, droplet size, stability.

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INTRODUCTION

Nanoemulsions are emulsions with droplet sizes ranging from 50-200 nm (Tadros *et al*., 2004). Due to the extremely small droplet size, some of the nanoemulsions are optically transparent and have high kinetic stability against sedimentation and creaming (Garcia-Celma *et al*., 2005). A wide range of applications for nanoemulsions can be found in the areas of pharmaceuticals, cosmeceuticals, food technology, *etc.* (Thiagarajan and Prakash, 2011). In the pharmaceutical area, nanoemulsions have gained increasing attention as a drugs and other bioactives carriers in a variety of delivery methods such as for parenteral, ocular, topical and transdermal (Burgess and Morais, 2012).

Nanoemulsions are classified as a non-equilibrium system and cannot be formed spontaneously. They need energy from either a mechanical source or a chemical potential of the components for their formation (Nguyen *et al*., 2008). The mechanical energy can be obtained from high

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shear mixing, high pressure homogenisation and ultrasonification to generate high energy forces that break up larger droplets into nano-sized droplets (Basri *et al*., 2013). Low energy emulsification can be obtained from the chemical potential of the components used in the formulations. This method takes advantage of the energy stored in the system to promote the formation of small droplets (Al-Sabagh *et al*., 2011).

To maximise resource utilisation and the probability of success, an initial study should focus on the use of long chain triglycerides or medium chain triglycerides as the oil phase (Floyd, 1999). PKOE is derived from triglycerides of palm kernel oil. It provides an excellent moisturising effect, less greasy and non-irritating behaviour (Basri *et al*., 2009). As for the surfactant, many synthetic surfactants continue to receive attention as they have shown promising characteristics that can be used in parenteral emulsion. Pluronics, for example, have gained much attention in recent years and have been used extensively in a variety of a pharmaceutical formulation (Kabanov, 2002).

In this study, PKOE nanoemulsion system using Pluronic F-127 as the surfactant and using high energy emulsification method was developed. The phase behaviour of PKOE and Pluronic F-127 was studied and the formulations of nanoemulsion system were prepared. The physico-chemical properties and stability of the nanoemulsions were also studied.

MATERIALS AND METHODS

Materials

Palm kernel oil was obtained from the Malaysian Palm Oil Board (MPOB) and was synthesised to PKOE in our laboratory (Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, Malaysia). Surfactant Pluronic F-127 was purchased from Sigma-Aldrich (UK). Deionised water was obtained using a Mili-Q water system (Milipore, USA).

Methods

Construction of ternary phase diagrams. PKOE was mixed with Pluronic F-127 in a series of ratios of PKOE to Pluronic F-127: 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 and 0:10 (w/w). Water was added to the oil phase from 5% (w/w) up to 100% increments to each ratio. The pre-emulsions were mixed using a vortex mixer from LMS Group model VTX-3000L (Japan) and then centrifuged using a EBA 20 centrifuge (Hettich Zentrifugen, USA) for 15 min at 4000 rpm. They were then visually observed under crossed polarised light. The possible physical appearance is isotropic phase; a monophasic optically transparent emulsion, homogenous phase; a cloudy emulsion and multiphase or else a biphasic or triphasic transparent/cloudy emulsion. The ternary phase diagram was constructed using the software, CHEMIX School, version 3.5 phase diagram plotter from Chemistry Software (UK).

Selection of pre-emulsions from the ternary phase diagram. Selected pre-emulsions for the formulation of nanoemulsions were determined from the homogenous region in the ternary phase diagram. The formulations were prepared via a low energy emulsification method where the oil phase was added drop-wise into the aqueous phase containing deionised water and Pluronic F-127. The formulations were then stirred with an overhead stirrer from IKA® model RM 20 digital (Germany) at 200 rpm for 1 hr.

Droplet size measurements. The droplet size of the formulations was measured by Nanophox, Sympatex (Germany) with Photon Cross Correlation Spectrometer (PCCS) using laser light scattering. Sample was diluted 500-fold with deionised water and then loaded onto a cuvette in a thermostated chamber. The measurement was carried out one day after formulation was formed. It was to ensure that all the droplets were in an equilibrium state after the stirring process. Each sample was measured five times and the average result was determined from the readings.

Preparation of nanoemulsion system. Further formulations of nanoemulsions were carried out in order to obtain nanometer range formulation. The nanoemulsion system was prepared using a high energy emulsification method. PKOE was added to the aqueous phase containing deionised water and Pluronic F-127 and was homogenised using IKA® T-50 high shear homogeniser (GERMANY) at 3000 rpm for 3 min. The formulation was subjected to a high pressure homogeniser (GEA Niro Soavi, Italy) at 1000 bar to produce a nanoemulsion system.

Stability study under storage with time. The formulations were stored at 5.0±0.5°C and 25.0±0.5°C for three months to observe any phase separation in these two different temperatures. Observations were made every day for the first week and once a week for the rest of the observation period. Particle size measurement was also carried out to access any particle growth of the nanoemulsion throughout the observation period.

Morphological characterisation - transmission electron microscope. The morphology of the formulation was determined using a high resolution

Hitachi H-1700 Transmission Electron Microscopy (TEM) (Japan). The emulsion was dropped onto a copper grid coated with formvar film and was stained with 2% phosphotungstic acid (PTA). The excess stain solution was gently wiped off with a filter paper and the emulsion sample was left to dry at room temperature. It was then characterised at a different magnification using the TEM.

RESULTS AND DISCUSSION

Analysis of the Ternary Phase Diagram

Figure 1 shows the ternary phase diagram of PKOEs/Pluronic F-127/water. One homogenous phase region appeared at the Pluronic F-127 rich apex and along the apex line of water and Pluronic F-127, and was designated as a homogenous phase (H). As the water content in the homogenous region was increased, the Pluronic F-127 was also increased showing that more water was needed to solubilise Pluronic F-127, as Pluronic F-127 is a hydrophilic surfactant. The rest of the phase diagram consisted of multiphase regions which were either two phases (2P) or three phases (3P) regions. These regions showed the instability of the formulations whereby the surfactant could not completely emulsify the oil in water.

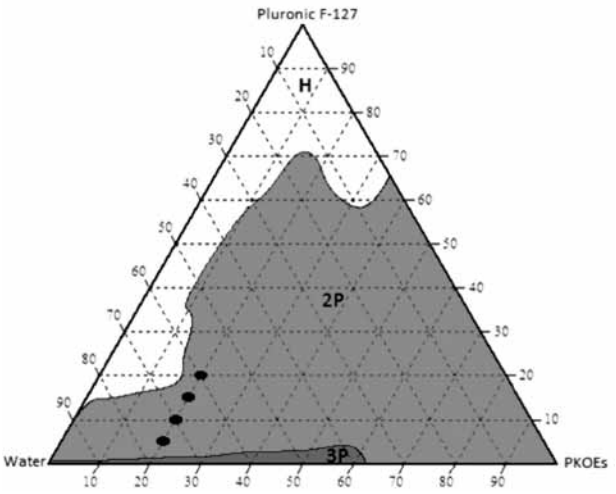


Figure 1. Ternary phase diagram of palm kernel oil esters (PKOE)/ Pluronic F-127/water. H=homogenous, 2P=two phases, 3P=three phases.

Selection of Pre-emulsions from the Ternary Phase Diagrams

The compositions of suitable pre-emulsions were determined from the ternary phase diagram. *Table 1* shows the compositions of the selected formulations. The position of these compositions at the phase diagram is shown in *Figure 1*. Formulations F1 and F2 remained stable while F3 and F4 were unstable showing two separate emulsion layers after being subjected with centrifugation force. As the oil phase was increased from F1 to F4, the Pluronic F-127 was not able to emulsify the oil in the water, thus causing the oil droplets to cream, as the oil has a lower density than water. The stable formulations of F1 and F2 were further characterised with respect to the droplet size in order to check the possibility of producing nanoemulsions by a low energy emulsification method.

Formation of Nanoemulsion Systems via the High Energy Emulsification Route

Figure 2 shows the droplet sizes of formulation F1 to F4 with two different emulsification methods, which are the low energy and high energy routes. The droplet sizes of F1-F4 were decreased to 102-115 nm after being subjected to high pressure homogeniser. There were no results for emulsion in the low energy method for F3 and F4 as they were not stable. However, after formulations F3 and F4 were subjected to the high energy emulsification method, nanoemulsions were successfully formed. As the emulsification energy increases, the emulsion droplets are broken up into smaller size and result in the total surface area to increase (Mooter *et al*., 2006). This activity leads to an increase in adsorption of surfactants between the oil and water layer, which results in an increase in the stability of the emulsion.

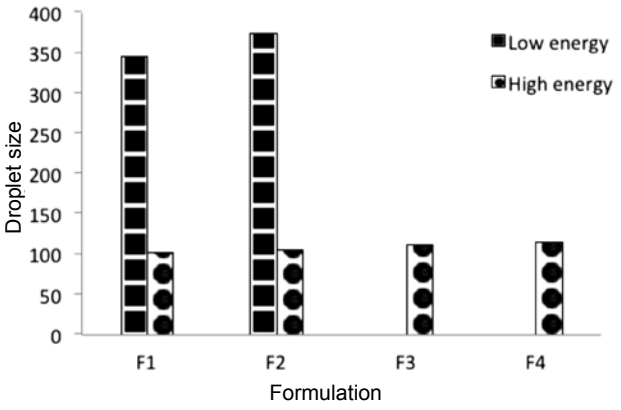


Figure 2. Droplet sizes of formulations with low energy and high energy emulsification method.

Storage Stability

Formulations F1 to F4 showed good stability over 90 days of observation and kept their milky appearance with no sign of creaming, flocculation or coalescence upon storage at 5.0±0.5°C. With regards to droplet growth (*Figure 3*), the nanoemulsions showed a slight increase in the droplet size from Day 1 to Day 90. There are two primary mechanisms that can lead an emulsion droplet to increase. The first is coalescence which is caused by the rupturing of films of the continuous phase and the fusion of two droplets into a single larger droplet. The second

TABLE 1. COMPOSITIONS OF SELECTED PRE-EMULSIONS AND STABILITY OF CENTRIFUGATION TEST

Formulations	Compositions, % (w/w)			
	PKOE	Pluronic F-127	Water	Centrifugation test
F1	5	20	75	Stable
F2	10	20	70	Stable
F3	15	20	65	Not stable
F4	20	20	60	Not stable

Note: PKOE – palm kernel oil ester.

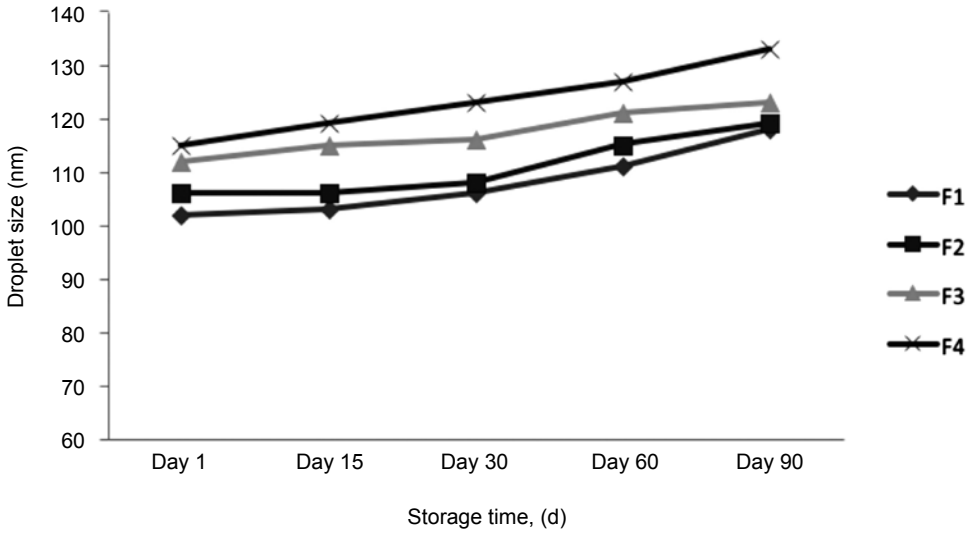


Figure 3. Droplet growth from Day 1 to Day 90.

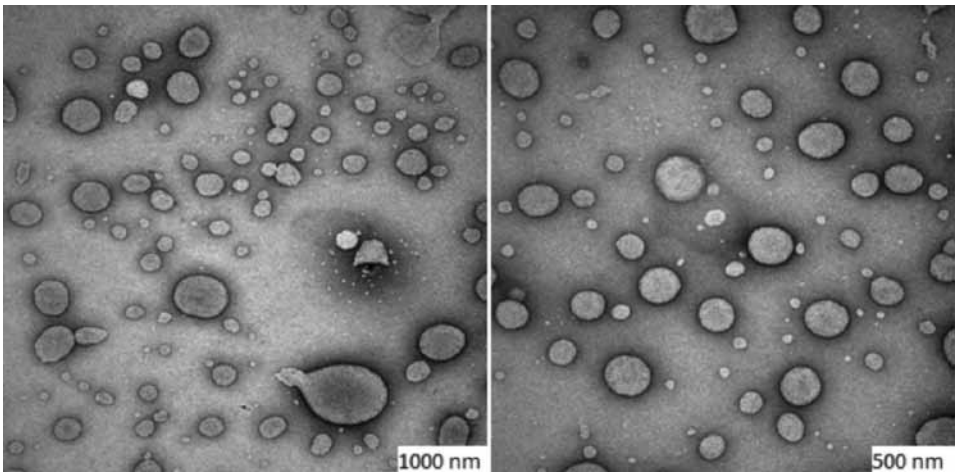
mechanism is the migration of the individual dispersed phase that has a relatively high solubility in the continuous phase from smaller to larger droplets (Mason *et al*., 2006). The rate associated with Ostwald ripening, coalescence, flocculation and creaming can be controlled by considering all the mechanisms and an emulsion that has no changes in droplet sizes over years can be formed. The increase in the droplet size in F1 to F4 in 90 days of storage is controllable since all of them are still in nano-size and despite the aging of the droplet size, the emulsions are stable and show similar physical characteristics from the beginning of the formulation.

Morphology Study

An analysis of the morphology study was carried out for formulation F1. The results presented in *Figure 4a*, showed that formulation formed by low-energy emulsification showed various sizes of spherical oil droplets distributed in the emulsion. *Figure 4b*, formulation formed by high-energy emulsification showed the spherical oil droplets appeared with a more uniform size. Comparing both emulsification methods, low emulsification produces emulsion droplets with various sizes and high emulsification produces emulsion with smaller and well distributed droplets. Some droplet sizes were also measured, as TEM is capable of point-to-point resolution and were found in agreement with the size analysis using photon cross correlation spectroscopy (Sinha *et al*., 2012).

CONCLUSION

Ternary phase diagrams were constructed and homogenous regions were successfully produced. Suitable pre-formulations were selected, then subjected to high energy emulsification, which produced nanoemulsion with less than 115 nm droplet sizes. Long-term stability studies proved that these nanoemulsions were stable for up to 90 days, and the droplets growth analysis showed slightly increasing in droplet size during the observation period. TEM images showed that the formulations with high energy emulsification appeared to be in a spherical shape and had well distributed droplets compared to low emulsification.



(a) (b)

Figure 4. Transmission electron microscopy (TEM) of formulation with (a) low energy method and (b) high energy method.

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