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Phase behavior of reverse microemulsions based on Peceol®

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ABSTRACT

The phase diagram of the four component system Peceol®/lecithin/ethanol/water was studied at 25 °C and at a fixed fraction of ethanol. It shows an isotropic W/O microemulsion phase, biphasic liquid system and Liquid crystalline phases. The stabilizing effect of lecithin with the fluidifying effect of ethanol on the microemulsion based on long chain glycerides provides an effective combination to solubilize a large amount of water. Some structural transitions in the phase diagram were investigated as a function of water content using conductivity, rheology, Karl Fisher titration, optical microscopy and SAXS measurements. The results show no change in the microstructure of the isotropic liquid upon phase separation in the liquid biphasic area. However, in the water rich region, migration of ethanol to the external aqueous phase at the expense of the saturated microemulsion promotes the formation of liquid crystalline phases. As a function of water content, the structural change to the liquid crystalline phases follows: isotropic phase L_2 → Inverted hexagonal phase H_2 → Inverted hexagonal H_2/lamellar L_α phases.

1. Introduction

Long chain glycerides such as oleate glycerides (C18:1 glycerides) are suitable polar lipids to prepare different drug delivery systems, with different supramolecular organizations as a function of water content. Among them glycerol monooleate has been widely explored in aqueous binary mixtures forming various isotropic and liquid crystalline phases [1–4]. The first publication of the phase diagram for Glycerol MonoOleate GMO was Lutton in 1965 [5]. Hyde et al. published again later a more detailed GMO/water phase behavior [6]. Then Larsson et al. did the most important work on polar lipids and on their use to prepare pharmaceutical formulations [7–9]. At room temperature, the Glycerol MonoOleate GMO forms reversed micellar (L_2), lamellar (L_α) and cubic (C) phases with increasing hydration. At high water concentration, the cubic phase co-exists in equilibrium with the excess water [3]. A modification of the phase diagram of the well-studied binary GMO–water system was observed when introducing additives [10–12] but also considering mixtures of GMO with different glycerides with new phases not present in the binary mixture GMO–water [13–17]. For example, the addition of triglycerides (glycerol trioleate) to the GMO–water mixture at less than 30 wt% leads to the formation of two liquid crystalline phases, a lamellar (L_α) and a reverse hexagonal (H_2) phases [14,16]. Interestingly the addition of a few weight percent of glycerol dioleate or oleic acid induces a transition from a cubic phase to an inverted hexagonal phase. These strong effects of additives result from a change in the microstructure, i.e. shape and size of the lipidic aggregates, that are best described by Israelachvili's and Ninham's theory of packing parameter [18]. In these systems, this is due mainly to the hydrophobic volume of these chemical entities that increases considerably the effective packing parameter of the system [4,11,13]. The characterization of the detailed supramolecular structure of such self-assembled systems requires the combination of techniques, such as small-angle X-ray (SAXS), neutron (SANS) scattering, Nuclear Magnetic Resonance (NMR), polarizing optical microscopy and cryo-TEM [13–17].

The pharmaceutical polar lipids based on glycerol monooleate (commercial name Peceol®) are the interesting oily vehicles used for oral liquid dosage formulations as self-emulsifying fluid being able to solubilize water insoluble drugs and to improve their oral bioavaibility [19,20,47]. Because of the complex composition of Peceol® (mixture of glycerol mono- and dioleate – 90 wt% – and a few percents of glycerol trioleate) and the strong influence of additives in GMO based microstructure, it is necessary to study the pseudo-ternary phase diagram for the mixtures of glycerides Peceol® in the presence of water and surfactant and cosurfactant. Lecithin was selected as a highly compatible surfactant and an important constituent of biological membranes [21–23]. However,

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due to the high lipophilicity of Peceol® and lecithin and their tendency to form liquid crystalline phases, addition of hydrophilic alcohol to the system was necessary to form a microemulsion. The ethanol was mainly used to destabilize the liquid crystalline structures and to promote the extension of the microemulsion region [24,25]. D'Antona et al. [26] found also that ethanol used as a cosolvent in the monoglycerides–water system reduces considerably its viscosity, resulting in the formation of stable microemulsions with optimal properties for drug delivery [27,28]. Coupling of the stabilizing effect of lecithin with the fluidifying effect of ethanol, are in favor of solubilization of a large amount of water with microemulsions based on long chain glycerides. The phase behavior of our system was partially determined and studied by means of various techniques, such as small-angle X-ray scattering, polarized microscopy, rheology and conductivity.

2. Materials and methods

2.1. Materials

Glycerol monooleate 40 (Peceol®) that consists of 45.3 wt% of monoglycerides, 44.5 wt% of diglycerides and 8.6 wt% of triglycerides (acid value 1.2, water 0.05 wt% and free glycerol 0.5 wt%) was supplied by Gattefossé (France). The fatty acid composition of Peceol® was oleic acid 81 wt%, linoleic acid 12 wt% and 7 wt% of saturated fatty acid. Soybean lecithin (Epikuron® 200) containing 94.5 wt% of phosphatidylcholine was purchased from Cargill, France. Anhydrous ethanol was purchased from Carlo Erba, France and high purity water, from SDS. 1.0 M of phosphate buffer solution was purchased from Sigma Aldrich. However, it has to be emphasized here that the composition of the commercial natural product Peceol® may vary slightly from batch to batch and thus impact the accurate position of the phase boundaries. Therefore a single batch was used throughout this study to insure the repeatability and exactitude of the observed results.

2.2. Sample preparation

For preparing samples, Peceol® was stirred gently at 37 °C in a mixture of lecithin and ethanol (prepared separately by weighing the surfactant and alcohol into a glass vial containing a magnetic stirrer until a transparent and homogenous yellow solution was obtained. The lipid mixture was ready for use immediately and contacted to water at various ratios to obtain a phase diagram. The total weight of the mixture studied per vial was 10 g and the samples were stored at least over night at 25 °C before further characterization in order to determine visually texture and phase boundaries accurately. All samples in the phase diagram contained a fixed fraction of ethanol that corresponded to 9 wt%.

2.3. Conductivity

Samples for conductivity measurements were prepared with a 0.01 M phosphate buffer solution at pH 7.4 instead of water, in order to increase the conductance of the aqueous phase. It was verified that the addition of this small amount of electrolyte to water had no impact on phase transitions before carrying out the conductivity measurements. The Crison conductivity meter was used (MultiMeter MM41) and conductivity measurements were performed at room temperature (25 ± 1 °C). The conductivity cell 5071 used was a platinum cell suitable for very low conductivities measurements. The measuring range was 0.05 μS/cm to $3 * 10^4$ μS/cm and the cell constant was 0.1 cm^{-1}. All measurements were repeated three times to ensure repeatability and accuracy of the results.

2.4. Polarizing optical microscopy

Polarizing light microscopy can be used to differentiate isotropic liquid (microemulsion or cubic phases) to liquid crystalline phases like hexagonal or lamellar lyotropic phases that are birefringent. Samples between a coverslip and a glass slide were observed at ambient temperature (25 °C) under a polarized light microscope (Axiolab, Zeiss) at 10× magnification and equipped with a Canon A620 camera.

2.5. Rheology

Rheology measurements were performed using TA instrument Rheometer (AR 2000 EX). A cone plate with a diameter of 4 cm and 6 cm and an angle of 2° and 1.1° respectively was used. Temperature was maintained at 25 ± 0.1 °C. Shear rate measurements were performed between 0.01 and 1000 s^{-1}. A sample volume of 1 ml was used.

2.6. Small-angle X-ray scattering (SAXS)

Scattering measurements were performed using a XENOCS instrument at ICSM. The wavelength of the incident X-ray beam was $\lambda = 0.71$ Å (Mo-radiation). The distance from the sample to the detector was 735 mm. The sample was sealed in a 2.0 mm glass capillary tube, and all measurements were performed at ambient temperature (25 °C). Azimuthal averaging of the isotropic 2D scattering images recorded using a Mar-Research 645 camera was applied using FIT2D software and normalization, taking into account transmission measurement, detector background of the detector and the empty cell subtraction. The scattered intensities were expressed versus the magnitude of scattering vector $Q = (4\pi/\lambda) \cdot \sin(\theta/2)$, where θ is the scattering angle. Experimental resolution was $\Delta Q/Q = 0.05$. Acquisition time was 15 min.

2.7. Karl Fischer titration

The water fraction in the isotropic liquid was determined by Karl Fischer titration (Karl Fischer Mettler Toledo). The HYDRANAL® Composit 5 KF reagent and methanol as alcoholic solvent were used. The sample was introduced directly into the measuring cell, and the amount of water in the test sample was calculated according to the volume of the consumed reagent. To minimize experimental error, all samples were measured three times.

3. Results and discussion

The phase behavior study of the four component system Peceol®/lecithin/ethanol/water was performed by establishing a pseudo-ternary phase diagram as shown in Fig. 1. Five different domains were characterized in the explored phase triangle at constant weight fraction of ethanol. Far from the lecithin rich corner a liquid monophasic region described as a microemulsion was observed at high Peceol® content (see Fig. 2A–D). Upon water dilution this phase demixes first into a clear liquid with excess water (see Fig. 2E–G) and then forms a turbid gel with excess water (see Fig. 2H–J) and finally a turbid liquid in the water rich region. In the lecithin rich region an opaque turbid gel and a small region with a clear gel were observed.

3.1. Phase diagram regions

According to the amphiphilic nature of Peceol® containing glycerides, this polar lipid can solubilize a small amount of water and form at room temperature a stable monophasic liquid. The