Development of Solid SEDDS, II: application of Acconon® C-44 and Gelucire® 44/14 as solidifying agents for self-emulsifying drug delivery systems of medium chain triglyceride.

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Original Article

ABSTRACT

Self-emulsifying drug delivery systems (SEDDS) are usually isotropic liquids consisting of drugs, lipids, surfactants and/or co-surfactants that spontaneously form fine oil-in-water emulsions in contact with water. Since a solid dosage form has better patient acceptance than a liquid, it was investigated whether liquid SEDDS containing medium-chain lipids (mono- or tri-glycerides) may be converted to solids or semisolids using lauroyl polyoxyl glycerides (Acconon® C-44, ABITEC, and Gelucire® 44/14, Gattefosse) as solidifying agents. Acconon® C-44 and Gelucire® 44/14 were melted at 65°C. The liquid lipids or the liquid lipid-surfactant mixtures, with and without dissolved drug (probucol), were mixed with the melts, and the hot liquid solutions were filled into hard gelatin capsules. The solutions solidified inside the capsules when cooled to room temperature. Acconon® C-44 and Gelucire® 44/14 had a greater propensity for solidifying the triglyceride of medium chain fatty acids (Captex® 355, ABITEC) rather than the monoglyceride. Powder XRD, DSC and microscopic analyses indicated that the lauroyl polyoxyl glycerides crystallized at room temperature, while the lipid or the lipid-surfactant mixtures present in the formulations remained interspersed in between solids as a separate liquid phase. The drug remained dissolved in the liquid phase and there was no crystallization of the drug. Although Acconon® C-44 and Gelucire® 44/14 are themselves surface active, the dispersion testing using the USP apparatus II at 50 rpm and 37°C using 250 ml of 0.01N HCl as the dispersion medium showed that a second surfactant (Cremophor® EL®, BASF) was required in the solid formulation to maximize drug release and dispersion. Formulations containing 1:1 and 3:1 w/w ratios of Captex® 355 and Cremophor® EL produced lipid particles in the range of 200 to 450 nm. Thus, a novel approach of preparing solid SEDDS resulting in submicron emulsions with particle size <500nm is presented.

KEY WORDS: Solid SEDDS, lauroyl polyoxyl glycerides, Acconon® C-44, Gelucire® 44/14, probucol, dispersion testing

INTRODUCTION

The majority of new chemical entities (NCE) that have emerged during the past two decades have been very insoluble in aqueous media, thus limiting their dissolution rate and oral bioavailability (1, 2). There has been a great interest in the development of lipid-based drug delivery systems to increase oral absorption of a water-insoluble drug solubilized in a mixture of

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lipid, surfactant and an optional cosurfactant (3-10). Lipid-based formulations are especially suitable for optimizing the oral delivery of drugs that are highly lipophilic (8). Depending on whether such solubilized formulations form emulsions or microemulsions in contact with gastrointestinal fluids after oral ingestion, they are referred to as either self-emulsifying drug delivery system (SEDDS) or self-microemulsifying drug delivery systems (SMEDDS) (11, 12). Despite a large number of NCEs that could potentially be formulated for oral absorption and increased bioavailability produced by lipid-based drug delivery, commercial applications of such systems have been limited (13, 14). Mullertz (15) reported that, as of 2010, there were only five drugs on the market (ciprofloxacin, cyclosporine A, lopinavir, ritonavir and tipranavir) to which the technology has been successfully applied to increase dissolution rates and bioavailability.

One, of the two major issues that limit a greater application of lipid-based delivery systems in the development of drug products, is the inadequate solubility of some of the NCEs in suitable lipids or lipid-surfactant mixtures. Another major issue with the lipid-based drug delivery system is, that they usually result in liquid formulations requiring packaging in bottles or encapsulation in soft gelatin capsules. However, the solid dosage form is the most preferred formulation approach for oral administration of drugs and more than 80% of all marketed drug products in the US are either tablets or hard gelatin capsules. Additionally, when the availability of the drug substance is limited and the time line is short at the early stages of the drug development, many formulators do not consider soft gelatin encapsulation as a possible dosage form as it often requires outsourcing of the manufacturing process. Recent research efforts have focused on the development of lipid-based formulations in solid or semisolid forms that may be filled into hard gelatin capsules, thus eliminating the need for liquid formulations.

There are several reports in the literature on the development of solid and semisolid dosage forms of lipids or lipid-like materials (16, 17). In general, such formulations utilized solid or semisolid amphiphilic lipids like lauroyl polyoxyl glycerides (Gelucire® 44/14) (18, 19) and d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) (5, 20) as vehicles for drugs. The drugs were first dissolved into molten vehicles at an elevated temperature and the hot solutions were filled into hard gelatin capsules, which then solidified once cooled to room temperature. However, since the vehicles crystallized out when they solidified at room temperature, it was possible that the drugs might separate into amorphous or crystalline forms (21). Therefore, such formulations were solid dispersions of amorphous (or crystalline) drugs rather than solutions of drugs in lipids (16, 22). Even when the drug initially remained amorphous in solid dispersions, it was possible that the drug could crystallize out during its expected shelf-life (21). All of these could defeat the purpose of developing a lipid-based formulation, where it is expected that the drug would remain in solution.

In 2009, Serajuddin *et. al.* (23) reported a novel approach of preparing solid lipid-based formulations where solutions of the drug in liquid lipid-surfactant mixtures were converted into solid forms by incorporating them into a solid polyethylene glycol (PEG) matrix. For this purpose, the formulations containing the drug, lipid, surfactant and PEG 3350 (m.p. 55–60°C) were heated to ~70°C, and the hot mixtures were filled into hard gelatin capsules. Upon cooling to room temperature, the formulation solidified, where the crystalline PEG 3350 formed the solid structure and the liquid lipid-surfactant mixture containing the dissolved drug dispersed in between PEG 3350 crystals as a separate phase. More recently, Shah and Serajuddin (24) developed another solid lipid-based formulation where instead of PEG 3350, poloxamer 188 was used as the solidifying agent. An added advantage of this formulation

was that poloxamer 188 also served as the emulsifying agent for the lipids used. It was, however, observed in both of these studies, that only the monoglycerides of medium chain fatty acids were amenable to solidification by PEG 3350 or poloxamer 188. The formulations could contain as much as 70-80% w/w liquid content. No such solidification of PEG 3350 and poloxamer 188, with relatively high lipid content, was observed when di- or tri-glycerides of medium chain fatty acids were used.

Since triglycerides of fatty acids, especially those of medium chain acids, are commonly used in lipid-based formulations, additional studies, which are reported here, were carried out to identify solidifying agents for formulations containing triglycerides. Preliminary studies indicated that lauroyl polyoxyl glyceride could serve as a suitable solidifying agent in formulations containing triglycerides. The present report describes the preparation and physicochemical characterization of the solid lipid-based formulations using lauroyl polyoxyl glycerides as the carrier that can be used to make solid dosage forms using hard gelatin capsules. The solid carrier is an amphiphilic (HLB ~14) and semisolid (m.p. ~ 44°C) excipient that is listed as lauroyl polyoxyl-32 glycerides in the United States Pharmacopoeia (USP-NF) and lauroyl macrogol-32 glycerides in the European Pharmacopoeia (EP). Since the excipient is obtained semi-synthetically from natural sources and contains multiple components, there could be variation in its properties depending on the sources. For this reason, materials obtained from two manufacturers (Acconon® C-44 , ABITEC; Gelucire® 44/14, Gattefosse) were examined to determine whether the source of the material affects the performance of the exipient as a solidifying agent for triglycerides. Probucol, which is a neutral molecule with an aqueous solubility of 0.002-0.005 μg/ml and an octanol-water logP value of 11 (25, 26), was used as a model drug to study the release of the drug from different oral solid dose formulations.

MATERIALS AND METHODS

Materials

Captex® 355 EP/NF (caprylic/capric triglyceride), Capmul® MCM NF (glyceryl caprylate/caprate), Capmul® PG-8 NF (propylene glycol monocaprylate) and Acconon® C-44 (lauroyl polyoxyl-32 glycerides, EP/NF) were supplied by ABITEC Corp., Columbus, OH, USA. Gelucire® 44/14 (lauroyl polyoxyl-32 glycerides, EP/NF) was obtained from Gattefosse Corp., Paramus, NJ, USA. Cremophor® EL (PEG-35 castor oil) was obtained from BASF Corp., Tarrytown, NY, USA. Probucol was purchased from Sigma Aldrich, St. Louis, MO, USA. All other chemicals and reagents used were of analytical grade or better.

Methods

Preparation of Formulation

Preliminary studies were conducted to determine whether lipids and the liquid surfactant were able to form solid systems with Acconon® C-44 and Gelucire® 44/14. Initially, the ability of Acconon® C-44 and Gelucire® 44/14 to solidify different lipids, such as Captex® 355 (triglyceride), Capmul® MCM (a monoglyceride) and Capmul® PG-8 (a propylene glycol monoester), at room temperature were determined. The samples were prepared at lipid to solidifying agent (Acconon® C-44 or Gelucire® 44/14) in ratios of 7:3, 6:4, 5:5, 4:6, and 3:7 w/w by melting the solidifying agents at ~65ºC and then mixing various lipids with them at the increased temperature. When the hot mixtures were cooled to room temperature (~20-25ºC), it was observed that only Captex® 355 solidified at all ratios with both Acconon® C-44 and Gelucire® 44/14, i.e., the lipid content was as high as 70% in the formulation. On the other hand, when Capmul® MCM and Capmul® PG-8 were used as lipids, the mixtures either did not solidify or

solidified only at low lipid to solidifying agent ratios. Therefore, only Captex® 355 was used here in the further studies carried out using Acconon® C-44 or Gelucire® 44/14.

Although the mixtures of Captex® 355 with Acconon® C-44 or Gelucire® 44/14 solidified at room temperature, the formulations did not disperse in the aqueous media to form microemulsions or emulsions. Instead there was a phase separation of Captex® 355 from the aqueous media. In other words, despite their amphiphilic nature, Acconon® C-44 and Gelucire® 44/14 were not able to emulsify the triglyceride. For this reason, one additional surface active agent, Cremophor® EL, which could also be solidified by Acconon® C-44 or Gelucire® 44/14, was incorporated into the system. Captex® 355 and Cremophor® EL were first mixed at 1:1 and 3:1 ratios, and the drug, probucol, was dissolved into them at a 60 mg/g concentration. The solutions were then mixed with molten Acconon® C-44 or Gelucire® 44/14 at the increased temperature of ~65ºC in 20-ml glass scintillation vials placed on hot plates. All samples were also vortexed for 2-3 minutes in the molten state to attain homogeneous mixing. The hot solutions were manually filled into #00 hard gelatin capsules (~1 gram). Before analysis, the capsules were stored at room temperature for at least 48 hours to ensure complete solidification of their contents. Formulations without the incorporation of drug were also filled in capsules to serve as controls.

Characterization of the formulation

All the solid preconcentrates containing Acconon® C-44 and Gelucire® 44/14 formed upon cooling, with and without the drug, were characterized by powder-X-ray diffractometry and differential scanning calorimetry. Selected solid systems were also observed using a microscope to observe their microstructures.

Powder X-ray diffractometry (P-XRD)

The P-XRD study was performed at room temperature using an X-ray diffractometer (X-ray Diffractometer XRD-6000, Shimadzu, Kyoto, Japan). The diffraction patterns were measured with a voltage of 40 kV and a current of 30 mA over a 2θ range of 10-80º using a step size of 0.02º at a scan speed of 4º/minute. The P-XRD intensities of solid preconcentrates were compared by measuring approximate peak intensities at 2θ = 23.1º and 23.2º, respectively, for Acconon® C-44 and Gelucire®44/14.

Differential scanning calorimetry (DSC)

The thermal characteristics of the solidifying agents (Acconon® C-44 and Gelucire® 44/14) and various formulations were determined by DSC (Pyris Diamond DSC-7, Perkin-Elmer, Waltham, MA, USA). The samples, accurately weighed within a range of 2 to 5 mg, were sealed into aluminum pans by crimping. The scans for all samples were recorded after holding for 5 minutes at the starting temperature (20ºC) and then heating from 20 to 60ºC with a heating rate of 5ºC/min under an extra dry nitrogen gas purge (20 ml/min).

Microscopic examination

Solid preconcentrates were analyzed using an optical microscope fitted with cross-polarizing lenses (Nikon Microscope Eclipse 50i, Morrell Instrument Co., Melville, NY, USA) and a confocal fluorescence microscope (Leica Microsystems Inc., Exton, PA, USA) with the wavelengths of 514 nm for excitation and 550 - 605 nm for emission using the bandpass filter of DD458-514 nm. The optical microscopic images were captured using a Nikon Digital Camera (DS 5000, Nikon Inc., Melville, NY, USA) with magnification of 100x. For fluorescence microscopy, Nile red, which is a fluorescent probe for lipids (27), was dissolved in a molten formulation. Two drops of the melt was placed on a glass slide and covered with a glass cover slip, and finally the edges of the cover slip were sealed with nail polish. The prepared slides were then allowed to cool in two different ways (a) under ambient condition (shock cooling) and (b) in an oven (GCA/

Precision Scientific, Chicago, IL, USA) from ~60 to 25ºC at a rate of 0.1ºC/min (controlled cooling).

Dispersion Testing

The efficiency of self-emulsification and dispersion of solid systems was assessed using the USP apparatus II (Paddle method; Distek Inc., NJ, USA) at 50 rpm and 37°C using 250 ml 0.01N HCL (pH~2) as the dispersion medium. Pipettes with siliconized tips were used to withdraw the aliquots from the dispersion vessels. Aliquots withdrawn from each vessel at 10, 15, 30, 45, 60, 120 and 180 minutes were placed in disposable plastic cuvettes (Beckman Coulter disposable cell, Beckman Coulter Inc., CA, USA) for particle size analysis using a Delsa Nano C Particle Analyzer (Beckman Coulter, Beckman Coulter Inc., CA, USA). Unfiltered samples were also analyzed for drug concentration in dispersion media. The aliquots were not filtered as the filtration could have reduced the drug concentration because some of the oil droplets in the dispersion fluid could be larger than the pore size of the 0.45 μm filter used. The volume of the dispersion medium in each vessel was kept constant by replacing the same volume of aliquot withdrawn with 0.01N HCl. To evaluate the effect of the drug loading on the emulsification of lipids from solid systems, the dispersion testing and particle size analysis were also carried out on the controls. All experiments were carried out in triplicate.

HPLC analysis

The HPLC analysis of probucol was carried out using a quaternary pump, an Agilent 1100 autosampler and a diode array detector (HP 1100 series, Agilent Technologies, Wilmington, DE, USA). The chromatographic column used was a C_8 Waters X-Bridge column (3.5μm), 4.6 mm x 150 mm. A methanol-water mixture (95:5 v/v) was used as the mobile phase at a rate of 1 ml/min, and the detection wavelength was set at 243 nm.

RESULTS AND DISCUSSION

Development of solid lipid-based formulations

The ability of lauroyl polyoxyl glycerides (Acconon® C-44 and Gelucire® 44/14) to solidify monoesters and triesters of medium chain fatty acids were compared in the present study. The monoesters used were glyceryl caprylate/caprate (Capmul® MCM) and PG monocaprylate (Capmul® PG-8), while the triester used was caprylic/capric triglyceride (Captex® 355). It was observed visually that Capmul® PG-8 could not be solidified by any of the solidifying agents used (Acconon® C-44 or Gelucire® 44/14), while Capmul® MCM could not be solidified by Gelucire® 44/14 at all and Acconon® C-44 could solidify it only at low lipid content in the mixture (40% w/w and lower). In contrast, the triester (triglyceride) Captex® 355 could be solidified at all lipids to surfactant ratios used (3:7 to 7:3, w/w), i.e., up to 70% w/w lipid could be solidified by Acconon® C-44 and Gelucire® 44/14. The powder XRD patterns of Captex® 355-Acconon® C-44 and Captex® 355-Gelucire® 44/14 mixtures in Figure 1 confirm the crystallinity of the solidifying agents in the mixtures. These results are contrary to what were observed earlier with PEG 3350 (23) and poloxamer 188 (24) as solidifying agents, which solidified monoesters and not the triesters. Thus, the results of the present study demonstrate a novel approach of solidifying triglycerides in dosage forms by using lauroyl polyoxyl glycerides as solidifying agents.

As mentioned earlier in the experimental section, the lipid (Captex® 355) did not disperse in aqueous media to form an emulsion or microemulsion when it was formulated alone with Acconon® C-44 or Gelucire® 44/14 and an additional surfactant was necessary to disperse the lipid in aqueous media. The powder XRD in Figure 2 shows that Cremophor® EL, which serves as an excellent dispersing agent for lipids in aqueous media (11, 12) could also be solidified by Acconon® C-44 and Gelucire® 44/14. Further, the 1:1-mixture of Captex® 355 and Cremophor® EL could be solidified by

Acconon® C-44 and Gelucire® 44/14 (Figure 3). The results similar to those in Figure 3 could be obtained when Captex® 355 and Cremophor® EL were used at other ratios (e.g., 3:1 and 1:3 w/w). These results, therefore, show that the lipid-based systems consisting of the triglyceride of medium chain fatty acids and a suitable surfactant may also be solidified by lauroyl polyoxyl glycerides (Acconon® C-44 or Gelucire® 44/14).

Physicochemical characterization of solid lipid-based formulations

Powder X-ray diffractometry (P-XRD)

Neat Acconon® C-44 and neat Gelucire® 44/14 are solid at room temperature. Their powder X-ray diffraction patterns showed similar crystalline peaks at 2θ = 19.1° and 23.1° (Figure 1). As the liquid Captex® 355, Cremophor® EL or the mixture of Captex® 355 and Cremophor® EL were added to the solid lauroyl polyoxyl glycerides, the consistency of the solids gradually changed from solid to semisolid, and visually, the liquid appeared to be

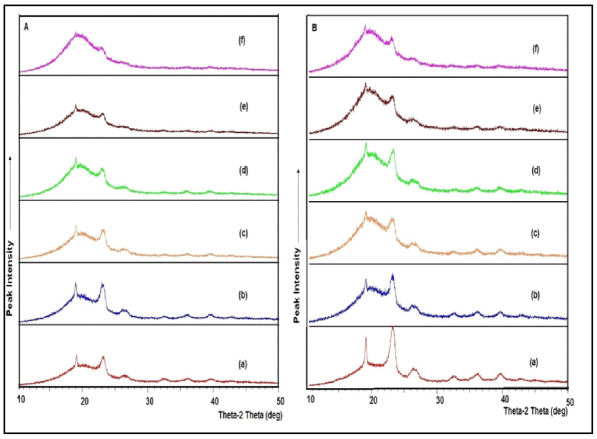

Figure 1 Powder XRD patterns of solid systems containing (A) Captex® 355/Acconon® C-44 and (B) Captex® 355/Gelucire® 44/14 mixtures. Key: (a) Neat lauroyl polyoxyl glyceride (Acconon® C-44 or Gelucire® 44/14), (b) Captex® 355/lauroyl polyoxyl glyceride (Acconon® C-44 or Gelucire® 44/14), 3:7, (c) Captex® 355/lauroyl polyoxyl glyceride (Acconon® C-44 or Gelucire® 44/14), 4:6 (d) Captex® 355/lauroyl polyoxyl glyceride (Acconon® C-44 or Gelucire® 44/14), 1:1 (e) Captex® 355/lauroyl polyoxyl glyceride (Acconon® C-44 or Gelucire® 44/14), 6:4 and (f) Captex® 355/lauroyl polyoxyl glyceride (Acconon® C-44 or Gelucire® 44/14), 7:3.

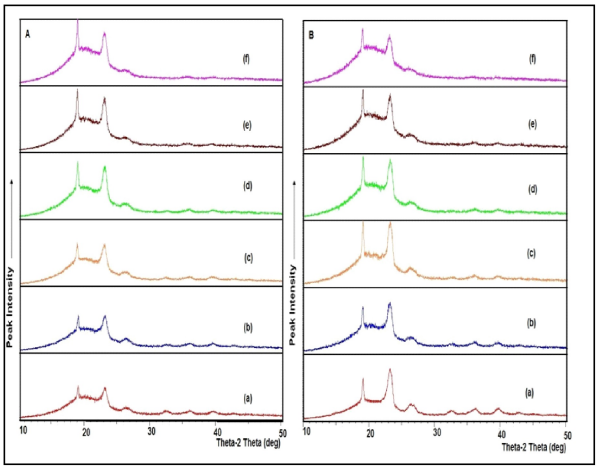

Figure 2 Powder XRD patterns of solid system containing (A) Cremophor® EL/Acconon® C-44 and (B) Cremophor® EL/Gelucire® 44/14 mixtures. Key: (a) Neat lauroyl polyoxyl glyceride (Acconon® C-44 or Gelucire® 44/14) (b) Cremophor® EL/lauroyl polyoxyl glyceride (Acconon® C-44 or Gelucire® 44/14), 3:7; (c) Cremophor® EL/lauroyl polyoxyl glyceride (Acconon® C-44 or Gelucire® 44/14), 4:6; (d) Cremophor® EL/lauroyl polyoxyl glyceride (Acconon® C-44 or Gelucire® 44/14), 1:1; (e) Cremophor® EL/lauroyl polyoxyl glyceride (Acconon® C-44 or Gelucire® 44/14), 6:4; and (f) Cremophor® EL/lauroyl polyoxyl glyceride (Acconon® C-44 or Gelucire® 44/14), 7:3.

uniformly distributed in the solid matrices as there were no phase separation or syneresis of liquids. Figures 1, 2 and 3 show the P-XRD patterns of Acconon® C-44 and Gelucire® 44/14 with increasing concentrations of, respectively, Captex® 355, Cremophor® EL, and the 1:1-mixture of Captex® 355 and Cremophor® EL. Characteristic powder XRD patterns of Acconon® C-44 and Gelucire® 44/14 were observed. The intensity of the peaks decreased and there were greater amorphous regions in the XRD patterns as the fraction of the liquid component (lipid, surfactant or their mixtures) in the solid or semisolid systems increased. The reduction in the intensity of the P-XRD peaks with the addition of the lipid, surfactant or the lipid-surfactant mixture also followed the change in the consistency of solids to semisolids. The consistency of the mixtures was sufficiently hard that they could be formulated as oral solid dosage forms in hard gelatin capsules.

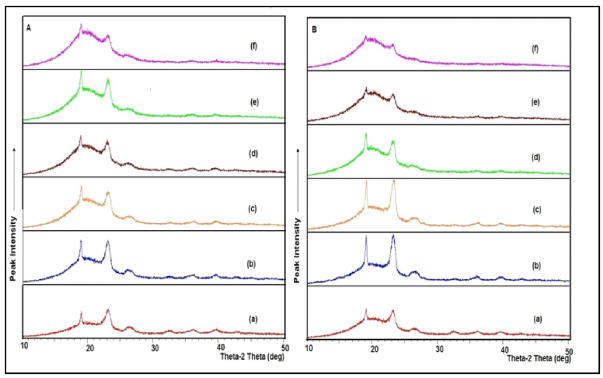

Figure 3 Powder-XRD patterns of solid systems incorporating liquid 1:1-mixture of Captex® 355 and Cremophor® EL in (A) Acconon® C-44 and (B) Gelucire® 44/14. Key: (a) Neat lauroyl polyoxyl glyceride (Acconon® C-44 or Gelucire® 44/14), (b) liquid lipid-surfactant mixture/lauroyl polyoxyl glyceride, 3:7; (c) liquid lipid-surfactant mixture/lauroyl polyoxyl glyceride, 4:6, (d) liquid lipid-surfactant mixture/lauroyl polyoxyl glyceride, 1:1; (e) liquid lipid-surfactant mixture/lauroyl polyoxyl glyceride, 6:4; and (f) liquid lipid-surfactant mixture/lauroyl polyoxyl glyceride, 7:3.

Figures 1, 2 and 3 represent powder XRD patterns of solid systems without any drug present. The presence of probucol in the solid systems (data not shown) showed no change in the XRD patterns, indicating that the presence of the drug did not have any impact on the crystallinity of Acconon® C-44 and Gelucire® 44/14.

There were also no drug peaks present. For some samples containing probucol, the powder XRD analysis was repeated after 3 months of storage at room temperature. No change in the XRD patterns was observed, indicating that the solid systems are physically stable. Additionally, the similar powder XRD patterns of neat Acconon® C-44 and Gelucire® 44/14 and their mixtures with lipid and surfactant shown in Figures 1, 2 and 3 indicate that both solidifying agents have similar crystallinity and behave similarly in oral solid dosage formulations.

Differential Scanning Calorimetry (DSC)

The results of the DSC study of neat Acconon® C-44 and Gelucire® 44/14 and the various formulations developed here are in agreement with the P-XRD analysis. Figure 4 shows DSC scans of Acconon® C-44 and Gelucire® 44/14 and their combinations with the Captex® 355-Cremophor® EL mixture. The melting points of the neat Acconon® C-44 and Gelucire® 44/14 were observed to be ~44ºC. Melting endotherms of the solids broadened and peak melting temperatures decreased as liquid Captex® 355, Cremophor® EL or the Captex® 355-Cremophor® EL mixture was added to them. Figure 4 shows the DSC scans obtained when increasing amounts of the 1:1-Captex® 355-Cremophor® EL mixture was added to Acconon® C-44 (Figure 4A) and Gelucire® 44/14 (Figure 4B), where endotherms, although broadened, were observed with the liquid component as high as 70% w/w. The lowering of the endothermic peaks is in agreement with the visual observation that Acconon® C-44 and Gelucire® 44/14 gradually changed from solid to semisolid with the addition of increasing amounts of liquid components. There was no change in DSC scans when the model drug probucol was incorporated into the formulations. In agreement with the P-XRD results, Acconon® C-44 and Gelucire® 44/14 maintained their crystallinity in presence of the liquid components in proportion to their concentrations in the formulations. The enthalpy of melting of Acconon® C-44 and Gelucire® 44/14 decreased linearly as concentrations of the solidifying agents decreased, i.e., the concentration of the lipid-surfactant component increased. Similar results have been reported previously by Li *et al.* (23).

Microscopic examination

The microstructure of solid lipid-based systems is shown in Figure 5. The optical microscopic images show that the liquid mixture (Captex® 355 and Cremophor® EL) exists as a separate phase trapped into solid lauroyl polyoxyl glyceride clusters (Figures 5B and C). To bolster the results of optical microscopy, the confocal fluorescence microscopic examination was performed using Nile red as the fluorescent probe to visualize the non-crystalline region of

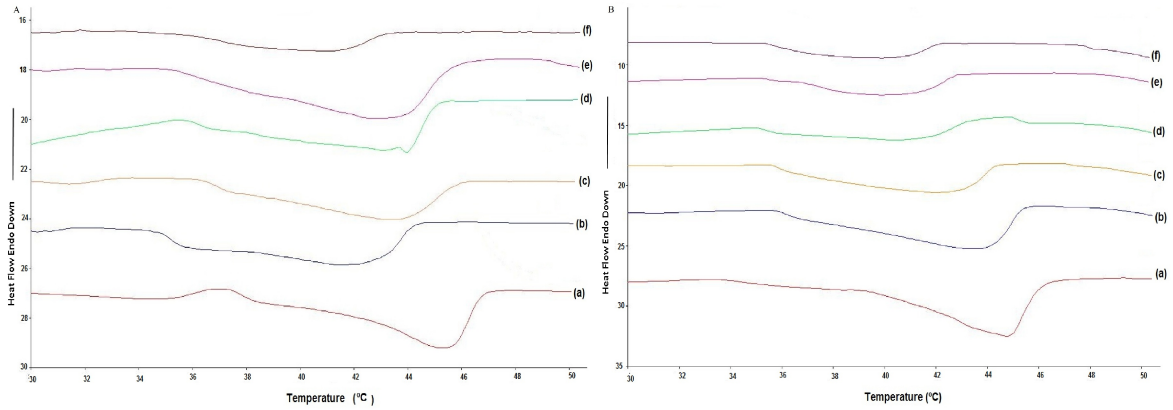

Figure 4 DSC scans of solid systems incorporating liquid 1:1-mixture of Captex® 355 and Cremophor® EL in (A) Acconon® C-44 and (B) Gelucire® 44/14. Key: (a) Neat lauroyl polyoxyl glyceride (Acconon® C-44 or Gelucire® 44/14), (b) liquid lipid-surfactant mixture/lauroyl polyoxyl glyceride, 3:7; (c) liquid lipid-surfactant mixture/lauroyl polyoxyl glyceride, 4:6, (d) liquid lipid-surfactant mixture/lauroyl polyoxyl glyceride, 1:1; (e) liquid lipid-surfactant mixture/lauroyl polyoxyl glyceride, 6:4; and (f) liquid lipid-surfactant mixture/lauroyl polyoxyl glyceride, 7:3.

the system. Again, two phases were detected with crystalline spherulites of polyoxyl glycerides (visualized in white) and a liquid mixture (visualized in red) located in between polyoxyl glyceride domains (Figure 5D).

Several reports in the literature show that solid polyethylene glycols (PEG) form crystalline spherulites upon cooling (28, 29, 30). As seen in Figure 5A, the cross-polarized optical microscope image of the neat lauroyl polyoxyl glyceride also shows a birefringent image of crystalline spherulites. There were only minimal gaps between the spherulites in Figure 5A, and with the addition of the lipid-surfactant mixture, the gap between the spherulites increased. It has been reported previously that polymeric materials like solid PEG may contain both crystalline and amorphous domains and a liquid may be trapped in the amorphous regions of the polymeric structures (31). It appears that a similar mechanism exists in the trapping or immobilization of the liquid lipid and surfactant by lauroyl polyoxyl glycerides (Figures 5B and C).

It has been reported in the literature that the cooling rate of the melts may have a major impact on the spherulite formation (32). Such an effect was also observed in the present study. Figure 5B shows the photomicrograph of a solid system containing 50% liquid (1:1 w/w Captex® 355 and Cremophor® EL) and 50% lauroyl polyoxyl glycerides, where the glass slide containing the molten formulation was suddenly exposed to room temperature. Since the quick cooling did not allow the spherulites to grow, irregular, needle-shaped crystals of lauroyl polyoxyl glycerides were observed in Figure 5B. In contrast, Figure 5C shows the microscopic image where the glass-slides were cooled at a controlled rate from 60ºC to room temperature (~ 25ºC) over a period of 4 hours in an oven to allow the spherulites to grow. Thus, when the molten preconcentrate was cooled slowly at a controlled rate, the spherulites were well-defined as shown in Figure 5C. Unlike the neat polyoxylglycerides, the spherulites were separated from each other due to the presence of a liquid phase in between them. The confocal fluorescent microscopic image in Figure 5D further differentiates the two phases in the solid system. Since the Nile red dye is soluble only in the lipid phase (27), the figure clearly shows that the lipid was trapped in the microstructure of lauroyl polyoxyl glycerides.

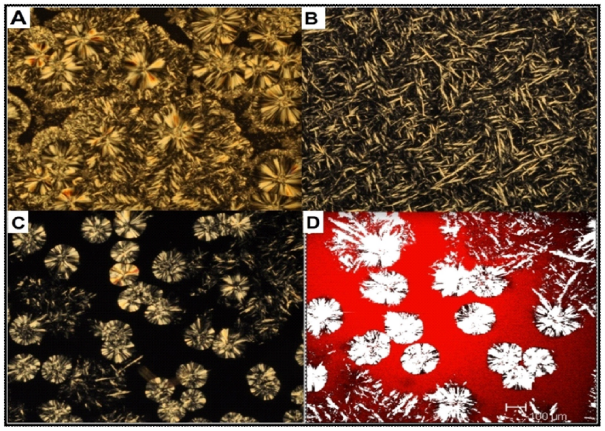

Figure 5 Photomicrographs of solid systems incorporating liquid 1:1-mixture of Captex® 355 and Cremophor® EL in lauroyl polyoxylglycerides (Acconon® C-44). Key: (A) Photomicrograph of neat lauroyl polyoxylglycerides obtained from cross-polarized optical microscope (controlled cooling at the rate of 0.1°C/min), (B) photomicrograph under cross-polarized optical microscope of 1:1 liquid mixture/lauroyl polyoxyl glyceride (1:1) cooled to room temperature by rapid cooling, (C) photomicrograph under cross-polarized optical microscope of 1:1 liquid mixture/lauroyl polyoxyl glyceride (1:1) cooled to room temperature at the controlled rate of 0.1°C/min over a period of 4 hours, and (D) confocal fluorescence photomicrograph of 1:1 liquid mixture/lauroyl polyoxyl glyceride (1:1) cooled to room temperature at the controlled rate of 0.1°C/min over a period of 4 hours, showing that the Nile red is solubilized in the liquid phase.

Dispersion Test

The dispersion test was performed to determine the ease of emulsification of the formulations and whether the drug precipitated during dispersion. Both Acconon® C-44 and Gelucire® 44/14 dispersed completely in aqueous media. However, when Captex® 355 alone was mixed with them, the lipid did not disperse in the aqueous media. Despite their amphiphilic nature, the solidifying agents did not have adequate surface activity to emulsify the lipid as large oil globules floating on surfaces of dispersion media were observed. Therefore, based on the results from previous studies (12), a second surfactant, Cremophor® EL, which has good emulsifying properties for Captex® 355 was incorporated into the system. The dispersion profiles of the solid systems of 1:1 and 3:1 mixture of Captex® 355 and Cremophor® EL produced by using Acconon® C-44 and Gelucire® 44/14, respectively, are shown in Figures 6 and 7. The exposure of the formulation containing Captex® 355-Cremophor® EL mixture to 0.01N HCl resulted in opaque emulsions within 35 to 40 minutes. In all formulations, the drug released was more than 80% at the end of 3 hours. During dispersion testing, a particle size analysis of each sample was performed, and the results are shown in the Figures 8 and 9.

In the solid systems containing the lipid, the particle sizes of the lipid globules produced in the dispersion media were in the range from 200 to 450 nm. The presence of the drug had no significant effect on the particle size.

Although only the dispersion test data at pH 2 (0.01N HCl) are reported here, separate tests at pH 6.8 showed that there was no effect of pH on the dispersion of probucol and the particle size of lipid globules. This is possibly because the drug as well as the lipids and the surfactant used are neutral and their physical and chemical properties are not pH-dependent.

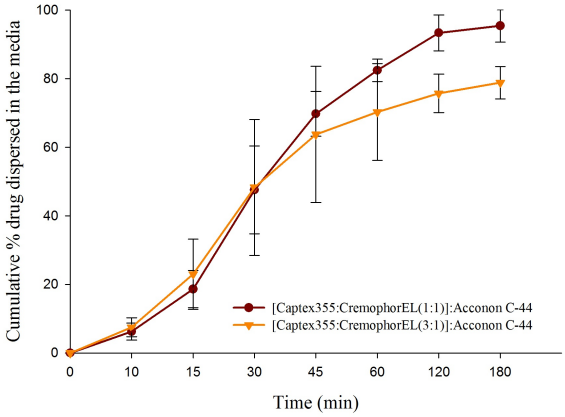

Figure 6 Dispersion profiles of solid formulations (hard gelatin capsule) containing 50% w/w of Acconon® C-44 and 50% w/w of liquid lipid-surfactant mixtures (Captex® 355 and Cremophor® EL at 1:1 and 3:1 w/w ratios) according to the USP apparatus II at 50 RPM using 250 ml 0.01N HCl as dispersion medium at 37 °C.

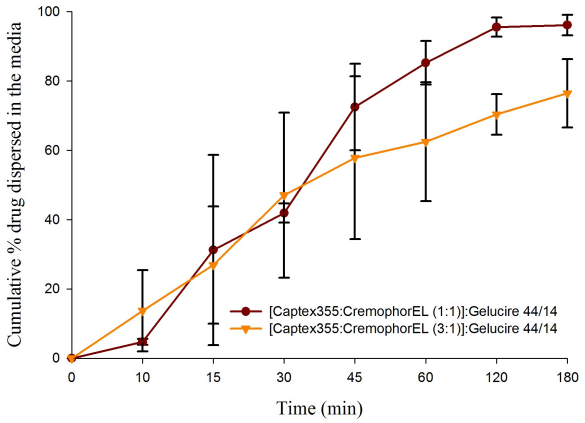

Figure 7 Dispersion profiles of solid formulations (hard gelatin capsule) containing 50% w/w of Gelucire® 44/14 and 50% w/w of liquid lipid-surfactant mixtures (Captex® 355 and Cremophor® EL at 1:1 and 3:1 w/w ratios) according to the USP apparatus II at 50 RPM using 250 ml 0.01N HCl as dispersion medium at 37 ºC.

Microemulsions were not formed in all the formulations. However, the particles were in the submicron range (<650nm). Particle size analysis of the solid system, after dispersion in the aqueous medium, was performed where the solidifying agent was kept constant at 30% and the remaining 70% liquid component had varying amounts of Captex® 355 and Cremophor® EL. These results indicated that the particle size of the oil globules decreased to microemulsion range with an increase in the concentration of Cremophor® EL.

During the dispersion testing several aliquots were examined using a microscope to detect any formation of birefringent drug crystals. However, no such crystals were observed. Nor did the centrifugation of the aliquots (up to 8000 RPM) show any separation of the solid phase. Further, as shown in Figures 8 and 9, there was no significant change in particle size with time during the dispersion test. These findings led to the conclusion that there was no precipitation of the drug from the solid formulations upon dispersion in aqueous media. However, if there were any, the drug was amorphous and had the same particle size as that of the emulsion globules.

CONCLUSION

The results of the present study provide a novel approach in developing solid self-emulsifying lipid-based drug delivery systems, where a liquid medium-chain triglyceride may be incorporated into the solid microstructure of lauroyl polyoxyl glyceride (Acconon® C-44 and Gelucire® 44/14). Although Acconon® C44

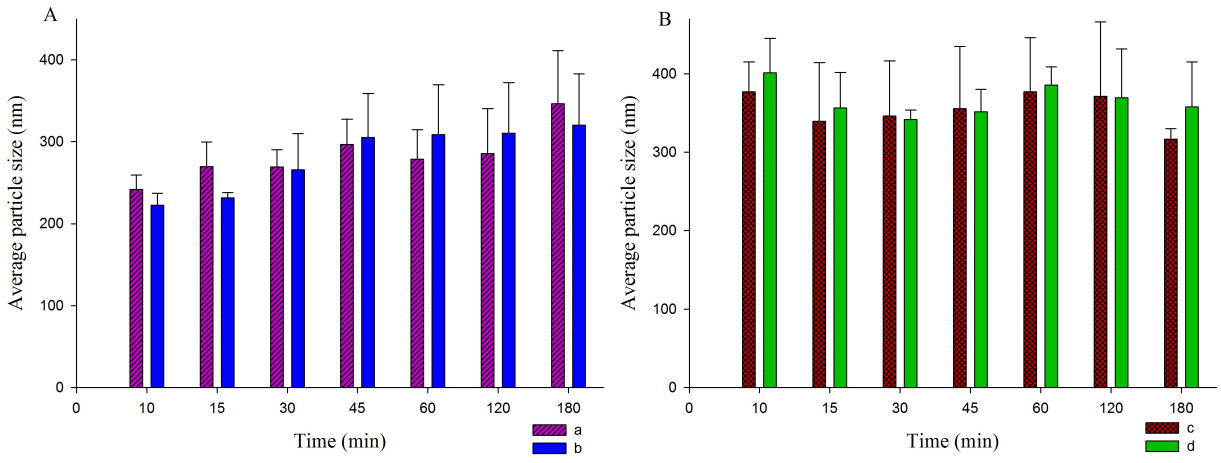

Figure 8 The average particle sizes of emulsion globules produced by (A) the 1:1 w/w mixture of Captex® 355 and Cremophor® EL and (B) the 3:1 w/w mixture of Captex® 355 and Cremophor® EL as a function of time, when both mixtures were converted to solid systems using Acconon® C-44 as the solidifying agent (50:50 w/w of liquid to Acconon® C-44). Key: (a) without the drug, (b) with the drug, (c) without the drug and (d) with the drug.

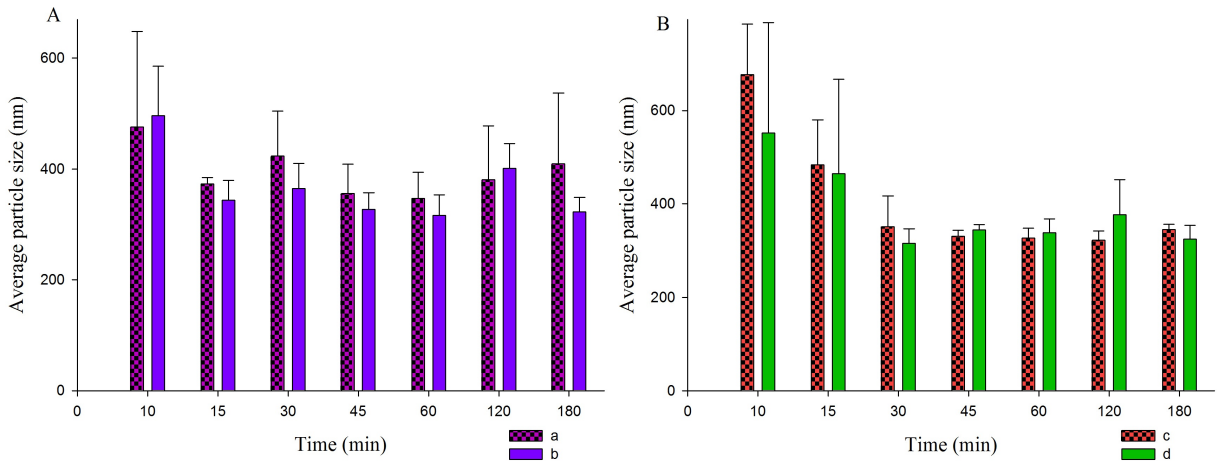

Figure 9 The average particle sizes of emulsion globules produced by (A) the 1:1 w/w mixture of Captex® 355 and Cremophor® EL and (B) the 3:1 w/w mixture of Captex® 355 and Cremophor® EL as a function of time, when both mixtures were converted to solid systems using Gelucire® 44/14 as the solidifying agent (50:50 w/w of liquid to Gelucire® 44/14). Key: (a) without the the drug, (b) with the the drug, (c) without the the drug and (d) with the the drug.

and Gelucire® 44/14 are surface active by nature, a co-surfactant was required to increase the dispersion rate and drug release from the system. Moreover, the solid may be filled into hard gelatin capsules providing the advantage that the drug is solubilized in the lipid. The model drug probucol remained in the solubilized state in lipids and no phase separation or crystallization of the drug was observed. The formulations dispersed in the aqueous media producing fine particles of lipids in a range from 200 to 450 nm with the drug remaining dissolved in the lipid globules.

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REFERENCES

1 Lipinski C.A., Lombardo F., Dominy B.W., Feeney P.J., Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug. Del. Rev., 23:3-25, 1997

2 Lipinski C.A., Drug-like properties and the causes of poor solubility and poor permeability, J. Pharmacol. Toxicol. Methods, 44:235-249, 2000

3 Charman S.A., Charman W.N., Rogge M.C., Wilson T.D., Dutko F.J., Pouton C.W., Self-emulsifying drug delivery systems: formulation and biopharmaceutic evaluation of an investigational lipophilic compound, Pharm. Res., 9:87-93, 1992

4 Shah N.H., Carvajal M.T., Patel C.I., Infeld M.H., Malick A.W., Self-emulsifying drug delivery systems (SEDDS) with polyglycolyzed glycerides for improving in vitro dissolution and oral absorption of lipophilic drugs, Int. J. Pharm., 106:15-23, 1994

5 Khoo S.M., Porter C.J.H., Charman W.N., The formulation of halofantrine as either non-solubilising PEG 6000 or solubilising lipid based solid dispersions: Physical stability and absolute bioavailability assessment, Int. J. Pharm., 205:65-78, 2000

6 Caliph S.M., Charman W.N., Porter C.J.H., Effect of short-, medium-, and long-chain fatty acid based vehicles on the absolute oral bioavailability and intestinal lymphatic transport of halofantrine and assessment of mass balance in lymph cannulated and non cannulated rats, J. Pharm. Sci., 89:1073-1084, 2000

7 Odeberg J.M., Kaufmann P., Kroon K.G., Höglund P., Lipid drug delivery and rational formulation design for lipophilic drugs with low oral bioavailability, applied to cyclosporine, Eur. J. Pharm. Sci., 20:375-382, 2003

8 Porter C.J.H., Trevaskis N.L., Charman W.N., Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs, Nature Rev. Drug Discov., 6:231-248, 2007

9 O'Driscoll C.M., Griffin B.T., Biopharmaceutical challenges associated with drugs with low aqueous solubility - the potential impact of lipid-based formulations, Adv. Drug. Del. Rev., 60:617-624, 2008

10 Serajuddin A.T.M., Li P., Haefele T.F., Development of lipid-based drug delivery systems for poorly water-soluble drugs as viable oral dosage forms, American Pharmaceutical Review, 11:34-42, 2008

11 Prajapati H.N., Dalrymple D.M., Serajuddin A.T.M., A Comparative evaluation of mono-, di-and triglyceride of medium chain fatty acids by lipid/surfactant/water phase diagram, solubility determination and dispersion testing for application in pharmaceutical dosage form development, Pharm. Res., 29:285–305, 2012

12 Prajapati H.N., Patel D.P., Patel N.G., Dalrymple D.M., Serajuddin A.T.M., Effect of difference in fatty acid chain lengths of medium-chain lipids on lipid-surfactant-water phase diagrams and drug solubility, J.Excipients Food Chem., 2(3):73-87, 2011

13 Hauss D.J., Oral lipid-based drug delivery—a case of implementation failing to keep up with innovation. American Pharmaceutical Review, September/October, 2006

14 Strickly R.G., Currently marketed oral lipid-based dosage forms: Drug products and excipients, in Hauss D (ed.), Lipid-based formulations for oral drug delivery: Enhancing bioavailability of poorly water-soluble drug; Informa Healthcare, New York, NY, pp. 1-31, 2007

15 Mullertz A., Ogbonna A., Ren S., Rades T., New perspectives on lipid and surfactant based drug delivery systems for oral delivery of poorly soluble drugs, J. Pharm. Pharmacol., 62:1622-1636, 2010

16 Vasanthavada M., Serajuddin A.T.M., Lipid-based self-emulsifying solid dispersions, in Hauss D (ed.), Lipid-based formulations for oral drug delivery: Enhancing bioavailability of poorly water-soluble drug; Informa Healthcare, New York, NY, pp. 149-183, 2007

17 Jannin V., Musakhanian J., Marchaud D., Approaches for the development of solid and semi-solid lipid-based formulations, Adv. Drug. Del. Rev., 60:734-746, 2008

18 Serajuddin A., Sheen P.C., Mufson D., Bernstein D.F., Augustine M.A., Effect of vehicle amphiphilicity on the dissolution and bioavailability of a poorly water soluble drug from solid dispersions, J. Pharm. Sci., 77:414-417, 1988

19 Aungst B.J., Nguyen N.H., Rogers N.J., Rowe S.M., Hussain M.A., White S.J., Shum L., Amphiphilic vehicles improve the oral bioavailability of a poorly soluble HIV protease inhibitor at high doses, Int. J. Pharm. 156:79-88, 1997

20 Varma M.V.S., Panchagnula R., Enhanced oral paclitaxel absorption with vitamin E-TPGS: Effect on solubility and permeability in vitro, in situ and in vivo, Eur. J. Pharm. Sci. 25:445-453, 2005

21 Serajuddin A.T.M., Solid dispersion of poorly water soluble drugs: early promises, subsequent problems, and recent breakthroughs, J. Pharm. Sci. 88:1058-1066, 1999

22 Vasanthavada M., Tong W.Q., Serajuddin A.T.M., Development of solid dispersions for poorly water-soluble Drug, in R.Liu (ed.), Water-insoluble drug formulations; Informa Healthcare, New York, NY, pp. 499-529, 2008

23 Li P., Hynes S.R., Haefele T.F., Pudipeddi M., Royce A.E., Serajuddin A.T.M., Development of clinical dosage forms for a poorly water soluble drug II: Formulation and characterization of a novel solid microemulsion preconcentrate system for oral delivery of a poorly water soluble drug, J. Pharm. Sci. 98:1750-1764, 2009

24 Shah A., Serajuddin A.T. M., Development of Solid SEDDS, I: Use of Poloxamer 188 as both solidifying and emulsifying agent, Pharm. Res., DOI 10.1007/s11095-012-0704-x (published online: 28 February, 2012)

25 Yagi N., Terashima Y., Kenmotsu H., Sekikawa H., Takada M., Dissolution behavior of probucol from solid dispersion systems of probucol-polyvinylpyrrolidone, Chem. Pharm. Bull., 44:241-244. 1996

26 Zaghloul A., Khattab I., Nada K.A., Al-Saidan S., Preparation, characterization and optimization of probucol self-emulsified drug delivery system to enhance solubility and dissolution, Pharmazie, 9: 654-660, 2008

27 Greenspan P., Fowler S.D., Spectrofluorometric studies of the lipid probe, Nile red, J. Lipid Res., 26: 781-9, 1985

28 Strawhecker K., Manias E., Crystallization behavior of poly(ethylene oxide) in the presence of Na montmorillonite fillers, Chemistry of Materials 15:844-849, 2003

29 Gines J., Arias M., Moyano J., Sanchez-Soto P., Thermal investigation of crystallization of polyethylene glycols in solid dispersions containing oxazepam, Int. J. Pharm., 143:247-53, 1996

30 Gránásy L., Pusztai T., Tegze G., Warren J.A., Douglas J.F., Growth and form of spherulites. Physical Review E, 72:011605, 2005

31 Morris K.R., Knipp G.T., Serajuddin A.T.M., Structural properties of polyethylene glycol-polysorbate 80 mixture, a solid dispersion vehicle, J. Pharm. Sci. 81:1185-1188, 1992

32 Sutananta W., Craig D.Q.M., Newton J.M., An investigation into the effect of preparation conditions on the structure and mechanical properties of pharmaceutical glyceride bases, Int. J. Pharm., 110:75-91, 1994