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ORIGINAL ARTICLE

Role of medium-chain fatty acids in the emulsification mechanistics of self-micro-emulsifying lipid formulations

Naser M.Y. Hasan *

School of Pharmacy, Taif University, Saudi Arabia

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KEYWORDS

SEDDS;
SMEDDS;
Lipid formulations;
Medium chain mono- and glycerides;
Poorly water-soluble compounds

Abstract *Purpose:* The objective of the present study was to design and develop stable o/w microemulsions comprising Miglyol 812, Imwitor 988 and Tagat TO as a non ionic surfactant. This was based on particle size measurements and phase behavior studies. The empirical role of incorporating medium-chain mono/di-glycerides in the lipid matrix in the mechanistic processes of emulsification was also established in various simulating physiological conditions.

Methods: The efficiency of self-emulsification was evaluated under conditions of varying key compositions in the lipid mixtures; oil, cosurfactant and surfactant. Droplet diameter was measured using laser diffraction and light scattering techniques. Equilibrium phase studies were performed and phase boundaries were determined for the lipid–water systems.

Results: Microemulsion systems were produced from blends of Miglyol 812, Imwitor 988 and Tagat TO. An optimized formulation consisted of {Miglyol 812/Imwitor 988} and Tagat TO spontaneously self-emulsified in water producing dispersions with droplet diameters of ~50 nm. Phase equilibrium diagrams have revealed significant enhancement in the water-solubilized region (L_2) without any presence of liquid crystalline materials.

Conclusions: Potential SMEDDS formulations for the bioavailability enhancement of poorly water-soluble compounds were developed by mixing blends of {Miglyol 812/Imwitor 988} and Tagat TO as a non-ionic surfactant. 'Diffusion and stranding' appears to be the dominant mechanism of emulsification.

1. Introduction

Approximately 40% of new APIs exhibit poor aqueous solubility and present a major challenge to modern drug delivery system because of their low bioavailability (Kyatanwar et al., 2010). Hence, one of the most persistent challenges faced by formulation scientists has been to find methods to improve

* Tel.: +966 501819552.
E-mail address: n.hasan@tu.edu.sa.

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the bioavailability of poorly water-soluble drugs. A drug must almost invariably be in solution within the gastrointestinal (GI) tract before it can cross the GI mucosa, thus poor water solubility may lead to incomplete and erratic absorption. Several approaches are being used to overcome these challenges such as, salt formations, micronization, solid dispersion, complexation with cyclodextrins, and incorporation of the poorly-water soluble active component into inert lipid vehicles such as oils, surfactant dispersions, self-emulsifying formulations and liposomes (Stegemanna et al., 2007).

Nonetheless, Lipid based technology as an approach to enhance the bioavailability of poorly water soluble compounds for oral application has received great attention by formulation scientists and hence, witnessed the introduction of successful products on the market including Neoral® (Cyclsoprin A, Novartis) (Murdandea and Gumkowskia, 2008), Kaletra® (Lopinavir and Ritonavir, Abbott) (Lugt et al., 2009), Fortovase® (Saquinavir, Roche) (Grovea and Mullertzb, 2006), however, in 2006 Fortovase was removed from the market due to lack of demand (Wadhwa et al., 2012), Targretin® (Bexarotene, Ligand) (Chen et al., 2013), Agenerase® (Amprenavir, GlaxoSmithKline) (Suman et al., 2009), Lipirex® (Fenofibrate, Sanofi-Aventis) (Singh et al., 2009), Rocaltrol® (Calcitriol, Roche) (Kubisaa et al., 2012) and Avodart® (Dutasteride, GlaxoSmithKline) (Choo et al., 2013)

Self-emulsifying drug delivery systems (SEDDS) are isotropic mixtures of oils and non-ionic surfactants which spontaneously emulsify in water upon gentle agitation producing fine oil in water (o/w) dispersions of droplets $< 5\,\mu m$ (Shah et al., 1994). On the other hand, self-micro-emulsifying drug delivery system (SMEDDS) is the name given to a drug vehicle consisting of oil or modified oils, surfactant and co-surfactant mixtures, solid or liquid at ambient temperature, which emulsifies spontaneously when mixed with water under gentle agitation forming a o/w microemulsion of droplets with diameters between 5 and 140 nm (Farah et al., 1993). Both SEDDS and SMEDDS can provide a reservoir of drug dissolved in the lipid matrix, which upon administration and making contact with gastrointestinal fluids spontaneously emulsify producing oil-in-water dispersions of small particle size with large surface area available for drug diffusion (Hasan, 2004). Thus, lipid based formulations represent an efficient vehicle for the *in vivo* administration of oral delivery of lipophilic drugs, with a possible alternative to tablets and capsules, provided however, that the drug has adequate solubility in the oil system.

However, In order to facilitate the prospect of formulation design using lipid based technology, lipid systems were classified by Pouton (2000, 2006) into Type I, II, III and IV based on various physicochemical factors such as, the hydrophilicity of the oil mixture, particle size of the resultant dispersion and the formulation digestibility (Table 1).

The lipid class system by Pouton (2006) has prompted the investigation of how key elements in the oil composite can influence the behavior of these systems and hence impact on the physical state of the drug after dispersion. Among these factors are included, type of oil, the use of co-surfactants, oil/co-surfactant ratio, the HLB of the surfactant and the inclusion of hydrophilic cosolvents (Hasan, 2004). Type I formulations comprise drug in solution in triglycerides and/ or mixed glycerides. Type II lipid formulations are defined as self-emulsifying formulations that consist of water insoluble components; the drug is dissolved in triglycerides and/ or mixed glycerides blended with non-ionic surfactants of HLB between 10 and 12. Type III systems are also called self-micro-emulsifying systems, as optical clarity can be achieved with these formulations. These systems besides triglycerides and/ or mixed glycerides contain hydrophilic components including hydrophilic nonionic surfactants (HLB > 12) and or hydrophilic co-solvents. Type III formulations are further sub-classified into Type IIIA and Type IIIB addressing the degree of hydrophilicity of this type. This renders Type IIIB relatively more hydrophilic than Type I, Type II or Type IIIA, which is likely to result in a colloidal solution of drug and oil in aqueous micelles and hence presents high risk of drug precipitation. Type IV formulations do not contain natural lipids and represent the most hydrophilic formulations.

An archetypal example of a Type III system is the reformulation of cyclosporine A as Neoral® (Pouton, 1999) (Table 2). The drug was more available from the Neoral than the earlier 'Sandimmune' formulation, which was a coarsely emulsifying system (Muller et al., 1994). This might be due to the fact that the coarse emulsion produced by the Sandimmune formulation could not be reduced to colloidal dimensions because of limited digestion (Pouton, 2000). An example of a Type IV formulation is the current capsule formulation of the HIV protease

Table 1 Typical properties of Type l, ll, lll and IV lipid formulations (Pouton, 2006).

	Increasing hydrophilic content →				
	Type I	Type II	Type IIIA	Type IIIB	Type IV
Typical composition (%)					
Triglycerides or Mixed glycerides	100	40–80	40–80	< 20	0
Surfactants	0	20–60 (HLB < 12)	20–40 (HLB > 12)	20–50 (HLB > 12)	0–20 (HLB < 12) 20–80 (HLB > 12)
Hydrophilic cosolvents	0	0	0–40	20–50	0–80
Particle size of dispersion (nm)	Coarse	100–250	100–250	50–100	< 50
Significance of aqueous dilution	Limited importance	Solvent capacity unaffected	Some loss of solvent capacity	Significant phase changes and potential loss of solvent capacity	Significant phase changes and potential loss of solvent capacity
Significance of digestibility	Crucial requirement	Not crucial but likely to occur	Not crucial but may be inhibited	Not required and unlikely to happen	Not required and unlikely to happen

Table 2 Composition of Neoral® compared with Sandimunn® (Lawrence, 1996).

Neoral®	Amount (mg)	Sandimunn®	Amount (mg)
Cyclosporin	100	Cyclosporin	100
1,2 propylene glycol	75	Ethanol	100
Ethanol	150	Maize oil	416
Partial glycerol transesterified corn oil	345	Transesterified triglyceride and Polyalkaline polyol (Labrafil M2126)	300
Cremophor RH 40	405		
Total amount per dose	1075	Total amount per dose	916

inhibitor amprenavir (Agenerase®) which contains TPGS as a surfactant and PEG 400 and propylene glycol as co-solvents (Suman et al., 2009).

This investigation focuses on the role of medium-chain mono/diglycerides in aiding the emulsification of lipid mixtures by conducting phase behavior studies to establish the mechanistic processes involved. It is also designed to elucidate the effects of mixture composition, emulsification temperature and media on the emulsification performance. As a result, potential self-microemulsifying formulations representing Type II systems composed of water dispersable materials have been developed to improve the bioavailability of poorly water-soluble compounds.

2. Materials and methods

2.1. Materials

Miglyol 812 (medium chain triglyceride) and Imwitor 988 (C_8/C_{10} mono/diglycerides) were supplied by Condea Chemie GmbH. Tagat TO (PEG-(25)-glyceryl trioleate) was supplied by Goldschmidt AG, Germany. The fatty acid distribution in Miglyol 812 according to the manufacturer is: caprylic (C_8): 50–65%, capric (C_{10}): 30–45%, caproic (C_6): $<2\%$ and Lauric acid C_{12}: $<3\%$. Imwitor 988 is approximately a 1:1 mixture of C_8/C_{10} mono/diglycerides with 2% free glycerol. Tagat TO is an ethoxylated triglyceride of commercial oleic acid; 65% oleic with the remainder mainly myristic C_{14}, palmitic C_{16}, stearic C_{18}, and linoleic C_{18}: 2. Simulated Gastric Fluid (SGF) without pepcin; 2.4 g NaCl and 7 ml HCl/1000 ml water, supplied by Sigma. All water used was Mili Q water.

2.2. Methods

2.2.1. Self-emulsification

Mixtures of oil and surfactant were produced by accurately weighing ingredients into screw-capped glass vials with tight closures followed by vortexing. Various oil blends of {Miglyol 812/Imwitor 988} were mixed with increasing concentrations of Tagat TO. An amount of 1gm of each mixture was introduced into 100 ml of Mili Q water in a 500-ml glass beaker held at 37 °C in a thermostatically controlled water bath. All materials were pre-equilibrated to the appropriate temperature. Agitation was provided by gentle shaking on a mechanical shaker at 100 oscillation per min.

2.2.2. Analysis of mean emulsion droplet diameter (MEDD)

Two techniques were used to measure the MEDD of the self-emulsified systems (with appropriate dilution). Low angle laser light diffraction (Malvern Mastersizer X) with small volume cell using 45 mm lens was used for emulsions with droplet distributions above 1 μm. Quasi-elastic light scattering (QELS, Malvern model LO-C photon correlation spectrometer) was used to analyze samples of submicron dispersions. For both techniques experiments were performed in triplicate and the size distributions of the resultant emulsions were obtained using the volume-average diameters of the 50 percentiles, D (v, 0.5). MEDD values are expressed as mean values of all data ± standard error.

2.2.3. Equilibrium phase studies

Phase behavior studies were conducted by two methods in order to construct pseudo-ternary and binary phase diagrams. (1) Static composition method: A series of phase compositions of oil, surfactant and water of 5 g mixtures were made up by weight in screw-capped glass vials. For construction of the whole ternary phase diagram, phase composition changes were made at 5%w/w intervals. Additional compositions were made at 2.5%w/w intervals where phase boundaries were observed. The various ternary and binary compositions were heated to 70 °C in a thermostatically controlled water bath for 15 min with intermittent mixing using a Fisons orbital vortex mixer until homogeneity was achieved. The mixtures were then allowed to cool to 25 °C and left undisturbed for 24 h for phase identification by visual observation using a crossed polarized viewer. (2) Dynamic composition method: For this method the weighed amounts and therefore the ratios of oil and nonionic surfactant in each mixture were constant, the only difference is the water content. Phase studies were performed using ~2 g samples of oil-surfactant mixtures diluted sequentially by the weighed addition of water. Formulations of interest were weighed into narrow bore glass ampoules. Milli-Q water was then sequentially added to the oil mixtures at 0.5%w/w intervals, ampoules were then sealed using an Adelphi ampoule sealer. The ampoules were then heated to 70 °C for 15 min to facilitate mixing using a vortexer. The ternary mixtures were equilibrated to the temperature of interest (4–65 ± 0.1 °C) for two hours and then thoroughly mixed for 5 min using a Fisons orbital whirlmixer. The ampoules were then returned to the thermostatically controlled water bath and left undisturbed for 24 h before identification of the phase type using a crossed polarized viewer.

2.2.4. Effect of emulsification temperature

Emulsions formed by mixtures of Miglyol 812/Imwitor 988 (10:0, 7:3, 6:4 and 5:5) containing 15% or 30%w/w Tagat TO were investigated over a temperature range between 5 and 60 °C. Emulsions were prepared under conditions of gentle agitation at the required temperature as described earlier.

Samples were then removed after 15 min and left to equilibrate at 25 °C in thermostatted water bath before particle sizing.

2.2.5. Effect of SGF on emulsification

Self-emulsifiable mixtures (1 g) of different oil systems were introduced into 100 ml of SGF in a 500-ml glass beaker held at 37 °C in a thermostated water bath and emulsified for 15 min as described earlier before particle sizing.

3. Results and discussion

3.1. Particle size analysis

Previous studies using binary systems containing medium-chain triglyceride oil (Miglyol 812) and Tagat TO have produced systems that exhibit optimum self-emulsifying behavior at 30 °C (Pouton et al., 1987; Wakerly et al., 1986). In contrast to the Tween 85-Miglyol 812 investigated by Pouton (1985), the Tagat TO-Miglyol 812 system produced by Wakerly et al. (1986), was shown to be capable of forming submicron emulsions by self-emulsification. In this study, the effect of incorporating, C_8/C_{10} mono/diglycerides (Imwitor 988) in the binary mixtures of Miglyol 812/Tagat TO on emulsification behavior has been investigated to establish the optimum requirements for good self-emulsifying systems. Imwitor 988 was included in the binary system (Miglyol 812/Tagat TO) by varying the ratio fraction of Miglyol 812/Imwitor 988 while gradually increasing the concentration of Tagat TO in each ratio.

The emulsification profile of Miglyol 812/Tagat TO system was chosen as the starting point to compare the emulsification behavior that results from the inclusion of Imwitor 988 in the binary mixtures. The particle size-surfactant concentration profiles obtained by laser diffraction analysis for the self-emulsified Miglyol 812/Imwitor 988-Tagat TO mixtures are shown in Fig. 1. The self-emulsification profile of Miglyol 812/Tagat TO presents three regions: (1) Low surfactant concentrations $<15\%$ (w/w) gave rise to a region of gross instability with high creaming rate. Microscopical examination of these crude emulsions showed that oil droplets in excess of 500 μm were present (Wakerly et al., 1986). (2) Concentration of surfactant between 20% and 45% resulted in sub-micron emulsions of improved stability. The MEDD apparently reached a minimum value at surfactant concentrations of 40–45%. The reduction in the MEDD in this second region probably resulted from increased interfacial stabilization by water soluble surface active components as the surfactant concentration was increased. (3) At higher surfactant concentrations ($>50\%$) the MEDD increased with a doubling of the polydispersity. The turbidity of self-emulsified systems decreased as the surfactant concentration was increased from 45% to 60%. The increase in the MEDD values for optically clear systems containing more than 45% surfactant may be explained by the large swollen microemulsion systems described by Gerbacia and Rosano (1973), where the refractive indices of dispersed and continuous phases are very similar.

The progressive inclusion of Imwitor 988 at $\leqslant 5$ parts in the oil blend (Miglyol 812/Imwitor 988 at ratios of 9:1, 7:3, 6:4 and 5:5) has improved the emulsification profiles with respect to the Miglyol 812/Tagat TO system as, relatively lower MEDD values were obtained at almost all surfactant concentrations (Fig. 1). On the other hand, less self-emulsifiable mixtures presented by an increase in the MEDD values with reference to Miglyol 812/Tagat TO were produced when Imwitor was included in the oil blend at >5 parts (Miglyol 812/Imwitor 988 at ratios of 3:7, 1:9 and 0:10). Optimum dispersions of near optical clarity which were classified as self-micro-emulsifying systems were obtained at Miglyol 812/Imwitor 988 ratios of 7:3 or 6:4 at Tagat TO concentrations between 30% and 40%w/w. These formulations were at the lower detection limit of the Mastersizer at surfactant concentration above 20% as they formed typical bluish clear microemulsions. Therefore,

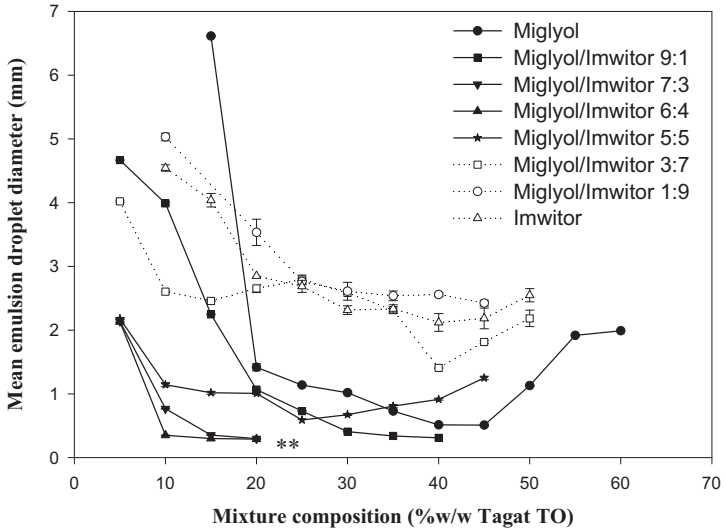

Figure 1 Mean emulsion droplet diameter (MEDD) profiles for self-emulsified systems containing different ratios of Miglyol 812/Imwitor 988 and increasing concentrations of Tagat TO. Lipid formulations were emulsified in water at 37 °C for 15 min. MEDD values were determined by laser diffraction method using the Mastersizer. Bars Represent Standard Errors ($n = 3$). Size droplets of dispersions of oil systems containing Miglyol/Imwitor ratios of 7:3 or 6:4 at Tagat TO concentrations $>20\%$ could not analyzed using the Mastersizer due to the size range cut off of the apparatus.

these systems were further characterized using PCS technique. Results obtained from PCS for emulsions formed from Miglyol 812/Tagat TO and {Miglyol 812/Imwitor} (6:4 or 7:3)-Tagat TO systems are shown in Fig. 2. Self-emulsifying mixtures of Miglyol 812/Tagat TO, produced a minimum MEDD of ≈220 nm at surfactant concentration between 25% and 30%. At surfactant concentrations between 20% and 40% for Miglyol 812-Tagat TO system, MEDD values measured by PCS method were lower than expected from the laser diffraction analysis using the Mastersizer. This may have been due to underestimation by PCS of the MEDD of this system which probably contained droplets >3 μm, which would have been excluded from any PCS analysis. Emulsions formed by Miglyol 812/Tagat TO mixtures containing, however, less than 20% surfactant were poor and therefore, was not possible to detect any scattered light using the PCS at the normal sensitivity level i.e. the MEDD of such emulsions were always out of the range covered by the apparatus.

As Fig. 2 depicts, the particle size-surfactant concentration profiles for {Miglyol 812/Imwitor} at ratios of 7:3 or 6:4 are of similar character exhibiting a minimum MEDD of ~50 to 60 nm at about 30–40% Tagat TO. Nonetheless, At surfactant concentrations >45% both systems 7:3 and 6:4 have produced though clear dispersions, yet, MEDD values have dramatically increased with a doubling of the polydispersity. This could be attributed to the formation of swollen microemulsion systems. Therefore, as Fig. 2 illustrates, the inclusion of only 30%w/w Imwitor 988 in the oil blend has significantly reduced the oil droplet size and resulted in producing self-micro-emulsifying system. This is evident from the contour plot representation of MEDD data depicted in Fig. 3 whereby, progressive inclusion of Imwitor 988 in the lipid mixture had caused a corresponding reduction in the oil droplet size of resultant dispersions. This was, however, up to a point where, further incorporation of Imwitor 988 caused increases in MEDD values. It is thought that Imwitor 988 which acts here as a co-surfactant can stabilize the interface by penetrating into the void spaces among surfactant molecules in the surfactant film around the oil droplet and hence lowering the interfacial tension and increasing the interfacial fluidity. At optimum concentrations of co-surfactant in the oil mixture, saturation at the O/W interface is achieved which results in producing lower particle size profiles. On including yet more cosurfactant in the system, the excess will disperse in the aqueous phase and as it has limited solubility in water, at this point particle size of the resultant dispersion is increased.

The significance of this robust self-microemulsyfying lipid system (Miglyol 812/Imwitor 988-Tagat TO at ratios of 70(70/30)30) is the ability to retain its solvent capacity for the drug after dispersion. This is due to it being comprised of water in-soluble components. Furthermore, this system is expected to have better solubilizing capacity for poorly-water soluble drugs as Imwitor 988 is apolar oil due to its monoglyceride content.

Medium-chain (C_6–C_{12}) fatty acids, mono-, di-, and tri-glycerides, particularly C_8/C_{10} mono/diglycerides, have been used in mixed micelle and emulsion formulations as absorption enhancers of a number of different drugs (Muranishi, 1990). In this investigation Imwitor 988 acts as a co-surfactant with a similar effect to short chain alcohols in aiding the emulsification process of oil based formulations.

3.2. Phase behavior: mechanistics of self-emulsification

Although self-emulsification is a dynamic non-equilibrium process involving interfacial phenomena, information about the self-emulsification can be obtained using investigations of equilibrium phase behavior (Charman et al., 1992). Emulsification efficiency of oil-surfactant systems (as determined from particle size) has been characterized and correlated with equilibrium phase diagrams.

Wakerly (1989) studied binary mixtures of Miglyol 812-Tagat TO in an attempt to elucidate the mechanistic processes of emulsification and to highlight the important involvement of liquid crystalline phases forming at the interface in such processes. Therefore, the initial work described here was

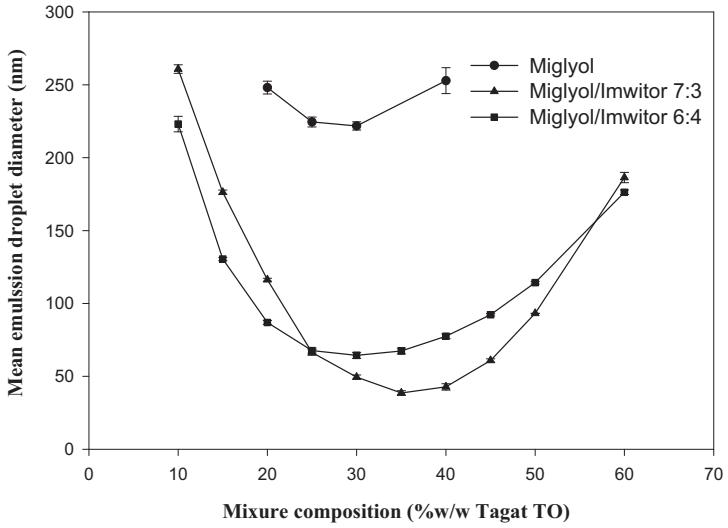

Figure 2 Mean emulsion droplet diameter profiles for Miglyol 812/Tagat TO and {Miglyol 812/Imwitor 988} (6:4, 7:3)-Tagat TO systems at increasing surfactant concentration. Oil formulations were emulsified in water at 37 °C for 15 min. MEDD values were measured by photon correlation spectrometer (PCS). Bars represent standard errors (n = 3).

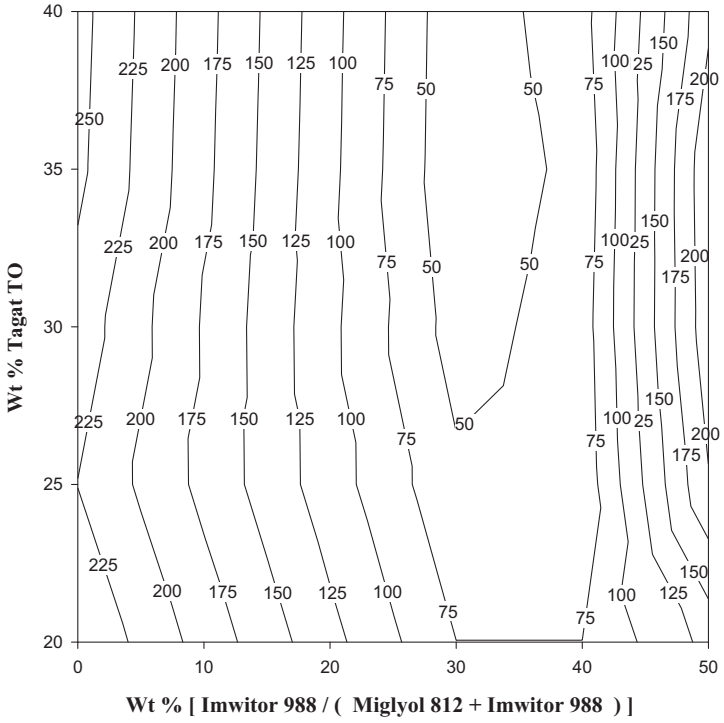

Figure 3 Contour plot representation of mean emulsion droplet size data (nm) for the emulsification of Miglyol 812/Imwitor 988-Tagat TO system. Optimum values were obtained at 30%w/w Imwitor 988 in the oil blend.

dedicated to studying the phase behavior of Miglyol-Tagat TO system as described by Wakerly. Hence, the effects of incorporating Imwitor 988 on the efficiency of emulsification of the binary mixtures will be established using equilibrium phase diagrams. Equilibrium phase behavior was studied in two ways (1) Static composition dilution method and (2) Dynamic composition dilution method.

In the phase diagrams, depicted in Figs. 4–6, aqueous-based liquids are denoted (L_1), oil-based liquids (L_2), and liquid crystalline phases (LC). Multiphasic mixtures were clearly distinguished by their turbidity, and were designated (L_1 + L_2) or (L_1 + L_2 + LC) when LC material was clearly present. Two types of LC phases were identified: (1) The LC phase, was viscous and exhibited ''white'' birefringence (2) The LC_a phase,

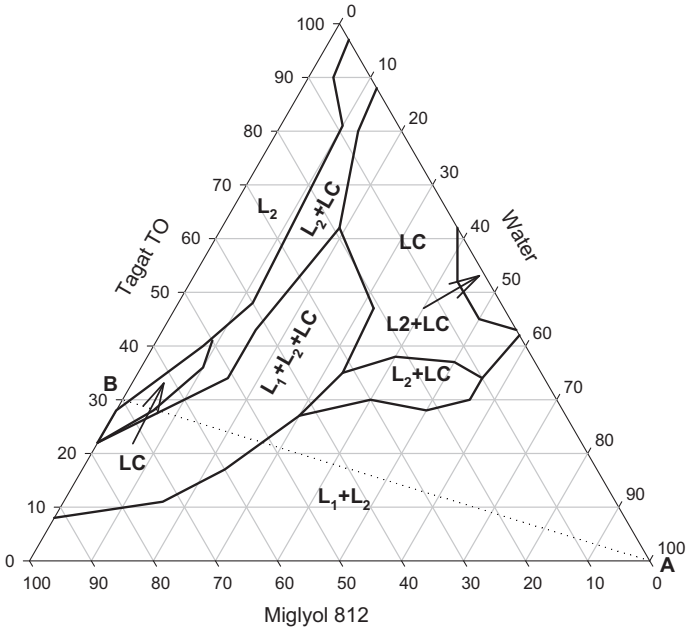

Figure 4 Triangular equilibrium phase diagram for the Miglyol 812-Tagat TO-water system at 25 °C. Line A–B represents the dilution of Miglyol 812-Tagat TO oil mixture of ratio 70/30 with water. Aqueous-based liquids are designated L_1, oil-based liquids (L_2) and liquid crystal phases ''white'' birefringence (LC).

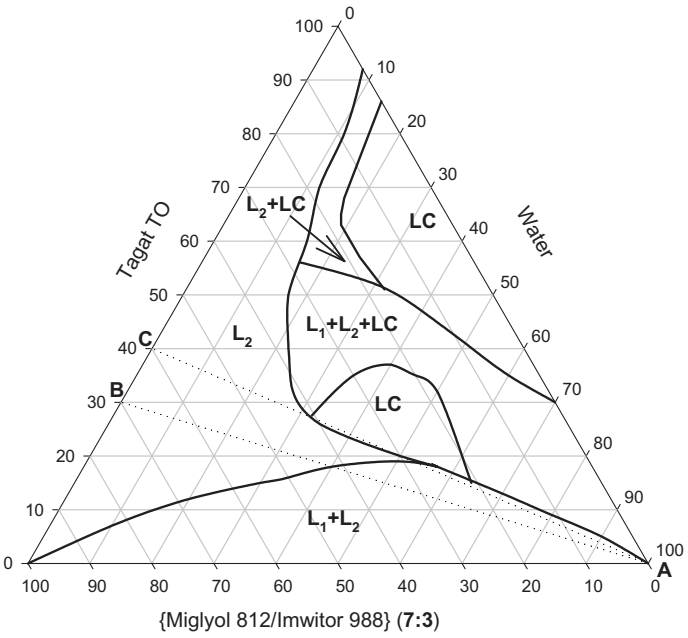

Figure 5 Triangular equilibrium phase diagram for the {Miglyol 812/Imwitor 988} (7:3)-Tagat TO-water system at 25 °C. Lines A–B and A–C represent the dilution of oil mixtures of compositions [70(70/30)30] and [60(70/30)40] with water, respectively.

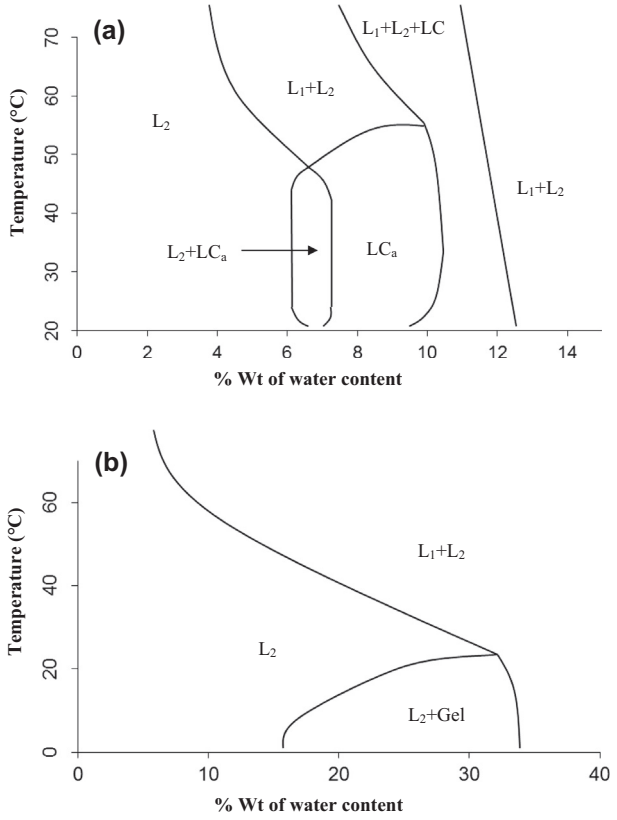

Figure 6 Equilibrium phase diagrams for Tagat TO-Miglyol 812 system at **30%** (w/w) surfactant on dilution with water **(a)** with no Imwitor 988 **(b)** with 30% Imwitor 988 in the oil blend, see text for the denotes.

transparent liquid crystalline dispersion exhibited low viscosity and "multicoloured" birefringence, typical of lamellar liquid crystalline phases. The presence of significant quantities of liquid crystalline material was established by strong birefringent patterns observed using the polarizing viewer.

3.2.1. Equilibrium phase behavior using static compositions

The triangular equilibrium phase diagram for the Miglyol 812-Tagat TO-water system at 25 °C is shown in Fig. 4. For simplicity no differentiation was made between the different liquid crystal phases in the initial equilibrium phase diagram studies. Considering the dilution of Miglyol 812/Tagat TO (70/30) with water at 25 °C, as depicted by the line B–A, the initial binary mixture (L_2 phase) could pass through $L_2 \rightarrow LC \rightarrow L_1 + L_2 + LC \rightarrow L_1 + L_2$ phases. However, during self-emulsification of the binary mixture non-equilibrium multiple phases could have been formed. Therefore, the complete route depicted by line B–A may not be followed. As Fig. 4 depicts, maximum solubilization of water (L_2) of only 10% was observed. In his study Wakerly [9] by constructing the triangular equilibrium phase diagrams for the Tagat-Miglyol-water system at various temperatures, 25, 30 and 40 °C, concluded that, the presence of a $L_1 + L_2 + LC$ region which only occurred to a great extent at 25 °C is not essential for self-emulsification. It is thought that the LC phase adjacent to the isotropic L_2 region probably controls self-emulsification.

The triangular phase diagram for {Miglyol 812/Imwitor 988} (7:3)-Tagat TO-water system is depicted in Fig. 5. Lines B–A and C–A represent the dilution with water of {Miglyol 812/Imwitor 988} (7:3) at Tagat TO ratios of 30 or 40, respectively. This region on the phase diagram, with respect to the Miglyol 812-Tagat TO-Water system, has shown significant enhancement in the L_2 region, and the complete routes of lines

B–A and C–A shown no areas of LC phases. Maximum solubilization of water as L_2 phase of around 40% or 50% was observed for oil mixture at ratios of 70(70/30)30 or 70(70/30)40, respectively (lines B–A and C–A depicted in Fig. 5). This indicates the fact that the enhancement in the L_2 region is of paramount importance in the mechanistics of emulsification when Imwitor 988 is included in the system, as the region is clear from any presence of LC phases. It is worth noting here that phase behavior studies for lipid mixtures containing > 30% of Imwitor 988 in the oil blind have shown relative reduction in the L2 region (data not shown). This correlates well with the particle size data whereby, 30% of Imwitor 988 in the oil blend is the optimum ratio for self-microemulsifiaction.

3.2.2. Equilibrium phase behavior using dynamic compositions

In order to highlight the important detailed areas on the phase diagrams in the region 0–40%w/w of water which appear to be crucial for the emulsification process, equilibrium phase diagrams with respect to water content and temperature described by Shinoda and Friberg (1986) were constructed. Comparative equilibrium phase studies were carried out on Miglyol-Tagat TO system with and without Imwitor 988. This enables insight into the mechanistics of the observed changes in self-emulsification behavior of these systems.

Fig. 6a displays the equilibrium phase diagram for a system containing 70% (w/w) Miglyol 812 and 30%w/w Tagat TO on dilution with water. Maximum water solubilization (L_2) extended to 7% at 45 °C. Moreover, large area of liquid crystal phase (LC_a and L_2 + LC_a) occurred on further dilution with water between 6 and 10%w/w at temperatures below 50. On the other hand, by incorporating three parts of Imwitor 988 in the binary mixture, as shown in Fig. 6b, substantial extension in the L_2 region occurred reaching a maximum of almost 33%w/w water at 20 °C and with no sign of LC phases. This is reflected in the fine dispersion obtained from the emulsification data of this system.

As depicted in these phase studies, there are two distinctive features that may correlate to the efficiency of emulsification; (a) a region of enhanced water solubilization typical of non-ionic surfactant systems and thought to be a phase inversion region (b) the formation of a lamellar liquid crystalline dispersion phase on further incorporation of water (Fig. 7a). The occurrence of such stable or metastable liquid crystal dispersions at the oil–water interface, based on loosely associated aggregation structures was suggested to allow rapid penetration of water and disruption of the interface (Pouton, 1982). The resulting disruption will promote rapid self-emulsification.

The inclusion of Imwitor 988 in the binary mixtures (Miglyol-Tagat TO) has substantially transformed the phase behavior of these oil systems. It has induced three major changes in the phase diagram structure of the binary mixtures, as summarized in Table 3. Firstly; changes in the L_2 region; progressive inclusion of Imwitor 988 has resulted in extensive enhancement in the water soubilisation region; including Imwitor 988 in the oil blend at ratios between 0%w/w and 30%w/w, extended the L_2 region from 7% to 32%. According to criteria

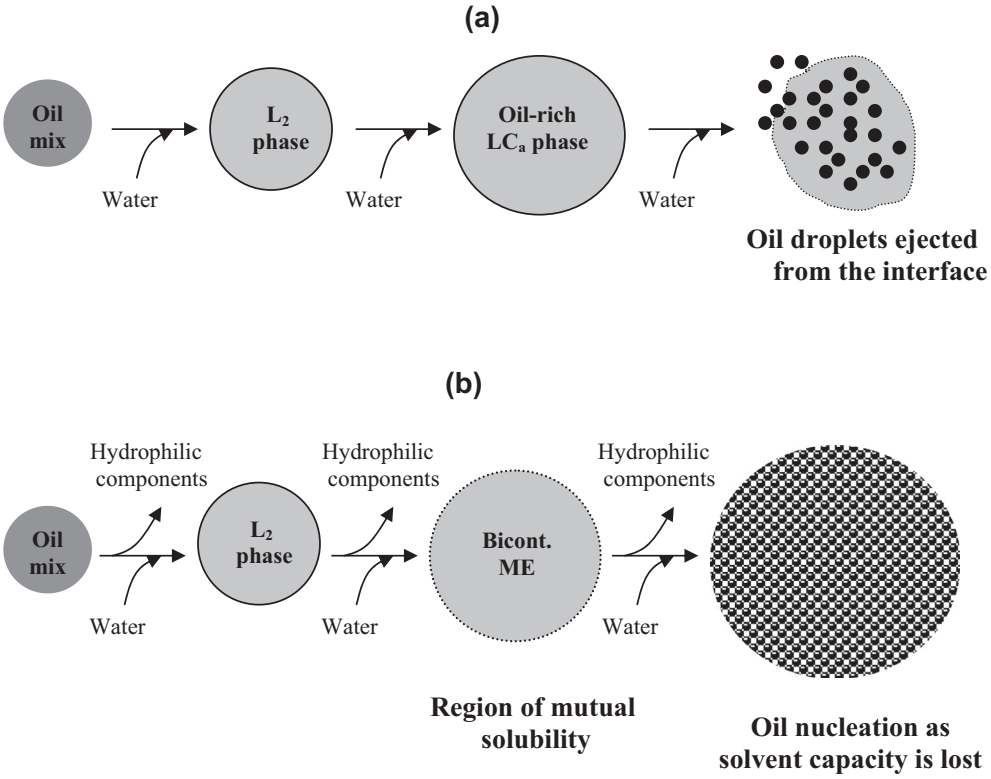

Figure 7 Mechanistics of self-emulsification process for (a) systems containing water-insoluble materials (e.g. Miglyol 812/Tagat TO system); this process involves the formation of liquid crystalline phases at the oil–water interface (b) systems containing a water-soluble component, e.g. {Miglyol 812/Imwitor 988}-Tagat TO system; 'diffusion and stranding' is thought to be the mechanism of emulsification for these systems which can lead to very fine dispersions.

Table 3 Summary of the changes in the phase behavior resulted from including various proportions of Imwitor 988 in the binary mixture of Miglyol 812/Tagat TO (70/30). L_2: oil-based liquids; LC_a: liquid crystalline phases; PBT: phase boundary temperature.

Description of phase Behavior	Wt% ratio of Imwitor 988 to Miglyol 812 included in the lipid mixture		
	0	10	30
L_2 max. (%)	7	12	32
LC_a	+ + +	+ +	–
Gel phase	–	–	+
PBT (°C)	45	30	20
Particle size (nm)	221.80	202.40	49.5

for the identification of microemulsions, L_2 phase in aqueous systems of polar lipids and triglyceride oil is w/o microemulsion. On the basis of an X-ray study obtained by cooling L_2 phases (Fontell et al., 1983), it was proposed that the structure consists of water lamellae separated by lipid bilayers corresponding to liquid–crystalline phase. Secondly; changes in the Phase Boundary Temperatures (PBT) which is referred to here as the minimum temperature at which phase transition from L_2/LC phase boundary to $L_1 + L_2$ occurs. This phase boundary temperature has a significant role which underlines the phase inversion temperature (PIT). It was evident from the phase behavior study that the inclusion of Imwitor 988 in the lipid mixture resulted in depression of the PBT; including Imwitor 988 in the oil blend at ratios between 0% and 30% caused depressions in the PBT from 45 to 20 (°C). This could be attributed to the fact that Imwitor 988 as a polar oil increases the solubility of the surfactant in the oil phase thus, less temperature is needed to exert phase inversion in the system. Thirdly; disappearance of liquid crystalline materials and the formation of gel phase instead, which was only observed when the ratio of Imwitor 988 in the oil blend exceeded 30%.

Based on a study by Rang and Millar (1998, 1999) investigating the emulsification of oils containing hydrocarbon, nonionic surfactant and *n*-octanol, the following mechanism for the self-emulsification of Miglyol 812-Imwitor 988-Tagat TO system can be proposed, see Fig. 7b. Addition of the oil mixture to water results in the formation of interface between the oil and aqueous-continuous phases. Solubilization of water with the oily phase results from aqueous penetration through the interface. This will occur until maximum solubilization (L_2) is reached close to the interface. Further aqueous penetration results in diffusion of the polar component of Imwitor 988 (1-Monocaprylate) and also to some extent Tagat TO into water. Subsequently, the system becomes supersaturated in oil and thus spontaneous nucleation of small oil droplets from the microemulsion occurs (i.e. phase transition from L_2 to $L_1 + L_2$). Further diffusion of Tagat TO and monocaprylate out of the L_2 phase forms a system that is ever more hydrophilic so that it eventually becomes miscible with water. The particle size of the nucleated oil droplets formed due to the diffusion process is expected to be related mainly to the interfacial and rheological properties of the L_2 phase; the larger the (L_2) area in the phase diagram the smaller the size of the nucleated oil droplets and vice versa. Optimal range of initial drop compositions is required to obtain an emulsion of small oil droplets. According to our findings spontaneous micro-emulsification yielding uniformly fine dispersions was achieved when the introduced drop was slightly lipophilic; i.e. (i) the ratio of Miglyol 812 to Imwitor 988 was slightly greater than that of the excess oil phase in equilibrium with a bicontinuous microemulsion at the experimental temperature, and (ii) when sufficient Tagat TO was used. The need for this slightly lipophilic oil composition is attributed to the relatively low HLB value of Tagat TO (HLB $\approx$ 11). However, in the case of surfactants with higher HLB values, less lipophilic oil composition is required.

3.3. The effect of emulsifying temperature and medium self-emulsification

3.3.1. Temperature variation

It is important from the stability prospective in emulsions to investigate the optimum temperature for stable emulsifications, the optimum hydrophilic chain length and the phase inversion temperature (PIT) of emulsifiers. Non-ionic surfactants often become increasingly more lipophilic at elevated temperatures. The change in micellar shape, size and eventual loss of aqueous solubility with increase in temperature is due to decreased hydration of the surfactant. Hydrogen bonding forces, which account for aqueous solubility, are reduced at elevated temperatures. This is analogous to reducing the HLB of the surfactant. The change in nonionic surfactant-oil–water system (nSOW) phase behavior with temperature can result in the phase transitions Winsor's type I to type III to type II. Therefore, the higher the emulsification temperature is than the PIT of the emulsifier, the larger the emulsion droplets will be. Emulsification at temperatures slightly below the PIT (about 2–4 °C below) affords optimum oil droplets of an o/w-emulsion type.

As Imwitor 988 is a polar oil that causes depressions in PIT which means that emulsion type inversion from o/w to o/w occurs at lower temperatures causing growth in oil droplet size. This action, however, is concentration dependent and moreover, is highly likely to be averted by using surfactants with relatively high HLB values (>12). Tagat TO has an HLB of 11 and hence, any reduction of its hydrophilicity due to temperature or electrolytes in the media will affect the emulsification performance of the lipid mixture. Nonetheless, oil systems containing 30% Tagat TO have shown no large increase in the MEDD at high emulsification temperatures comparable to the particle size increase observed when these systems contained 15% surfactant. For {Miglyol 812/Imwitor 988}-Tagat TO at ratios of 70{70/30}30, almost clear microemulsion dispersions of minimum particle size around 50 nm were produced at temperatures between 35 and 40 °C. Dispersions turned turbid at temperatures above 45 °C and yet, on cooling to the room temperature for size measurement they became clear again. This phenomenon has been observed by many researchers and considered to be one of the distinctive criteria for microemulsions.

3.3.2. Emulsification media

Since the luminal environment in the proximal GI tract varies considerably with site and meal ingestion, it is essential to consider the use of several different sets of emulsification conditions to assess the dissolution behavior of the oil formulations with and without drugs. The United States Pharmacopoeia (1995) calls for the Simulated Gastric Fluid (SGF) to simulate dissolution in the stomach. SGF simulates pH conditions in the fasted stomach. In the fed state, however, composition in the stomach will be highly dependent on the ingested meal. Long-life milk (Macheras et al., 1986) and clinical nutrition products, e.g. Ensure® HN (Ashby et al., 1989) have been suggested as media suitable for simulating fed state in the stomach.

Generally, electrolytes present in the emulsification media, as the variation in temperature, can affect the emulsification performance of lipid formulations. Due to salting out effect the preferential aqueous solubility of the surfactant is shifted to the oil phase inducing phase separation. This effect, however, can be counteracted by using surfactants with relatively high HLB values, as any reduction of the surfactant hydrophilicity incurred by electrolytes will not be sufficient to induce phase separation.

A significant increase in the MEDD values was observed for lipid mixtures containing >20% of Imwitor 988 after emulsification in SGF versus water. Lipid mixtures comprising {Miglyol 812/Imwitor 988}-Tagat TO at ratios of 70{70/30}30 produced dispersions of oil droplet size of 150 nm on emulsification in SGF vis-à-vis 50 nm in water. This almost threefold increase in the MEDD value can be explained by the effect of Imwitor 988 on reducing the PIT and also the loss of surfactant's affinity for the aqueous phase because of the electrolytes present in the media. This may suggest a phase inversion at temperatures lower than the preferable optimum temperature for emulsification (37 °C). For use *in vivo* it would obviously be preferable to have optimum emulsification at 37 °C yet this might be achieved by using slightly more hydrophilic surfactants such as, Cremophor RH 40 and Tween 80. Nonetheless, this does not preclude the use of this system *in vivo* since particles around 150 nm are still achievable. This increase in the oil droplet size may minimize the effect of water in 'squeezing out' the drug, if it is to be delivered with this system, by minimizing the direct contact with the aqueous phase. In this case, the drug will be sequestered within the oil phase and hence, crystallization is prevented. Furthermore, it is worth noting that *in vitro* emulsification using SGF does not bring about a full picture of what is going on in the stomach. Hence the media excludes the human gastric lipase which initiates gastric lipolysis; a process that aids the emulsification as it facilitates the hydrolysis of triglycerides to diglycerides and fatty acids. It has been suggested (Dressman et al., 1998) that a suitable surfactant be added to this media better simulate the surface tensions typical of the fasted stomach. However, *in vivo* studies are worth being carried out to verify the performance of this genuine system in a suitable animal model.

4. Concluding remarks

This present study was designed to develop stable o/w microemulsions comprising Miglyol 812 (medium chain, C_6–C_{12}, triglycerides), C_8/C_{10} mono/diglycerides (Imwitor 988) and non-ionic surfactant polyoxyethylene-25-glyceryl trioleate (Tagat TO). This type of formulation is referred to as type II and is likely to retain its solvent capacity for the drug after dispersion as it is comprised of water in-soluble components. The resulting emulsions as assessed by particle size analysis are shown to be dependent on the glycerides blend {Miglyol 812/Imwitor 988}, surfactant concentration, emulsification temperature and media. Manipulation of these parameters can result in emulsion formulations of controlled droplet size and hence surface area. Such considerations are important when the partition of lipophilic drugs into the aqueous phases and drug release rates are considered. Equilibrium phase behavior studies have shown that the amount of water solubilized as w/o (L_2 phase) is of paramount importance for the observed good emulsification in these systems.

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