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The influence of cosurfactants and oils on the formation of pharmaceutical microemulsions based on PEG-8 caprylic/capric glycerides

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Abstract

In the present study the effect of type and concentration of a cosurfactant and oil on the ability of nonionic surfactant PEG-8 caprylic/capric glycerides (Labrasol®) to solubilize both oil and water phases was evaluated. Seven different cosurfactants (polyglyceryl-6 dioleate (Plurol Oleique®) (PO), polyglyceryl-6 isostearate (Plurol Isostearique®) (PI), polyglyceryl-4 isostearate (Isolan® GI 34) (IGI34), octoxynol-12 (and) polysorbate 20 (Solubilisant gamma® 2421) (SG2421), octoxynol-12 (and) polysorbate 20 (and) PEG-40 hydrogenated castor oil (Solubilisant gamma® 2429) (SG2429), PEG-40 hydrogenated castor oil (Cremophor® RH 40) (CRH40) and diethyleneglycol monoethyl ether (Transcutol®)) and six oils (isopropyl myristate, ethyl oleate, decyl oleate, medium chain triglycerides, mineral oil and olive oil) were used in phase behaviour studies of a quaternary system Labrasol®/cosurfactant/oil/water. The amount of surfactant required to completely homogenize equal masses of oil and water to form a single phase microemulsion (termed as balanced microemulsion) (Smin, %w/w), the minimal concentration of the surfactant/cosurfactant blend required to produce a balanced microemulsion (SCoSmin, %w/w) as well as the maximum concentration of water solubilized in investigated surfactant/oil and surfactant/cosurfactant/oil mixtures (W_{max}, %w/w) were determined. The obtained results indicated that Labrasol® showed a good efficiency in the presence of lower molecular volume fatty acid esters with a preferred chemical structure such as isopropyl myristate (Smin 56.14% (w/w); W_{max} 12.28% (w/w)). Oils with high molecular volume (olive oil and mineral oil) do not result in microemulsion formation. Transcutol® decreased the capacity of Labrasol® for solubilization of oil and water phases. The tendency of Labrasol® to solubilize both, water and oil phases, was favoured by polyglycerol-6 ester type of cosurfactants (PO and PI) while the influence of the polyglycerol-4 ester (IGI34) as well as of polyoxyethylene type of cosurfactants (CRH40, SG2421 and SG2429) on the surfactant efficiency, was not significant. Furthermore, the results revealed the significant influence of the surfactant/cosurfactant mass ratio (K_m) on synergistic effect between polyglyceryl-6 esters and Labrasol® in the formation of microemulsions using isopropyl myristate as oil phase. Optimized microemulsion systems were stabilized with Labrasol®/polyglyceryl-6 esters blend at K_m 5:5 (SCoSmin, 27.5% (w/w) and W_{max}, 71.43% (w/w) for PI; SCoSmin, 29.18% (w/w) and W_{max}, 65.00% (w/w) for PO) and the electrical conductivity measurement results for optimized balanced microemulsions showed that their structures were highly conductive indicating a bicontinuous microstructure.

Keywords: Microemulsions; SMEDDS; The efficiency of a surfactant; Labrasol®; Polyglycerol esters; Polyoxyethylene surfactants

1. Introduction

Microemulsions are thermodynamically stable and optically isotropic transparent colloidal systems consisting of water, oil and amphiphiles (surfactant, usually in combination with a cosurfactant). When a sufficient amount of an appropriate surfactant is added to solubilize oil and water completely, single phase systems (Winsor IV microemulsions) are formed (Winsor, 1948). Single phase microemulsions are currently of interest to the pharmaceutical scientist as potential drug delivery vehicles due to their long term stability, ease of preparation, and considerable capacity for solubilization of a variety of drug molecules (Lawrence and Rees, 2000; Malmstein, 1999). These systems often require high surfactant concentrations in order to provide very low interfacial tension ($\leq 10^{-3}$ mN/m) and sufficient interfacial coverage to microemulsify entire oil

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and water phases (Wennerström et al., 2006). However, high surfactant levels are often not acceptable because of bioincompatibility, performance or economic reasons. Therefore, surfactants and their concentrations are of great interest to the formulators. It is well known that the incorporation of another amphiphile, as cosurfactant, enables the adjustment of the surfactant efficiency as well as concentration of a surfactant required to form single phase microemulsions (Hiuberts and Shah, 1997; Sagitani and Friberg, 1980). Interest in using nonionic tensides both, as a surfactant and as a cosurfactant (so-called non-alcohol cosurfactants), is increasing due to high stability, low toxicity, low irritancy and biodegradability of many nonionic surfactants (Lawrence and Rees, 2000; Malmstein, 1999). In spite of a substantial amount of investigations of various microemulsion systems based on nonionic surfactants as potential drug delivery vehicles, usually they have been involved in only a few individual vehicles each. This has hampered general conclusions and development of guidelines to formulators, in particular which surfactants and oils form microemulsions at which concentrations. Indicators of a surfactant phase behaviour and suitability such as hydrophyle–lipophile balance (HLB) (Griffin, 1949) or critical packing parameter (CPP) (Israelachvilli et al., 1976) are empirical and most widely used for surfactant selection. It is important to note that compositional variables (oil, presence of other amphiphiles, hydrophilic molecules (i.e. glycerol, sorbitol) or electrolytes) as well as temperature may have an influence on hydrophilic and hydrophobic properties and the geometry of the surfactant molecule and the efficiency of a surfactant to generate microemulsion (Kahlweit, 1999; Lawrence and Rees, 2000; Sjöblom et al., 1996). Additional aspect associated with the rational selection of amphiphiles is based on the fact that commercially available surfactants are mixtures of homologous substances with different lipophilic chain length and with different degree of polymerization in hydrophilic part of a molecule. For this reason the relationship between physico-chemical characteristics of nonionic surfactants and their phase behaviour in ternary (oil/water/surfactant), pseudo-ternary (oil/water/surfactant/cosurfactant) or even more complex systems, such as microemulsion-based drug delivery systems, is still unclear. Therefore, it would be desirable to have a screening procedure to optimize oil and cosurfactant in order to maximize the efficiency of a given surfactant to form a microemulsion with as little surfactant as possible. During the past decade numerous studies concerning the formulation and characterization of microemulsions based on nonionic surfactant PEG-8 caprylic/capric glycerides (Labrasol®, Gattefosse, France) for oral (Ghosh et al., 2006), transdermal (Alvarez-Figueroa and Blanco-Méndez, 2001; Delgado-Charro et al., 1997; Djordjevic et al., 2004, 2005; Escribano et al., 2003; Kreilgaard et al., 2000; Parikh and Ghosh, 2005; Rhee et al., 2001; Špiclin et al., 2003; Zhao et al., 2006) and intranasal (Zhang et al., 2004) delivery of drugs have been published. The frequently used cosurfactants in the preparation of microemulsions with Labrasol® were nonionic tensides polyoxyethylene-40 hydrogenated castor oil (Rhee et al., 2001; Zhao et al., 2006), polyglyceryl esters (Alvarez-Figueroa and Blanco-Méndez, 2001; Delgado-Charro et al., 1997; Djordjevic et al., 2004, 2005; Escribano et al., 2003; Ghosh et al., 2006; Kreilgaard et al., 2000; Špiclin et al., 2003) or diethyleneglycol monoethyl ether (Escribano et al., 2003; Parikh and Ghosh, 2005; Zhang et al., 2004). However, despite the fact that the majority of these studies demonstrated the importance of the microemulsion composition for phase behaviour and drug delivery rate, the studies have not been systematic or consistent, and there are no general conclusions about the effect of type and concentration of a cosurfactant on the efficiency of Labrasol® to solubilize oil and water phases.

The aim of this paper was firstly to identify physico-chemical characteristics of an oil phase for which Labrasol® has high solubilizing capacity. Secondly, this study aims to investigate and compare the effects of polyoxyethylene and polyglycerol types of the nonionic surfactants on phase behaviour of a pseudo-ternary system, with respect to their use as cosurfactants for Labrasol®. Furthermore, important purpose of this work was to determine influence of the surfactant to cosurfactant mass ratio (K_m) on the efficiency of Labrasol® as well as to optimise type and concentration of a cosurfactant in order to form microemulsions capable of solubilizing a high percentage of both oil and water.

2. Materials and methods

2.1. Materials

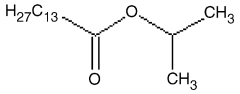
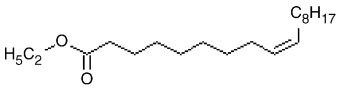
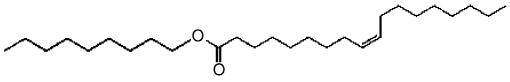
Surfactant, PEG-8 caprylic/capric glycerides (Labrasol®), was kindly donated by Gattefosse, France. Table 1 lists the cosurfactants used in this study.

Isopropyl myristate (Crodamol® IPM), ethyl oleate (Crodamol® EO), medium chain triglycerides (Crodamol® GTCC), and olive oil (Cropure Olive®) were purchased from Croda Chemicals Europe, England. Decyl oleate (Tegosoft® DO) was obtained from Degussa, Germany. Mineral oil was of Ph. Eur. grade. All chemicals were used as received without further purification. Water was double-distilled with conductance less than 3 μS/cm.

Table 1
Cosurfactants used in this study

Compound	Trade name	Supplier
Polyglyceryl-6 dioleate	Plurol Oleique®	Gattefosse, France
Polyglyceryl-6 isostearate	Plurol Isostearique®	Gattefosse, France
Polyglyceryl-4 isostearate	Isolan® GI 34	Goldschmidt, Germany
Octoxynol-12 (and) polysorbate 20	Solubilisant gamma® 2421	Gattefosse, France
Octoxynol-12 (and) polysorbate 20 (and) PEG-40 hydrogenated castor oil	Solubilisant gamma® 2429	Gattefosse, France
PEG-40 hydrogenated castor oil	Cremophor® RH 40	BASF, Germany
Diethyleneglycol monoethyl ether, purified	Transcutol® P	Gattefosse, France

Table 2
Chemical structures, relative molecular masses (Mr), density (d) (at 20 °C) and calculated molecular volumes (v) of oils investigated

Oil	Chemical structure	Mr (g/mol)	d (g/cm^3)	v (cm^3/mol)
Isopropyl myristate		270.45	0.854	525.7
Ethyl oleate		310.52	0.868	593.9
Decyl oleate		421.54	0.862	811.8
Medium chain triglycerides	As specified by manufacturer	554.00	0.945	973.2
Mineral oil	As specified in Ph. Eur.	–	0.858	–
Olive oil	As specified by manufacturer	–	0.909	–

2.2. Phase behaviour study

The efficiency of a surfactant usually represents as the amount of an amphiphile required to completely homogenize equal quantities of oil and water. It is typically determined at equal oil to water weight fractions in order to avoid effects of domain curvature on the surfactant efficiency measurements (Kahlweit, 1999; Sjöblom et al., 1996). In the present study, the efficiency of Labrasol® was determined at experimental temperature of 25 ± 1 °C which corresponds to common conditions of preparation, storage and application of pharmaceutical microemulsions, and expressed as the minimal concentration of the surfactant required to obtain a single phase microemulsion (Smin, %w/w).

2.2.1. Screening of oils

In order to find an appropriate oil for which Labrasol® has good solubilizing capacity and, thus, that can be used as the oil phase in microemulsion, the amount of surfactant required to completely homogenize equal masses of oil and water to form a single phase microemulsion (Smin) was determined for different oils. Oils employed were olive oil, mineral oil, medium chain triglycerydes and fatty acid esters (ethyl oleate, decyl oleate and isopropyl myristate). The relevant physico-chemical properties of investigated oils are presented in Table 2. The molecular volumes (v) were calculated as $v = 1.66(\mathrm{Mr}/d)$ (Richardson et al., 1997), where Mr represents the relative molecular mass (in g/mol) of the oil and d is its density (in g/cm^3). The values of Mr and d were taken from the manufacturers specifications. Smin was determined by adding Labrasol® to the oil/water mixtures which were prepared by weighing the appropriate amounts of the oil and water at fixed oil to water weight ratio (1:1) at 25 ± 1 °C. Labrasol® was added drop by drop to the oil/water mixture and during the titration, samples were magnetically stirred in order to reach the equilibrium quickly. The amount of added surfactant that corresponds to Smin was determined by observing visually the changes of the sample appearance from turbid to transparent. Furthermore, the water solubilization capacity of Labrasol/oil mixtures at constant surfactant to oil mass ratio 1:1 (W_{max}, %w/w) was determined by titrating surfactant/oil mixtures with water, to the water solubilization limit which was detected visually as the transition from transparent to turbid system, upon addition of excess of water. The transparent samples containing Smin or W_{max} were allowed to equilibrate for a minimum of 72 h and then examined visually for transparency and through cross polarizers for optical isotropy. Clear, isotropic, one-phase systems were designated as a microemulsion. Based on obtained Smin and W_{max} values for the investigated oils, isopropyl myristate was selected and used as an oil in further investigations.

2.2.2. Optimization of the cosurfactant and K_m value

Further experimental work was performed in order to investigate the effect of different cosurfactants and K_m value on the efficiency of Labrasol® to produce single phase microemulsions. Three polyglycerol esters, three ethoxylated nonionic surfactants and diethyleneglycol monoethyl ether were used as cosurfactants (Table 1). The influence of the type and concentration of cosurfactant on surfactant efficiency was determined by varying K_m value from 9:1 to 1:9, as shown in Table 3. HLB values of surfactant/cosurfactant mixtures are calculated from HLB values of individual tensides (Labrasol® (HLB 14), polyglyceryl-6 dioleate (HLB 10), polyglyceryl-4 isostearate (HLB 5), PEG-40 hydrogenated castor oil (HLB ∼ 15), polyglyceryl-6 isostearate (HLB ∼ 9)) and presented in Table 3. HLB values of Solubilisant gamma® 2429 and

Table 3
K_m and HLB values calculated for Labrasol®/cosurfactant mixtures investigated

K_m	Cosurfactants			
	Plurol Oleique®	Isolan® GI 34	Cremophor® RH 40	Plurol Isostearique®
9:1	13.6	13.1	14.1	13.5
8:2	13.2	12.2	14.2	13.0
7:3	12.8	11.3	14.3	12.5
6:4	12.4	10.4	14.4	12.0
5:5	12.0	9.5	14.5	11.5
4:6	11.6	8.6	14.6	11.0
3:7	11.2	7.7	14.7	10.5
2:8	10.8	6.8	14.8	10.0
1:9	10.4	5.9	14.9	9.5

Solubilisant gamma® 2421 were not available in manufacturers specifications. Assessment criteria to evaluate the optimum K_m were the minimal concentration of the surfactant/cosurfactant blend required to produce a balanced microemulsion (SCoSmin, %w/w) as well as the maximum concentration of water solubilized in investigated surfactant/cosurfactant/oil mixtures (W_{max}, %w/w). The values of SCoSmin were determined using titration method. Previously, mixtures of the surfactant and cosurfactants in a predetermined weight ratios (K_m) (Table 3) were made. Each surfactant/cosurfactant mixture was added to mixtures of oil and water (the water/oil ratio 1:1 was kept constant), in a drop-wise manner under the mixing of a magnetic stirrer, at the experimental temperature of 25 ± 1 °C. After each addition, the samples were stirred to reach the equilibrium. During the titration, following the dilution line (see Fig. 1), the mixture was visually observed for clarity. When a sufficient amount of the surfactant/cosurfactant mixture is added to completely solubilize the equal masses of oil and water (SCoSmin), single phase microemulsion is formed. The transparent samples were maintained at 25 ± 1 °C for a minimum of 72 h, to complete the equilibrium of the system, before visual inspection, crossed polarized light microscopy and conductivity measurements.

Additionally, the influence of the type of the cosurfactant and K_m value on the water solubilization capacity of oil/surfactant/cosurfactant mixtures was investigated. Labrasol® and the cosurfactant were mixed to give the selected K_m values, with corresponding HLB (Table 3). The obtained surfactant/cosurfactant mixtures and isopropyl myristate were then mixed at the weight ratio of 1:1. These mixtures were titrated dropwise with water, under moderate magnetic stirring at constant temperature (25 ± 1 °C). After addition of each aliquot of water, the samples were stirred to reach the equilibrium and checked visually. The transitions from turbid mixture to optically clear system, or from clear system to turbid dispersion or separate phases, were sharp. During the titration, the samples were left to equilibrate and then characterized by visual inspection and polarized light microscopy. The boundaries of the microemulsion domains were determined by titrating the isopropyl myristate/Labrasol®/cosurfactant mixtures with water, to the water solubilization limit (W_{max}, %w/w), which was detected as the transition from the isotropic single phase system to a two phase system (sample became turbid), upon addition of small amount of excess of water (see Fig. 1). It should be noted that, in the present study, no distinction has been made between a microemulsion and a dispersion of micelles.

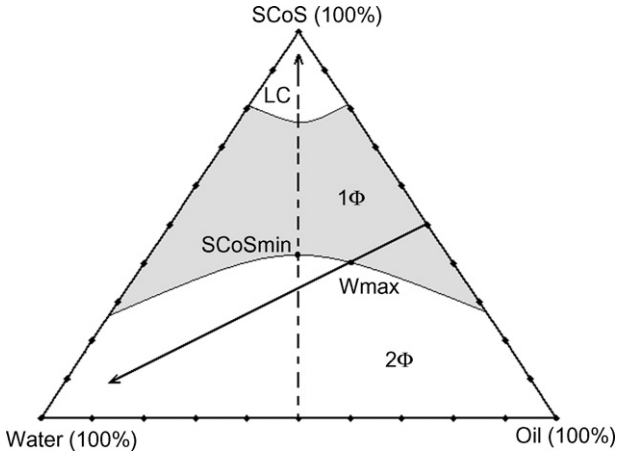


Fig. 1. A hypothetical pseudo-ternary phase diagram of an oil/water/surfactant/cosurfactant system (two corners of the diagram represent 100% (w/w) of the particular component (oil and water) and the third corner represents a 100% (w/w) of a binary mixture of surfactant and cosurfactant (SCoS), including the dilution lines for investigation of: (a) the SCoS efficiency to solubilize the equal masses of oil and water (SCoSmin) (the dashed line) and (b) the water solubilization capacity of a surfactant/cosurfactant/oil mixture at constant SCoS/oil weight ratio 1:1 (W_{max}) (the full line). At insufficient percentages of SCoS as well as at water concentrations higher than W_{max}, two phase systems (2Φ) are observed; for SCoS concentrations above SCoSmin and at water concentrations lower than W_{max}, there is a single phase region (1Φ); at very high SCoS concentrations liquid crystals (LC) are formed.

2.3. Polarized light microscopy

In order to verify the isotropic nature of microemulsions, samples were examined using cross-polarized light microscopy (Leitz Wetzlar 307-083.103 514652, Germany). A drop of sample was placed between a coverslip and a glass slide and then observed under cross-polarized light (the polarizer and the analyzer are aligned such that their vibrational directions are at 90°). Isotropic material, such as microemulsion, in contrast to anisotropic liquid crystals, will not interfere with the polarized light (Friberg, 1990) and the field of view will remain dark because the analyzer absorbs light passing through the polarizer.

2.4. Electrical conductivity measurements

Electrical conductivity (σ) of the samples was measured using a conductivity meter CDM 230 (Radiometer, Copenhagen, Danmark), at the frequency of 94 Hz. The measurements were performed in triplicate at 25 ± 1 °C.

3. Results and discussion

3.1. Influence of oils on the efficiency of the surfactant (Smin) and the water solubilization capacity (W_{max})

Labrasol® is a well defined mixture of 30% mono-, di- and triglycerides of saturated C6–C14 fatty acids (predominantly C8 and C10 fatty acids), 50% of mono- and di- fatty acid esters of polyethylene glycol (PEG) and 20% of free PEG400 (Kreilgaard et al., 2000) which corresponds to monographs caprylocaproyl macrogolglycerides (European Pharmacopoeia, 1998) and caprylocaproyl polyoxylglycerides (Pharmacopeia, 2005). It had been employed as a pharmaceutical excipient for the solubilization of hydrophobic drugs (Cyclosporine A (Tran et al., 1999), RP 69698 (Sheen et al., 1995), piroxicam (Karataş et al., 2005; Yüksel et al., 2003), and coenzyme Q10 (Kommuru et al., 2001)). Also, this excipient was used to enhance the intestinal absorption of unstable or/and poorly absorbable drugs such as hydrophilic drug gentamicin (Hu et al., 2001, 2002; Ito et al., 2005; Koga et al., 2006; Rama Prasad et al., 2003a,b), high molecular weight drugs such as vancomycin (Rama Prasad et al.,

Table 4
The surfactant efficiency (Smin) and the water solubilization capacity (W_{max}) for oils investigated

Oil	Smin (%, w/w)	W_{max} (%, w/w)
Isopropyl myristate	56.1	12.3
Ethyl oleate	54.6	4.8
Medium chain triglycerides	68.1	4.8
Decyl oleate	83.1	5.7
Mineral oil	>90	<1
Olive oil	>90	<1

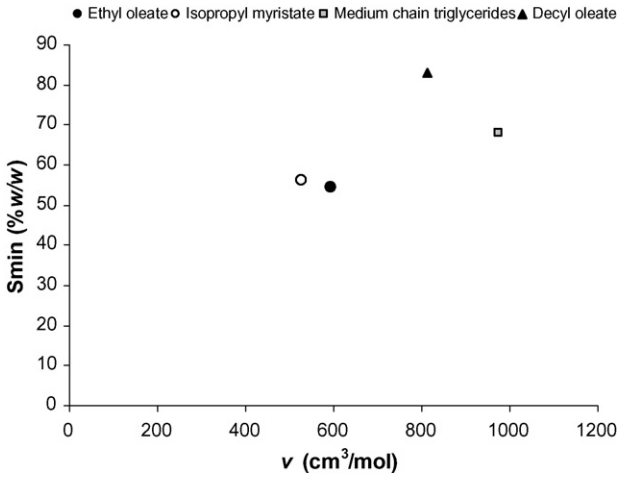


Fig. 2. The surfactant efficiency (Smin) as a function of molecular volume (v) of oils investigated.

2003a,b), protein drugs (erythropoietin (Venkatesan et al., 2005, 2006a,b), insulin (Eaimtrakarn et al., 2002)), and heparin (Ito et al., 2006; Rama Prasad et al., 2004). Furthermore, Labrasol® has been in use as a surfactant in self-microemulsifying drug delivery systems (SMEDDS) (Hu et al., 2002; Ito et al., 2005; Sha et al., 2005). Another area of growing interest is the use of Labrasol® for the stabilization of microemulsion vehicles for cutaneous delivery of drugs (Alvarez-Figueroa and Blanco-Méndez, 2001; Delgado-Charro et al., 1997; Djordjevic et al., 2005; Kreilgaard et al., 2000; Parikh and Ghosh, 2005; Rhee, 2001; Špiclin et al., 2003; Zhao et al., 2006), which had a low potential to induce skin irritation and it has been observed penetration enhancing effect which appears to be attributable to the Labrasol®. In formulation of microemulsions which will be used as drug delivery systems, it is very important to determine with which oils a given surfactant form microemulsions. The most often used oils as pharmaceutical excipients for microemulsion formulation are long-chain triglycerides (i.e. vegetable oils), medium chain triglycerides and fatty acid esters. The choice of the oil phase is sometimes difficult because it is important for both the area of existence of a microemulsion and the solubility of the drug (Lawrence and Rees, 2000; Malmstein, 1999). Smin and W_{max} values determined for oils investigated are shown in Table 4. All the samples were found to be clear isotropic liquids as confirmed by polarized light microscopy. The determined amount of Labrasol® required to completely solubilize equal masses of oil and water was the highest for decyl oleate (83.1%, w/w), followed by medium chain triglycerides (68.1%, w/w), isopropyl myristate (56.1%, w/w) and ethyl oleate (54.6%, w/w). In general, the efficiency of the Labrasol® was found to decrease with increasing molecular volumes (v) of oils investigated (Table 2, Fig. 2). Attempts to obtain microemulsions using mineral oil or olive oil were not successful even at extremely high surfactant concentrations (Smin > 90%, w/w). Mineral oil is a mixture of high molecular weight hydrocarbons. Olive oil contains predominantly long-chain triglycerides of oleic acid and, in contrast to mineral oil, it is polar. However, molecular weight of both of oils is most probably too high to assist in the formation of a microemulsion. Medium chain triglycerides were solubilized with lower surfactant concentration than olive oil. These results could be explained by the fact that in medium chain triglycerides about 95% of fatty acids are made of 8–10 carbon atoms and therefore their molecular volumes are smaller than for long-chain triglycerides in olive oil. The obtained results are consistent with already published observations that in some cases smaller molecular volume triglycerides could be solubilized by nonionic surfactants of polyoxyethylene *n*-alkyl ethers, to a greater extent than the larger molecular volume triglycerides (Warisnoicharoen et al., 2000a,b). Low molecular volume fatty acid esters, isopropyl myristate and ethyl oleate, were solubilized completely with a lower concentration of Labrasol®, in contrast to the higher molecular volume oil decyl oleate. In general, it was concluded for both triglycerides and fatty acid esters, that lower the molecular volume of the oil, the greater the surfactant efficiency. In addition, it was observed that efficiency of Labrasol® to solubilize decyl oleate is much lower than for medium chain triglycerides, in spite of lower molecular volume for this fatty acid ester. Also, in case of two other fatty acid esters, and, unexpectedly, Labrasol® shows a maximum efficiency for solubilization of ethyl oleate, instead of isopropyl myristate in which the molecular volume was lower. It was considered that efficiency of Labrasol®, beside molecular volume, depends to a great extent on chemical structure of oils investigated.

The second goal was to identify the oil which may be used to formulate microemulsion systems dilutable with water. In all Labrasol®/oil mixtures tested, values of W_{max} were relatively small (Table 4). Water was solubilized to the greatest extent, namely 12.3% (w/w) in the Labrasol®/isopropyl myristate mixture, whereas in the mixtures of the surfactant with the larger molecular volume oils, maximum concentrations of water incorporated were 4.8% (w/w) for ethyl oleate and medium chain triglycerides, 5.7% (w/w) for decyl oleate, and <1% (w/w) for mineral oil and olive oil. Isopropyl myristate, due to a very low molecular volume, probably penetrated the intefacial monolayer and interacted with Labrasol® incresing its efficiency to solubilize oil and water. High molecular volume of other investigated oils, hindered their interactions with Labrasol®. Surprisingly, molecular volumes of ethyl oleate and isopropyl myristate are very similar, but the water solubilization capacity of Labrasol®/ethyl oleate mixture was comparable to the mixtures containing large moleculare volume oils investigated. These observations were not quite consistent with the previous

considerations for ethyl esters of fatty acids to act as cosurfactants by penetrating the hydrophobic chain region of the surfactant monolayer (Warisnoicharoen et al., 2000a,b). The obtained results indicated that Labrasol® showed a good efficiency and water solubilizing capacity in the presence of lower molecular volume fatty acid esters with a preferred chemical structure such as isopropyl myristate.

3.2. Influence of cosurfactants on the efficiency of the surfactant (SCoSmin) and the water solubilization capacity (W_{max})

The effect of seven different cosurfactants (Table 1) on the efficiency of Labrasol® was investigated. The minimum concentration of Labrasol®/cosurfactant mixture required to produce a balanced microemulsions (SCoSmin) was determined varying surfactant/cosurfactant mass ratio (K_m) and obtained results were presented in Fig. 3. SCoSmin is presented as a function of Labrasol® percentage in investigated surfactant/cosurfactant mixtures. The efficiency of Labrasol®, as it was previously determined in this study, in the system containing equal masses of water and isopropyl myristate, was 56.1% (w/w). In general, as concentration of Labrasol® was varying in the range of investigated K_m values, there was no significant difference in the surfactant efficiency compared to the cosurfactant free system, when the polyoxyethylene surfactants were used as the cosurfactants (SCoSmin values were 49.0–57.6% (w/w) for RCH40, 51.8–58.2% (w/w) for SG2421 and 49.5–55.1% (w/w) for SG2429). Addition of Transcutol® for all investigated K_m values, resulted in a decrease of surfactants efficiency. SCoSmin values of the Labrasol®/Transcutol® mixtures were found to increase from 61.2% (w/w) to 78.7% (w/w) with increasing concentration of Transcutol® in the blend from 10 to 60% (w/w) (Fig. 3), despite an expectation of surfactant efficiency enhancement based on the published reports (Escribano et al., 2003; Parikh and Ghosh, 2005; Zhang et al., 2004). It can be assumed that Transcutol®, as an excellent solvent, modified the microenvironment of Labrasol® and led to a decrease of their solubilization capacity for oil and water phases. Efficiency of Labrasol® was shown to be significantly affected using investigated polyglycerol esters (Fig. 3). As the concentration of Labrasol® decreases from 90 to 40% (w/w) in the mixture with IGI34, polyglycerol ester with four glycerol groups, S/CoSmin becomes enlarged, from 60.9% (w/w) at 20% (w/w) of IGI34, reaching values >90% (w/w) at concentrations of IGI34 ≥ 60% (w/w). Two investigated polyglyceryl-6 esters, PI and PO, decrease SCoSmin compared to the Smin as their concentrations in the mixture with Labrasol® increased from 10 to 50% (w/w) and high similarity was observed between these cosurfactants (Fig. 3). The total SCoS concentration in the system decreased significantly from 51.0% (w/w) at 10% (w/w) of the cosurfactant to 29.2% (w/w) and 27.5% (w/w) in the presence of 50% (w/w) of PO and PI, respectively. Interestingly, microemulsion formation was not facilitated with further increase in concentration of polyglyceryl-6 esters. A comparable effect has been reported previously (Ho et al., 1996) when different polyglycerol fatty acid esters with 4–10 glycerol groups in a hydrophilic chain were investigated as a surfactant and it was observed that microemulsions could only be formed in the presence of a cosurfactant (ethanol, 1-propanol or 1-butanol). Although, the use of mixed surfactants usually increases the water maximum solubilization, indicating a synergistic effect (Hiuberts and Shah, 1997; Sagitani and Friberg, 1980), it was observed that the mixtures of Labrasol® and polyoxyethylene surfactants as cosurfactants did not display significantly increased surfactant efficiency, compared to Labrasol® itself. The simultaneous attraction of the surfactant mixture for the oil and aqueous phases is usually described by HLB value. Labrasol® is a commercially available surfactant mixture, which, according to the manufacturers, has HLB 14. CRH40 induced only minor increase in HLB (14.1–14.9) at investigated K_m values (Table 3). Although HLB of Solubilisant gamma® 2429 and Solubilisant gamma® 2421 were not available, it was rationalized that HLB values of their mixtures with Labrasol® are ranged between 14 and 16.7, because HLB of individual constituents of these two cosurfactants are 14.4 (octoxynol-12), 16.7 (polysorbate 20) and ~15 (PEG-40 hydrogenated castor oil). The obtained results could be explained by the weak influence of polyoxyethylene cosurfactants on HLB value in a blend with Labrasol® which was inadequate to obtain the better microenvironment for the stabilization of the microemulsions with high content of oil and water phase. In contrast, when polyglycerol esters were used as cosurfactants, HLB of surfactant/cosurfactant mixtures were shown to be decreased. But, the individual effects of investigated polyglycerol esters on SCoSmin were different. For polyglyceryl-6 esters optimal HLB value for the production of balanced microemulsions with as little surfactant as possible was around 12. But, the HLB concept was insufficient to explain decrease in surfactant efficiency when polyglyceryl-4 isostearate was used, even at HLB 12.2 (K_m 8:2, SCoSmin 60.9% (w/w)). The results indicated that the chemical structure of the polyglyceryl esters

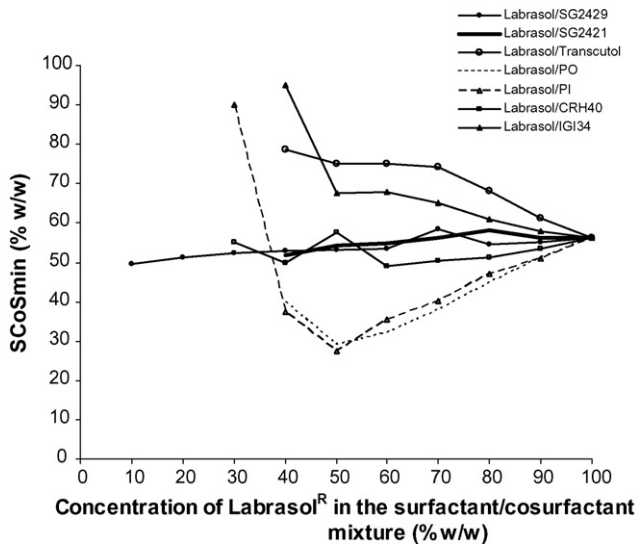


Fig. 3. The effect of K_m value (expressed as a concentration of Labrasol® in the surfactant/cosurfactant mixture) on the SCoS efficiency (SCoSmin) determined for the cosurfactants investigated.

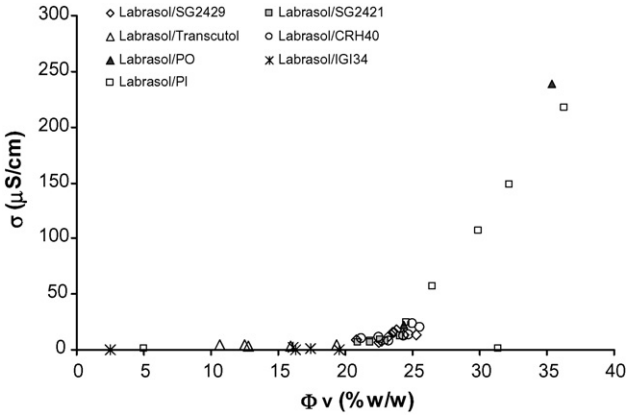


Fig. 4. Electrical conductivity (σ) of the balanced microemulsions obtained at SCoSmin for cosurfactants investigated as a function of water content (Φ_w).

as well as K_m value were very important for the SCoSmin value. The HLB concept was not quite applicable because raw materials for synthesis of surfactants investigated are usually mixtures of fatty acids with different hydrocarbon chain lengths and, furthermore, polyoxyethylene- and polyglycerol-nonionic surfactants are mixtures of oligomers with different number of oxyethylene or glycerol groups in hydrophilic chain, respectively (Porter, 1994). Also, these surfactants usually contain a large fraction of unreacted polyethylene glycols or polyglycerols, respectively, which may influence their phase behaviour (Fukuda, 2005; Izquierdo et al., 2006; Kunieda et al., 2002).

Additionally, the electrical conductivity measurements were conducted on the obtained balanced microemulsions containing SCoSmin. The obtained results are represented in Fig. 4. All balanced microemulsions formed at the water content up to 25% (w/w) possessed a low electrical conductivity (<25 μS/cm). Only polyglyceryl-6 esters produce microemulsions with higher water content. As the water concentration increased to 36.3% (w/w) (PI) and 35.4% (w/w) (PO), a noticeable increase in electrical conductivity of the samples was observed (217.7 μS/cm and 238.6 μS/cm for PI and PG, respectively) implying that these cosurfactants enhanced a synergistic effect between surfactant and cosurfactant in the formation of balanced microemulsions most likely with a high conductive bicontinuous structure. These results correlate well with our previous observations that the electrical conductivity of Labrasol®/polyglyceryl-6 dioleate/isopropyl myristate/water system increased as the dispersed water phase volume increased, leading to attractive interactions between the microemulsion droplets and subsequent percolation (Djordjevic et al., 2004). These results also confirm the synergistic effect between polyglyceryl-6 esters and Labrasol® in the formation of balanced microemulsions using isopropyl myristate as oil phase. The strong similarity in the electrical conductivity results obtained for samples containing PI and PG, may explain by almost the same water content in investigated systems.

Furthermore, the influence of the type of the cosurfactant and K_m value on the water solubilization capacity of the Labrasol®/cosurfactant/isopropyl myristate mixtures was

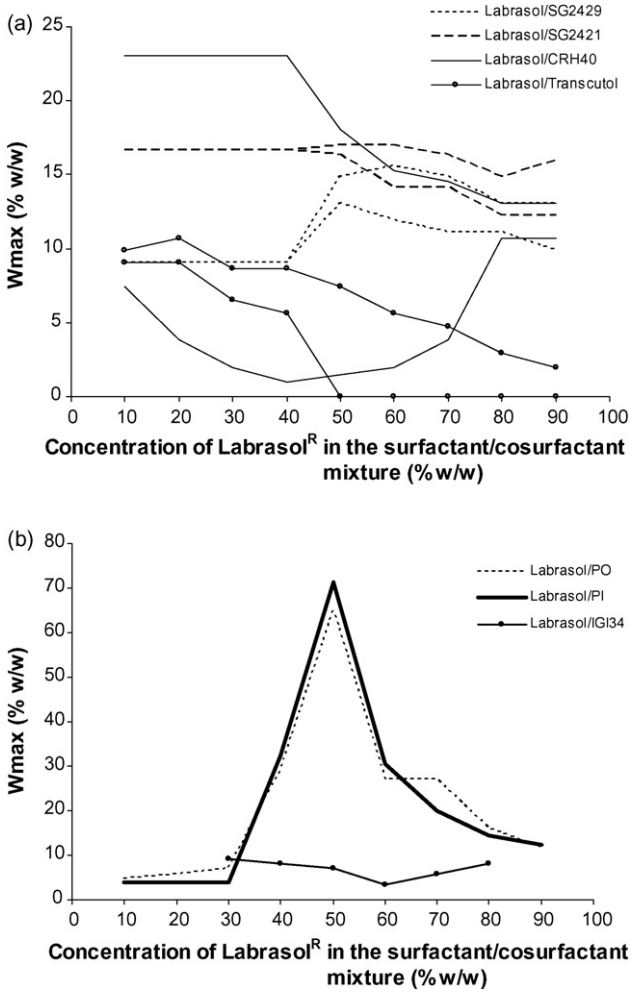


Fig. 5. The effect of K_m value (expressed as a concentration of Labrasol® in the surfactant/cosurfactant mixture) on the water solubilization capacity (W_{max}) determined for cosurfactants investigated: (a) Solubilisant gamma® 2421 (SG2421), Solubilisant gamma® 2429 (SG2429), Cremophor® RH 40 (CRH40) and Transcutol®; (b) Plurol Oleique® (PO), Plurol Isostearique® (PI) and Isolan® GI 34 (IGI34).

investigated. In the absence of a cosurfactant, a maximum of 12.9% (w/w) water can be solubilized by the mixtures of the Labrasol® and isopropyl myristate at constant oil to surfactant weight ratio 1:1. The effect of varying K_m on the maximum water solubilized for each cosurfactant investigated was plotted using pseudo-binary phase diagrams (Fig. 5). The construction of pseudo-binary phase diagrams makes it easy to directly follow the variation of the microemulsions existence area at the wide range of K_m values. The area inside the curves assigned on the phase diagram showing the microemulsion region. The area outside the curves indicates a turbid region with multiphase systems. Fig. 5a shows the pseudo-binary phase diagram for the systems containing CRH40, SG2421, SG2429 or Transcutol® as the cosurfactant. Addition of Transcutol®, at all K_m investigated, resulted in a decrease in the W_{max} compared to the cosurfactant-free system. Furthermore, as the concentration of Transcutol® increased, the water solubilization capacity as well as the area of microemulsion formation in the phase diagram decreased, especially at K_m values less than 5:5

(Fig. 5a). These observations were consistent with the results for decreasing effect of Transcutol® on efficiency of Labrasol® (Fig. 3). Labrasol®/cosurfactant/isopropyl myristate mixtures which contained cosurfactants SG2421 and SG2429, exhibited an one-phase microemulsion region at $K_m \geq 5:5$ (Fig. 5a). However, at lower Labrasol® concentrations (<50.0%, w/w) microemulsions only formed in an extremely narrow water range (around 16% (w/w) for SG2421 and around 9% (w/w) for SG2429). For CRH40, as shown in the same figure, as K_m decreases, the microemulsion existence area becomes enlarged, reaching a maximum at K_m 4:6 (23.1%, w/w). However, this trend was reversed using SG2421 or SG2429, where water was solubilized to the greatest extent at K_m 6:4 (15.6% (w/w) and 17.0% (w/w) for SG2429 and SG2421, respectively). Fig. 5b shows the phase diagrams, constructed to determine the optimum K_m for the water solubilization in the systems consisting of Labrasol®, isopropyl myristate and polyglycerol fatty acid esters. It could be noted that the area of microemulsion region obtained for polyglycerol-6 esters (PI and PO), was considerably large compared to the cosurfactants of polyoxyethylene type and Transcutol® as well as to the polyglycerol-4 ester (IGI34). In the case of polyglycerol-6 esters, strong similarity between PG and PI was observed for water solubilization. As K_m decreases, water solubilization capacity increases, reaching a maximum at K_m 5:5 (W_{max} 65.0% (w/w) for PO and 71.4% (w/w) for PI), while only 7.0% (w/w) of water, could be solubilized at the same K_m for IGI34 (Fig. 5b). These results were in agreement with observations of Kunieda (Kunieda et al., 2002) and Fukuda (Fukuda, 2005) that hydrophile–lipophile properties of polyglycerol ethers, a class of surfactants which have chemical structure generally similar to polyglycerol esters, depend significantly on their hydrophilic chain length so that with increase in the number of glycerol groups in hydrophilic chain, increases their affinity to form hydrogen bonds with surrounding water molecules. With further increasing in concentration of polyglycerol-6 esters in the blend ($K_m < 5:5$), the water solubilization capacity was found to decrease. These results were consistent with the decreasing influence of polyglycerol-6 esters on the efficiency of Labrasol® to solubilize oil and water phases.

To summarise, the obtained results suggest that the solubilization of a water is easier in the systems containing the mixture of the polyoxyethylene surfactant (Labrasol®) and polyglycerol-6 esters as cosurfactant, than in the strictly polyoxyethylene based systems. These results follow the observations recorded in the literature (Fukuda, 2005; Kunieda et al., 2002) that surfactants containing glycerol groups can solubilize water and oil to a much greater extent than nonionic surfactants with oxyethylene groups. From a microemulsion formulation point of view, as polyglycerol fatty acid esters have been suggested as *orally safe* surfactants as well as isopropyl myristate has marked as a good enhancer of drug permeation through the biological membranes, the increased efficiency of Labrasol® using polyglycerol-6 esters as cosurfactant increases the attraction of this type of microemulsions as colloidal drug delivery vehicles with improved pharmaceutical relevance.

4. Conclusions

The efficiency of Labrasol® to generate microemulsions could be influenced by type and concentration of cosurfactants and oils investigated. Despite all the complexity, it was possible to identify favourable physico-chemical properties for an oil and a cosurfactant included in this study. Low molecular volume oils, such as fatty acid esters and triglycerides with medium chain lengths are preferred instead of high molecular volume oils. The phase behaviour study revealed that the maximum proportion of water could be incorporated in Labrasol® based microemulsions containing isopropyl myristate as oil phase. Furthermore, a significant difference regarding the water solubilization capacity between investigated oils with similar molecular volumes (isopropyl myristate and ethyl oleate) suggested that the chemical structure of oil is also very important factor in microemulsion formation. In systems containing Transcutol® as well as IGI34 as cosurfactant, the efficiency of Labrasol® was reduced for all investigated K_m values. The enhancement of the polyoxyethylene type of cosurfactants on the surfactant efficiency, compared to two investigated polyglyceryl-6 esters, as well as to the cosurfactant free system, was not significant in the range of investigated K_m values. The tendency of Labrasol® to solubilize both, water and oil phases, was favoured by polyglyceryl-6 esters, PI and PO, reaching a maximum at K_m value 5:5. Balanced microemulsions stabilized with Labrasol®/polyglyceryl-6 esters blend contained a high content of both oil and water and their structure was likely bicontinuous. Systematic experiments carried out in this study including the experimental procedure developed in order to characterize the efficiency of the nonionic surfactant PEG-8 caprylic/capric glycerides (Labrasol®) to generate microemulsions with high percentage of oil and water phase, as well as microemulsion systems dilutable with water, may be useful in laying down the basis for understanding of the role of formulation variables (such as an oil and a cosurfactant) on the efficiency of complex mixtures of nonionic surfactants to produce balanced microemulsions and SMEDDS.

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