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Formulate-ability of ten compounds with different physicochemical profiles in SMEDDS

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ABSTRACT

In order to gain a better understanding of the reasons of successful self-microemulsifying drug delivery systems (SMEDDS) formulation, ten poorly water-soluble drugs, exhibiting different physicochemical properties, were selected. The solubility of the compounds was determined in various oils (long and medium chain) and surfactants (HLB > 12 and HLB < 10). The best performing excipients were selected for SMEDDS formulation. The droplet size and zeta potential of SMEDDS were measured in the absence and the presence of drug. Media, time and the presence of drug showed little or no influence on droplet size of most systems. Some systems displayed a different zeta potential in the presence of drugs. *In vitro* pharmaceutical performance of the SMEDDS formulations was investigated using the dialysis bag method in reverse mode next to conventional *in vitro* release methodology. The results suggested that the measured concentration of the compounds inside the dialysis bag corresponded to solubility of the compound in the release medium, which suggested that the formation of micelles inside the dialysis bag was delayed or disturbed. Conventional *in vitro* release methodology with pH change from acidic to neutral appeared as a simple method which gives valuable information about the dispersion and the solubilization ability of the SMEDDS formulation at different pHs. In general, formulate-ability in SMEDDS was found to depend on the solubility of the drugs in the excipients and log *P* of the compounds (the optimal log *P* was found between 2 and 4).

1. Introduction

The advent of combinatorial chemistry and high throughput screening has resulted in the rapid identification of many potent new chemical entities. Coincident with the increasing use of these technologies, there has been a developing trend towards the identification of lead compounds with higher molecular weights and lower water solubilities (O'Driscoll and Griffin, 2008). As solubility is considered one of the prerequisites to intestinal absorption, many of these drugs exhibit poor and variable oral bioavailability (Amidon et al., 1995). Many approaches to improve oral bioavailability have been investigated by enhancing drug solubility or increasing the surface area available for dissolution using, e.g. salts, solid dispersions, silica-based materials or water-soluble drug complexes (i.e. cyclodextrins).

Dosing drug substances that exhibit poor water solubility but sufficient lipophilic properties, in a predissolved state, for example in lipid-based formulations, are beneficial since the energy input associated with a solid–liquid phase transition is avoided, thus overcoming the slow dissolution process after oral intake (Charman et al., 1992; Humberstone and Charman, 1997; Craig, 1993; Porter et al., 2004). Lipid-based formulations include lipid solutions, emulsions, microemulsions, self-emulsifying drug delivery systems (SEDDS) or self-microemulsifying drug delivery systems (SMEDDS) (Pouton, 2000; Vetter et al., 1985; Kang et al., 2004). A SMEDDS is a mixture of an oil, a surfactant and sometimes a co-solvent or co-surfactant, that spontaneously forms a transparent (or at least translucent), stable microemulsion upon dilution with water (Charman et al., 1992; Constantinides, 1995; Greiner and Evans, 1990; Shah et al., 1994). Lipid-based formulations have been shown to enhance oral absorption of lipophilic drugs (Pouton, 2000). Although the exact mechanisms responsible for this enhanced

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absorption are not fully known, it is believed that various factors including improved drug solubilization, increased membrane permeability and lymphatic transport may contribute significantly (O'Driscoll, 2002; Porter et al., 2007).

Self-emulsifying systems have gained considerable interest after the successful development of lipid-based formulations of cyclosporine A (Neoral Sandimmun®), and two HIV protease inhibitors, ritonavir (Norvir®) and saquinavir (Fortovase®), which are commercially available as SMEDDS formulations (Grevel et al., 1986; Vanderscher and Meinzer, 1994; Tarr and Yalkowsky, 1989; Uede et al., 1984). Although there are increasing number of papers reporting on successful SMEDDS formulation, the formulation process is still based on trial and error.

In contrast to oral solid dosage forms, the drug in a SMEDDS formulation is already in a dissolved state. Moreover, the absorption mechanism for SMEDDS and other lipid-based formulations is rather complicated (Wassan, 2001; Porter and Charman, 2001; Charman, 2000). Thus, the multiple roles of *in vitro* dissolution testing, which have been recognized for oral solid dosage forms, such as guiding the drug development process, a preliminary test for the detection of possible bioinequivalence between products, a quality control tool and so forth, can be questioned for SMEDDS formulations (Chen, 2008). Because a standard protocol for *in vitro* release testing of lipid-based formulations has not been established, different authors have been using different methods in their studies. The *in vitro* digestion has been used in different studies as, in theory, the digestion products can enhance and affect drug solubilization and dissolution (Porter et al., 2007; Gershanik and Benita, 2000). However, this method is rather complicated, indirect and according to a recent study of Goddeeris et al., drug release did not seem to be driven by the digestion of the oil phase (Goddeeris et al., 2007). Although, *in vitro* dissolution profiles in biorelevant media could better estimate the plasma profile for lipophilic drugs than the profiles obtained with compendial media (Nicolaides et al., 2001), using FaSSIF and FeSSIF still did not result in appropriate release from lipid-based preparations when used in conjunction with the conventional paddle method (Jantratid et al., 2008). The dialysis bag method, in normal or reverse mode, has also been used in different studies of SMEDDS formulations (Shen and Zhong, 2006; Woo et al., 2007; Chen et al., 2008). Both are relatively easy methods to carry out. However, the normal dialysis bag method has a limited volume, which can hinder the emulsifying process of the SMEDDS formulation. The dialysis bag method in reverse mode is claimed better in simulating the *in vivo* circumstances (Chen et al., 2008), but no further evidence for the claim has been shown.

In this study, ten structurally diverse, poorly water-soluble drugs were selected in order to gain better understanding of the mechanism underlying successful SMEDDS formulation. The resulting formulations were investigated in terms of physical nature and *in vitro* pharmaceutical performance. In addition, the dialysis bag method in reverse mode, which has been increasingly used for evaluation of SMEDDS formulations, was investigated and compared to the conventional *in vitro* release methodology.

2. Materials and methods

2.1. Materials

Ten poorly water-soluble compounds were selected based upon their physicochemical profile, in order to obtain a test series with a high degree of diversity (Table 1). Carbamazepine was purchased from PharmInnova (Waregem, Belgium); indomethacin and griseofulvin from Certa (Eigenbrakel, Belgium); nifedipine, danazol and fenofibrate from Indis (Aartselaar, Belgium) and methylprednisolone, phenytoine and diazepam from Fagron (Waregem, Belgium). Itraconazole was kindly donated by Janssen Pharmaceutica (Beerse, Belgium). Captex 200P (propylene glycol mono- and dicaprylate and mono- and dicaprate), Capmul MCM (glyceryl mono- and dicaprate) and Caprol 3GO (polyglycerol-3 oleate) were kindly supplied by Abitec Corp. (Janesville, WI, USA). Soybean oil was purchased from Sigma–Aldrich Chemie (Steinheim, Germany). Sodium chloride was purchased from Fisher Scientific UK Limited (Loughborough, Leicestershire, UK), and hydrochloric acid 1N and trisodium phosphate were bought from VWR International (Leuven, Belgium). Regenerated dialysis membranes (Cellu-Sep membrane), MWCO 3500 D, were purchased from Micron Separation Europe (Braine-l'Alleud, Belgium). Demineralized water was used for all experiments (Elga, maxima ultra pure water, ≥18 MΩ).

2.2. Methods

2.2.1. Solubility studies of compounds in various excipients

The solubility of compounds in various lipids and surfactants was determined. An excess amount of compound was introduced to 1 ml of each excipient and the mixture was shaken in an eppendorf vial at 37 °C for 48 h (equilibrium). The eppendorf vial was centrifuged at 15,000 rpm for 15 min using a Hettich Mikroliter centrifuge (Tuttlingen, Germany). The concentration of the compound was determined by HPLC analysis.

2.2.2. Screening of candidate SMEDDS

The screening of candidate SMEDDS was done by mixing one selected surfactant with one selected oil in different ratios; subsequently water was added so that the concentration of the mixture surfactant–oil was 0.5% (w/v). If the emulsions were clear or slightly opaque, they were selected for further characterization.

2.2.3. Preparation of SMEDDS formulations

The formulations were prepared by initially mixing oil with surfactant at 50–60 °C. Drug compounds were then dissolved into the mixture of surfactant and oil by constant stirring and kept at 50–60 °C until a clear solution was obtained. All mixtures stayed clear at room temperature.

Table 1
Overview of the properties of the ten model compounds.

	1	2	3	4	5	6	7	8	9	10
Molecular weight (g/mole)[a]	236.27	337.46	284.74	360.83	352.77	357.79	705.63	364.34	374.47	252.27
pKa[a]	–	–	2.92	–	–	3.80	3.92	–	–	9.12
log *P* est.[a]	2.25	4.21	2.70	5.19	1.92	4.23	6.16	1.82	2.50	2.16
log *P* Exp.[b]	2.45	–	2.82	–	2.18	4.27	5.66	–	2.20	2.47
Zeta potential (mV)[c]	−12.6	−40.7	−45.2	−52.4	−29.6	−55.1	−37.0	−36.6	−31.7	−39.9

1: carbamazepine; 2: danazol; 3: diazepam; 4: fenofibrate; 5: griseofulvine; 6: indomethacine; 7: itraconazole; 8: methylprednisolone; 9: nifedipine; 10: phenytoine.
[a] Data obtained by using free Marvin software download from website (http://www.chemaxon.com/marvin/index.html).
[b] Data from the literature.
[c] Experimental data.

Table 2
Overview of HPLC methods for analysis of the model compounds.

Compound	Column	UV detection	Mobile phase
Carbamazepine	1	284 nm	Methanol:12.5 mM phosphate buffer, pH 6.5 (45:55)
Griseofulvine	1	294 nm	Methanol:25 mM phosphate buffer, pH 3.5 (50:50)
Indomethacine	1	266 nm	Acetonitrile:25 mM ammonium acetate buffer, pH 3.5 (40:60)
Diazepam	2	230 nm	Acetonitrile:25 mM phosphate buffer, pH 5.5 + 30 mM triethylamine (45:55)
Fenofibrate	2	287 nm	Acetonitrile:25 mM ammonium acetate buffer, pH 3.5 (65:35)
Phenytoine	2	225 nm	Methanol:water (48:52)
Nifedipine	2	340 nm	Acetonitrile:25 mM ammonium acetate buffer, pH 3.5 (65:35)
Danazol	3	288 nm	Acetonitrile:water (60:40)
Methylprednisolone	3	248 nm	Acetonitrile:water (33:67)
Itraconazole	3	260 nm	Acetonitrile:tetrabutylammonium hydrogen sulfate 0.01N (50:50)

1: Hypersil BDS C18 5 μm 100 mm × 4 mm.
2: Lichrospher 60 RP8 select B 5 μm 125 mm × 4.6 mm.
3: Lichrospher 100 RP8 5 μm 125 mm × 4.6 mm.

2.2.4. Physicochemical evaluation

2.2.4.1. Droplet size measurement. The droplet size of the emulsions was determined at 2% (w/v) concentration by photon correlation spectroscopy using a CGS-3 spectrometer (Malvern Instruments, Worcestershire, UK) equipped with a goniometry, auniphase 22 mV He–Ne laser operating at 632.8 nm, an avalanche photodiode and detector and an ALV-5000/EPP multi-angle tau correlator. Light scattering was monitored at 90°.

2.2.4.2. Zeta potential measurement. The zeta potential of the emulsions was measured at 2% (w/v) concentration by a Zetamaster instrument (type ZEM 5000, Malvern Instruments Limited, Worcestershire, UK). The zeta potential was determined 10 times for each sample and averages and standard deviations were calculated.

2.2.5. In vitro drug release

2.2.5.1. Dialysis bag method in reverse mode. The dialysis bag method in reverse (DR) was performed in combination with the USP 24 method 2 (paddle method) in a Hanson SR8 plus dissolution apparatus (Chatsworth, CA, USA). 500 ml of simulated gastric fluid without pepsin (SGF_{sp}) was used as dissolution medium at a temperature of 37 °C and a paddle speed of 100 rpm. Prior to the test, five dialysis bags containing 3 ml blank medium were placed into the release medium for 2 h. Subsequently, SMEDDS formulations were put directly into the release medium, one dialysis bag was taken out at 30, 60, 120, 180 and 240 min and the drug concentration was determined. After 2 h, the pH of the medium was adjusted to 6.8 by the addition of trisodium phosphate.

2.2.5.2. Conventional in vitro release method with pH change. The conventional *in vitro* release was performed based on the USP 24 method 2 (paddle method) in a Hanson SR8 plus dissolution apparatus (Chatsworth, CA, USA). 500 ml of SGF_{sp} was used as dissolution medium at a temperature of 37 °C and a paddle speed of 100 rpm. After 2 h, the pH of the medium was adjusted to 6.8 by the addition of trisodium phosphate. SMEDDS formulations were put directly into the release medium. Samples were taken at 5, 10, 20, 30, 45, 60, 90, 120, 130, 180 and 240 min, and then filtered using 0.45 μm pore size membrane filters.

2.2.6. HPLC method

The HPLC analysis was performed using a Merck Hitachi pump L-7100, an autosampler L-7200 and a UV–VIS detector L-7420. Columns, mobile phases and detection wavelengths used to analyze the ten compounds are listed in Table 2. All the mobile phases were used at flow rate of 1.0 ml/min. 20 μl sample volume was injected into the system.

2.2.7. Statistics

The Student's *t*-test (95% confidence interval) was employed when comparing two mean values, namely to compare the droplet size at different time points or in different media to that of at time 0 in water, to compare the zeta potential value of systems with that of surfactant used in the systems, and to compare the dissolution profile when using the dialysis bag method. One-way analysis of variance (ANOVA) was used when comparing droplet size and zeta potential value of drug loaded systems and those of placebo systems and different dissolution profiles using the conventional *in vitro* release method.

3. Results and discussion

3.1. Solubility studies of compounds in various excipients

Compounds should be soluble in the excipients of the SMEDDS formulation, and therefore suitable oils and/or surfactants need to be selected. Lipids commonly used in lipid-based formulations are medium- or long-chain glycerides. Different medium- and long-chain glycerides, both polar and non-polar, were used in this study, including Caprol 3GO (i.e. C8:C10 triglyceride), Capmul MCM (i.e. C8:C10 mono-, diglyceride), Captex 200P (propylene glycol diesters of saturated fatty acids, mainly of caprylic and capric acid), Miglyol 812 (caprylic/capric triglyceride), soybean oil (i.e. C18 mono-, di- and triglyceride with oleic fatty acids) and corn oil (mono-, di- and triglyceride with mainly oleic and linoleic acids).

The efficiency of self-microemulsification is related to the hydrophilic–lipophilic balance (HLB) value of the surfactant. Generally, surfactants with HLB 12–15 are regarded as being of good efficiency. Considering the safety and biocompatibility of the excip-

Table 3
The selected candidate SMEDDS after screening.

System	Ratio (w/w)	System	Ratio (w/w)
Tween 80–Caprol 3GO	5–1 6–1 7–1	Cremophor EL–Caprol 3GO	8–1 9–1
Tween 80–Captex 200P	2–1 3–1 4–1	Cremophor EL–Captex 200P	3–1 4–1 5–1
Tween 80–Capmul MCM	3–1 4–1 5–1	Cremophor EL–Capmul MCM	2–1 3–1 4–1
Tween 80–Soybean oil	7–1 8–1 9–1	Labrasol–Caprol 3GO	5–1 6–1 7–1

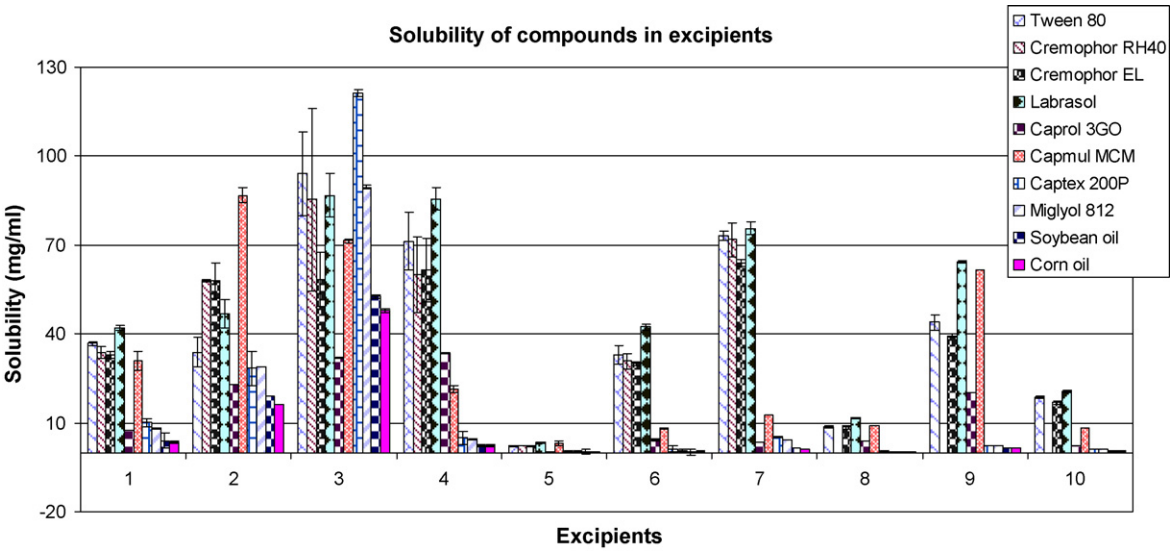

Fig. 1. Solubility of compounds in different excipients. 1—danazol; 2—diazepam; 3—fenofibrate; 4—indomethacin; 5—itraconazole; 6—phenytoine; 7—nifedipine; 8—methylprednisolone; 9—carbamezepine; 10—griseofulvine ($n = 3$). Error bars indicate standard deviation.

ient, we selected several non-ionic surfactants, namely Cremophor EL (HLB 12–14), Cremophor RH40 (HLB 12–14), Tween 80 (HLB 15) and Labrasol (HLB 14). As the surfactants and oils are usually a mixture of components, and the exact ratio among those components is not available, exact figures of different properties of excipients such as log *P*, pKa, molecular volume and so forth, like the one for the drug compounds is not possible.

The solubility of different compounds in the various excipients at 37 °C was determined (by HPLC) and is presented in Fig. 1. In most cases, the following order in solubility was observed: surfactants > medium-chain oils > long-chain oils. The compounds could be divided into different groups according to their solubilities. Diazepam and fenofibrate belong to the group with relatively good solubility (≥20 mg/ml) in both surfactants and oils: the solubility of diazepam is lowest in corn oil (16.4 mg/ml) and highest in Capmul MCM (86.8 mg/ml). Fenofibrate had highest solubility in Captex 200P (121.3 mg/ml) and lowest in Caprol 3GO (31.8 mg/ml). The solubility of carbamazepine, danazol,

Table 4
The mean droplet size of placebo SMEDDS in different media ($n = 5$).

System	Ratio (w/w)	Mean droplet size (nm) in different media					
		Water			HCl 0.1 M	NaH_2PO_4 0.01 M, pH 6.8	NaH_2PO_4 0.001 M, pH 6.8
		Time 0 h	Time 1 h	Time 6 h			
Tween 80–Captex 200P	2–1	32.6 ± 5.9	50.0 ± 3.1[a]	63.4 ± 6.2[a]	16.0 ± 4.8[a]	17.1 ± 5.1[a]	17.0 ± 3.7[a]
	3–1	8.8 ± 3.2	10.7 ± 3.7	35.4 ± 5.1[a]	9.2 ± 3.1	9.5 ± 2.5	9.4 ± 4.7
	4–1	7.9 ± 2.9	9.7 ± 1.6	35.9 ± 4.7[a]	8.4 ± 3.9	9.5 ± 3.2	8.8 ± 3.5
Tween 80–Capmul MCM	3–1	175.6 ± 10.1	192.6 ± 10.2[a]	215.8 ± 11.9[a]	195.3 ± 11.1[a]	152.0 ± 8.7[a]	154.2 ± 9.4[a]
	4–1	79.8 ± 7.2	78.9 ± 6.3	91.8 ± 6.7[a]	107.0 ± 11.9[a]	104.5 ± 9.7[a]	91.5 ± 10.1
	5–1	11.1 ± 1.4	9.8 ± 1.1	8.4 ± 1.0[a]	11.9 ± 1.3	20.4 ± 1.8[a]	17.1 ± 1.5[a]
Tween 80–Caprol 3GO	5–1	181.5 ± 10.3	159.0 ± 10.1[a]	151.4 ± 10.0[a]	219.6 ± 9.7[a]	145.3 ± 9.8[a]	260.6 ± 9.2
	6–1	160.2 ± 4.1	167.0 ± 3.6[a]	126.6 ± 9.2[a]	191.2 ± 10.2[a]	163.8 ± 9.8[a]	179.8 ± 7.2[a]
	7–1	185.5 ± 2.5	161.6 ± 4.1[a]	158.6 ± 7.3[a]	126.9 ± 7.5[a]	185.8 ± 9.3	137.4 ± 6.4[a]
Tween 80–Soybean oil	7–1	6.3 ± 0.5	6.8 ± 0.2	6.6 ± 0.2	7.2 ± 0.2[a]	8.2 ± 0.9[a]	7.9 ± 0.5[a]
	8–1	6.1 ± 0.2	7.3 ± 0.9[a]	6.5 ± 0.3[a]	6.8 ± 0.2[a]	7.4 ± 0.4[a]	7.1 ± 0.3[a]
	9–1	6.0 ± 0.2	6.3 ± 0.2	6.1 ± 0.1	6.8 ± 0.2[a]	8.0 ± 0.1[a]	6.7 ± 0.1[a]
Cremophor EL–Captex 200P	3–1	12.8 ± 3.1	13.2 ± 3.2	12.1 ± 2.8	11.8 ± 3.0	13.7 ± 3.6	12.7 ± 2.7
	4–1	11.0 ± 2.6	11.8 ± 3.5	10.8 ± 4.7	10.2 ± 2.7	10.6 ± 2.5	10.6 ± 3.2
	5–1	8.93 ± 1.3	10.6 ± 2.6	9.2 ± 1.3	9.6 ± 2.8	9.2 ± 1.3	10.0 ± 1.2
Cremophor EL–Capmul MCM	2–1	217.8 ± 12.6	189.7 ± 11.7[a]	165.4 ± 12.1[a]	165.4 ± 17.1[a]	184.0 ± 13.5[a]	134.4 ± 12.1[a]
	3–1	54.9 ± 5.2	53.8 ± 6.1	63.2 ± 2.3[a]	80.6 ± 5.4[a]	–	88.4 ± 4.5[a]
	4–1	17.0 ± 1.6	12.7 ± 1.7[a]	15.6 ± 1.5	27.7 ± 1.8[a]	44.8 ± 2.0[a]	41.6 ± 1.9[a]
Cremophor EL–Caprol 3GO	8–1	85.6 ± 4.9	81.1 ± 4.1	79.0 ± 4.5	60.8 ± 1.1[a]	59.0 ± 3.9[a]	43.6 ± 2.6[a]
	9–1	39.1 ± 1.8	37.5 ± 1.8	35.1 ± 1.7[a]	37.6 ± 1.4	44.4 ± 1.6[a]	26.5 ± 1.5[a]
Labrasol–Caprol 3GO	5–1	76.5 ± 8.7	75.7 ± 6.3	73.7 ± 7.3	2143 ± 73.7[a]	107.3 ± 6.3[a]	58.3 ± 5.6[a]
	6–1	74.4 ± 7.0	75.8 ± 7.1	77.5 ± 6.8	647.9 ± 12.7[a]	177.2 ± 3.9[a]	48.1 ± 2.5[a]
	7–1	84.6 ± 6.4	82.6 ± 6.1	78.5 ± 5.3	490.8 ± 9.7[a]	184.7 ± 3.9[a]	40.5 ± 4.7[a]

[a] Significantly different (95%) from the droplet size at time 0 in water.

indomethacine, nifedipine and phenytoine in surfactants was all above 30 mg/ml, while in non-polar oils it was always below 10 mg/ml. Griseofulvine and methylprednisolone had medium solubility (20 mg/ml ≥ solubility ≥ 10 mg/ml) in surfactants and poor solubility (≤5 mg/ml) in oils. Itraconazole had poor solubility in both surfactants and oils. It can also be seen that for example, griseofulvine and methylprednisolone having comparable solubility in surfactants and poor solubility in oils, have some similarity in log *P*. However, in the case of diazepam and fenofibrate, although both have good solubility in surfactants and oils, no similarity in their properties can be discerned. There is no clear link between the physicochemical properties of the model drugs and their ability to be dissolved in the selected excipients.

3.2. Screening of candidate SMEDDS

All tested surfactants showed almost the same solubilizing capacity for the drug compounds; therefore they were all candidates for the screening study of possible SMEDDS formation. As Cremophor EL and Cremophor RH 40 are similar and Cremophor EL was easier to handle, Cremophor RH 40 was not further investigated.

Both polar oils Caprol 3GO and Capmul MCM were selected because neither of the two gave better solubility to all compounds in this study. Captex 200P, a non-polar, medium-chain oil, and soybean, a long-chain oil, were chosen over Miglyol 812 and corn oil as they gave higher solubility of the drugs under investigation.

In general, a pseudo-ternary phase diagram is constructed from one selected surfactant with one selected oil and/or one co-solvent (co-surfactant) when studying the development of a SMEDDS formulation. However, since few surfactants and oils were selected in this study, a full screening of possible SMEDDS was carried out instead of a construction of a phase diagram. Only the systems which gave slightly opaque or clear emulsions were selected for further studies. The selected systems with their compositions are shown in Table 3.

3.3. Assessment of the physical properties of selected SMEDDS

3.3.1. The placebo SMEDDS

The droplet size and zeta potential are important characteristics of an emulsion when evaluating its stability. Therefore, the droplet size of placebo SMEDDS was determined in different media, i.e., in demineralized water, hydrochloric acid 0.1 M and sodium dihydrogenphosphate 0.01 and 0.001 M, pH 6.8. The droplet size was also measured as a function of time, i.e., after 0, 1 and 6 h. The results are shown in Table 4.

The mean droplet size of all systems ranged from about 6 nm in the case of Tween 80–soybean oil systems to a maximum of 217.8 nm in the case of Cremophor EL–Capmul MCM (2–1). Time had little influence on droplet size of most of the systems, only for Tween 80–Captex 200P and Tween 80–Capmul MCM (3–1) systems, the mean droplet size did increase significantly in time. No or little effect of media on droplet size was expected, as non-ionic surfactants are less affected by changes in pH and ionic strength compared to ionic surfactants. This assumption was confirmed statistically for some systems, for example Cremophor EL–Captex 200P systems showing no significant differences in droplet size. For most systems, however, a *t*-test showed a significant (95% confidence interval) difference between the droplet sizes when the medium was altered. Nevertheless, for these systems, droplet size ranges are usually lower than the maximum limit (150 nm) for droplet size of self-microemulsifying systems. In some systems, though, a change of medium was able to increase the droplet size above 150 nm, e.g. for the Labrasol–Caprol 3GO systems, the mean droplet size in the case of ratio 7–1 increased from 84.6 nm in water to 490.8 nm in acidic medium or from ca. 76 to 2143 nm in the case of ratio 5–1. The increase in the droplet size of Labrasol–Caprol 3GO systems in acidic medium might arise due to the fact that oil is present at the fixed aqueous layer of the micelle (this will be elaborated below). However this explanation cannot account for the absence of an increase in droplet size with Tween 80–Caprol 3GO and Cremophor EL–Caprol 3GO systems.

Table 5
Zeta potential values of placebo SMEDDS.

System	Ratio (w/w)	ζ (mV)
Tween 80–Caprol 3GO	5–1	−26.9 ± 0.7[a]
	6–1	−24.8 ± 0.7[a]
	7–1	−27.2 ± 1.1[a]
Cremophor EL–Caprol 3GO	8–1	−23.3 ± 0.4[a]
	9–1	−21.2 ± 0.5[a]
Labrasol–Caprol 3GO	5–1	−31.4 ± 3.4[a]
	6–1	−29.1 ± 2.1[a]
	7–1	−29.9 ± 1.1[a]
Tween 80–Captex 200P	2–1	−7.0 ± 0.6
	3–1	−7.9 ± 0.6
	4–1	−6.1 ± 0.6
Cremophor EL–Captex 200P	3–1	−14.6 ± 2.7[a]
	4–1	−15.4 ± 3.7[a]
	5–1	−11.4 ± 2.9[a]
Tween 80–Capmul MCM	3–1	−8.8 ± 0.4
	4–1	−6.5 ± 0.7
Cremophor EL–Capmul MCM	2–1	−4.8 ± 1.1
	3–1	−4.8 ± 1.0
	4–1	−5.6 ± 0.2
Tween 80–Soybean oil	9–1	−12.9 ± 8.2

[a] Significantly different (95%) from the zeta potential value of surfactant.

The zeta potential of these systems in demineralized water was also measured and the results are shown in Table 5. If systems with the same surfactant are ranked and compared, different zeta potential values can be observed. This value was from −6.1 to −7.9 mV; −6.5 to −8.8 mV; −24.8 to −27.2 mV and −12.9 mV for Tween 80 with Captex 200P, Capmul MCM, Caprol 3GO and soybean oil, respectively. For systems containing Cremophor EL, the zeta potential values were also different.

On the other hand, if systems with the same oil component are compared, some trends are visible. For Caprol 3GO, the zeta potential values ranged from −21.2 to −31.4 mV and differed from that of the surfactants (the measured ζ values of Tween 80, Cremophor EL, Labrasol are -7.5 ± 2.1, -4.1 ± 1.6, and -23.2 ± 3.9 mV, respectively). They did not seem to depend strongly on the type of surfactant but only on the oil. For the Capmul MCM and Captex 200P–Tween 80 systems, the zeta potential values were similar to those of the surfactants. The oil did not appear to have much influence on these systems. The values of the zeta potential also seemed not to depend strongly on the size of the micelles. In general, the zeta potential value of a micelle depends partly on the properties of its fixed aqueous layer. If the fixed aqueous layer of the micelles contained only surfactant, the zeta potential value of a micelle should be similar to that of the surfactant. The results that showed an influence of the oil on the zeta potential value of the system seem to point towards an interaction between oil and surfactant; in this case, the oil is assumed to be present in the fixed aqueous layer of the micelle.

As mentioned above, measurement of droplet size and zeta potential value is instrumental for the evaluation of the stability of emulsions and selecting the potential SMEDDS; elucidating the structures of the micelles formed is beyond the scope of this paper.

Table 6
Drug incorporation in different formulations.

Compound	Formulation			
	Tween 80–Caprol 3GO (5–1)	Tween 80–Captex 200P (3–1)	Tween 80–Capmul MCM (5–1)	Tween 80–Soybean oil (7–1)
Carbamazepine	3%	2.5%	3%	3%
Diazepam	3%	3%	3%	3%
Indomethacine	5%	5%	5%	5%
Nifedipine	3%	3%	3%	3%
Phenytoine	2%	2%	2%	2%
Methylprednisolone	–	0.5%	0.5%	–
Danazol	–	2%	–	–
Fenofibrate	–	5%	–	–
Griseofulvin	–	–	–	–
Itraconazole	–	–	–	–

3.3.2. Incorporation of drugs into the selected systems

Although there was no clear difference in droplet size and zeta potential values, Tween 80 systems were preferred to Cremophor EL systems as Tween 80 could form microemulsions with all the trial oils. As Labrasol–Caprol 3GO systems were not stable in acidic medium, they were not selected. The ratio between surfactant and oil was chosen based on the size and the stability of droplet size of the systems: those with the same surfactant and oil which gave the smallest droplet size were selected. Moreover, the amount of surfactant was kept as low as possible as surfactants may cause irritation inside the GI tract. Therefore, for systems having similar droplet sizes, those with the lowest amount of surfactant were selected. Finally, four systems were selected for further testing: Tween 80–Captex 200P (3–1; w/w); Tween 80–Capmul MCM (5–1; w/w); Tween 80–Caprol 3GO (5–1; w/w) and Tween 80–soybean oil (7–1; w/w).

Table 6 shows the results of drug incorporation into the different systems. As the amount of itraconazole and griseofulvine that could be loaded into the systems was very low, no SMEDDS could be formed. Their solubility was medium or low in surfactants, both low in oils. Methylprednisolone, fenofibrate and danazole could form SMEDDS with only one or two systems. Like griseofulvine, methylprednisolone had medium solubility in surfactants and low in oils and both had log *P* values lower than 2. Both fenofibrate and danazol had high solubility in surfactants and their log *P* values were higher than 4. The other five drugs which could form SMEDDS with all 4 systems, all displayed high solubility in surfactants and their log *P* was about 2 except for indomethacine.

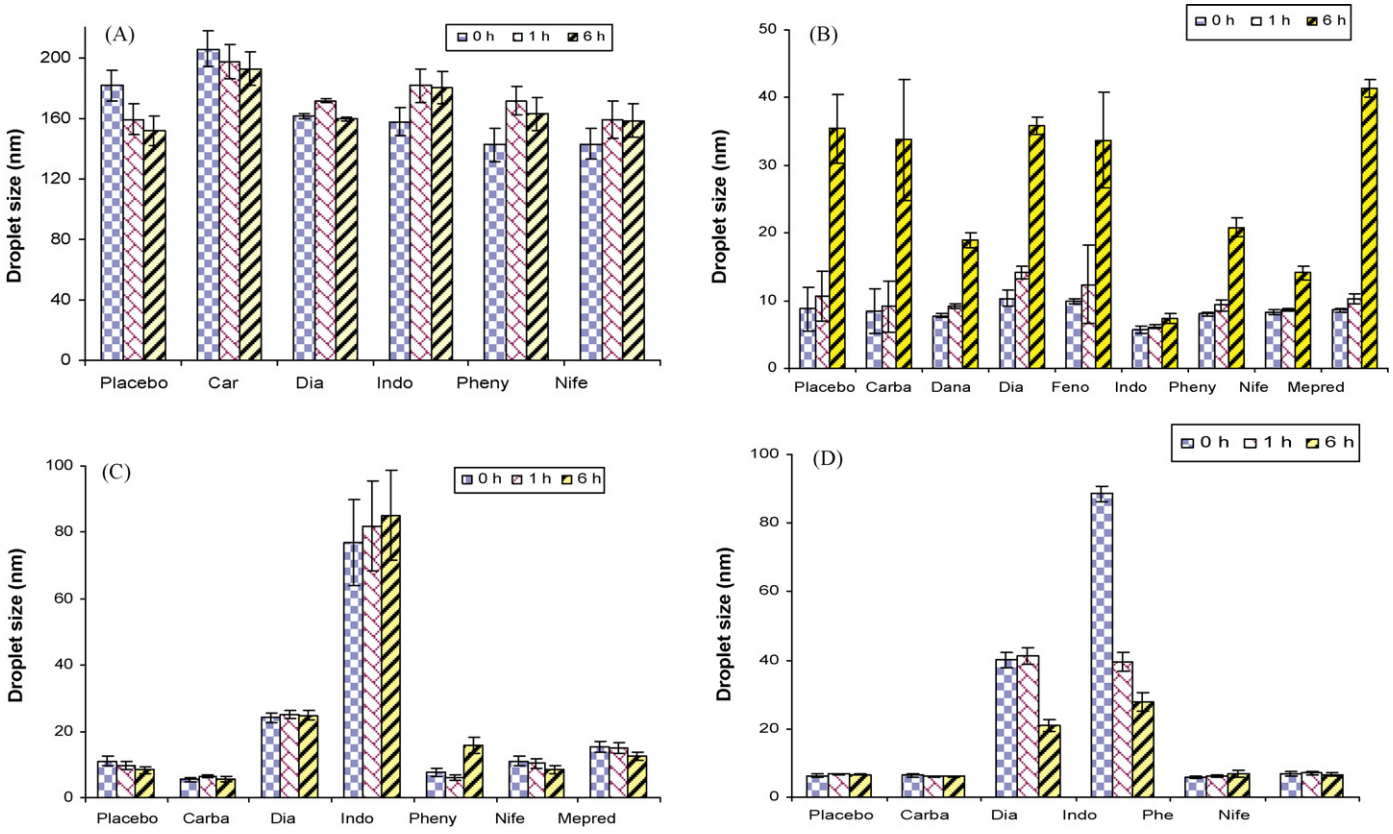

Fig. 2. The influence of drug loading on droplet size of system: (A) Tween 80–Caprol (5–1), (B) Tween 80–Captex (3–1), Tween 80–Capmul (5–1) and Tween 80–soybean oil (7–1). Placebo: system without drug; Carba: carbamazepine; Dana: danazol; Dia: diazepam; Feno: fenofibrate; Indo: indomethacine; Mepred: methylprednisolone; Nife: nifedipine; Pheny: phenytoine ($n = 5$). Error bars indicate standard deviation.

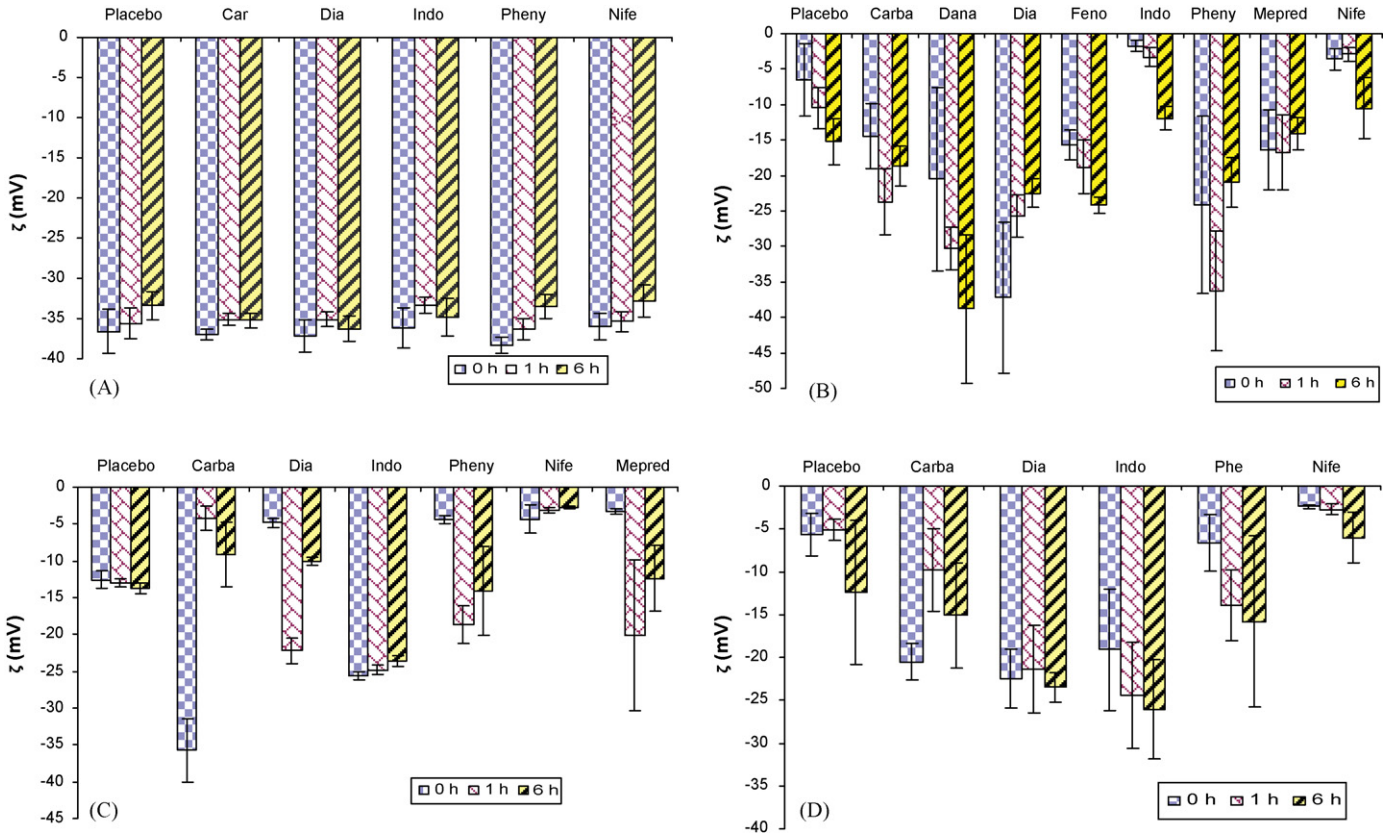

Fig. 3. The influence of drug loading on zeta potential values of system: (A) Tween 80–Caprol (5–1), (B) Tween 80–Captex (3–1), Tween 80–Capmul (5–1) and Tween 80–soybean oil (7–1). Placebo: system without drug; Carba: carbamazepine; Dana: danazol; Dia: diazepam; Feno: fenofibrate; Indo: indomethacine; Mepred: methylprednisolone; Nife: nifedipine; Pheny: phenytoine ($n = 10$). Error bars indicate standard deviation.

It is obvious that the drug compounds should first be able to dissolve in surfactants and oils to formulate a SMEDDS. However, the log *P* value of the drug compounds seemed also to play a role to form a SMEDDS. The drug compounds with log *P* higher than 4 (except for indomethacine) and lower than 2 did not successfully form SMEDDS, even in the case of fenofibrate, which had good solubility in both surfactants and oils. Only drug compounds with log *P* values between 2 and 4 could successfully be formulated as SMEDDS.

3.3.3. *Evaluation of SMEDDS*

In a next set of experiments, the influence of drugs on the droplet size and zeta potential of the systems in demineralized water was studied. Results are shown in Figs. 2 and 3. For the Tween 80–Caprol 3GO system, the mean droplet sizes and zeta potential values of SMEDDS were similar to those of the placebo system. The droplet size remained stable from 1 to 6 h.

For Tween 80–Captex 200P, the loaded drug systems had similar droplet size compared to those without drug. The droplet size remained stable until 1 h at about 10 nm. Afterwards, the size of the droplet increased till a maximum of about 40 nm. Unlike the droplet size, the zeta potential values of Tween 80–Captex 200P loaded drug system did not exhibit the same trend. They varied from about −2 to almost −40 mV.

For the Tween 80–Capmul MCM system, most of the drugs did not cause an increase in droplet size, except for diazepam and indomethacine where droplet size increased to about 25 and 80 nm, respectively. The droplet sizes of all systems stayed stable for 6 h. The zeta potential values of unloaded drug system and loaded with indomethacine and nifedipine remained almost the same for 6 h at about −13, −25 and −3 mV, respectively. The systems loaded with diazepam, methylprednisolone and phenytoine displayed a similar change in zetapotential values. They shifted from ca. −4 mV at time 0 to −20 mV at time 1 h and at time 6 h, they were ca. −13 mV, like that of the unloaded drug system.

An increase of the droplet size of the Tween 80–soybean oil systems was also observed when diazepam and indomethacine were loaded into the system.

Possible patterns of drug association with a micelle depend on the drug hydrophobicity. Completely water-soluble, hydrophilic drugs can only be adsorbed within the micelle corona compartment, while completely insoluble hydrophobic molecules can only be incorporated into the micelle core compartment. Drug molecules with intermediate hydrophobic/hydrophilic ratio will have intermediate positions within the micelle particle (Torchilin, 2001). Drug loading does not influence the micelle size significantly, indicating that the hydrophobic drug located in the hydrophobic core space of the micelles has almost no impact on the whole droplet size (Mu et al., 2005). Thus, the change of droplet size of the drug loaded systems is possibly due to the position of the drug in the micelles. The zeta potential value depends on different factors including the size and the surface properties of a particle. The change of zeta potential of drug loaded systems in this study did not seem to give a correlation with the change in the droplet size. A possible explanation is that some model drugs changed the properties of the surface of the micelles by their presence at the fixed aqueous layer of the micelles. However, as already mentioned above, structure elucidation of the micelles is beyond the scope of

this paper. The different position of a drug in different micellar systems leads to diverse effects on the zeta potential value which could explain why a link between the zeta potential of the different drugs and the zeta potential of the final SMEDDS could not be established in this study.

3.4. *In vitro* drug release

3.4.1. *Dialysis bag method in reverse mode (DR)*

The DR has been used as an alternative method for the normal dialysis bag method in drug release tests of SMEDDS formulations. In the normal dialysis bag method, the formulation is placed inside the bag and drug will release from the bag to the outside medium. Conversely, with the DR, the formulation is placed in the medium, outside the dialysis bag. Drug will move from the outside medium into the bag.

In this study, the DR was tested with three SMEDDS formulations: Tween 80–Captex (3–1) loaded with carbamazepine 2.5%, danazol 2% and indomethacine 5%. Samples were taken both inside and outside the dialysis bag. The pH of the SGF_{sp} was changed to 6.8 after 2 h. The results are shown in Fig. 4.

For the carbamazepine formulation, the concentration of compound was comparable both inside and outside the dialysis bag. In contrast, the concentration of danazol outside the dialysis bag reached ca. 90%, while inside the dialysis bag danazol concentration was almost equal to zero. Interestingly, the indomethacine formulation gave another different results. Outside the dialysis bag, the drug concentration was maintained above 80% during 4 h, while inside the bag, only traces of indomethacine were found during the first 2 h. When the pH of the medium was changed, the concentration of indomethacine inside the dialysis bag increased dramatically. The *in vitro* dissolution of pure indomethacine using DR was carried out also. In the first 2 h, both outside and inside the dialysis bag, only traces of indomethacin were found. Those concentrations of indomethacine also increased after changing the pH of the medium. The profile of drug release of pure indomethacine inside the dialysis bag was the same as that of the indomethacine formulation.

These results suggest that the measured concentration of compound inside the dialysis bag is just the solubility of that compound in the medium. The low solubility of indomethacine in acidic medium, which is even lower than 1 μg/ml, explained why only traces of indomethacine were found inside the dialysis bag in the case of formulation and both in- and outside the bag in the case of pure drug. When the pH of the medium was increased to 6.8, as the pKa of indomethacine is 4, indomethacine solubility is higher and an increase of indomethacine concentration was observed. Because of the solubilizing ability of SMEDDS, indomethacine formulation could maintain the high drug concentration despite the pH change in the medium. For danazol, the solubility is low and does not depend on the pH of the medium explaining the low concentration of danazol inside the dialysis bag. The solubility of carbamazepine in water and acidic medium is 225 and 242 μg/ml, respectively. The concentration of compounds that were used in this dialysis bag method in reverse was kept at 50 μg/ml. As the solubility of carbamazepine is higher than the concentration used in the experiment, the concentration of carbamazepine inside the dialysis bag was also high.

The results of this experiment indicate that using the dialysis bag method in reverse, one simply measures the solubility of compound in the release medium. In general, there is equilibrium between the micelles, formed by the SMEDDS formulation, and free surfactants, free oils and free drug in medium. Initially the inside of the dialysis bag only contains release medium, and the free molecules will move from the outside to the inside of the dialysis bag. Theoretically, the micelles will be then formed again inside the dialysis bag. The whole process will continue until equilibrium inside and outside the dialysis bag is reached. Thus, the concentration of drug inside and outside the dialysis bag should be comparable. Our results suggest that the formation of micelles inside the dialysis bag was delayed or disturbed. This is in contrast to some recently published studies, which claim that the dialysis method in reverse was better in simulating the *in vivo* circumstances for the drug. There were two possible reasons for that claim. Firstly, in those studies, the authors used higher MWCO dialysis membranes than those in our study (Jantratid et al., 2008; Shen and Zhong, 2006). With high MWCO membranes, it is possible that not only free drug, free surfactant, free oil but also the micelles of SMEDDS formulation can enter inside the dialysis bag. This was not the case for the low MWCO used in our study. The low MWCO membrane assured that only free drug, free surfactant and free oil molecules could enter inside the dialysis bag. Secondly, the concentration of compound in the dissolution test of those studies was lower than the solubility of that compound like in the case of carbamazepine in our study.

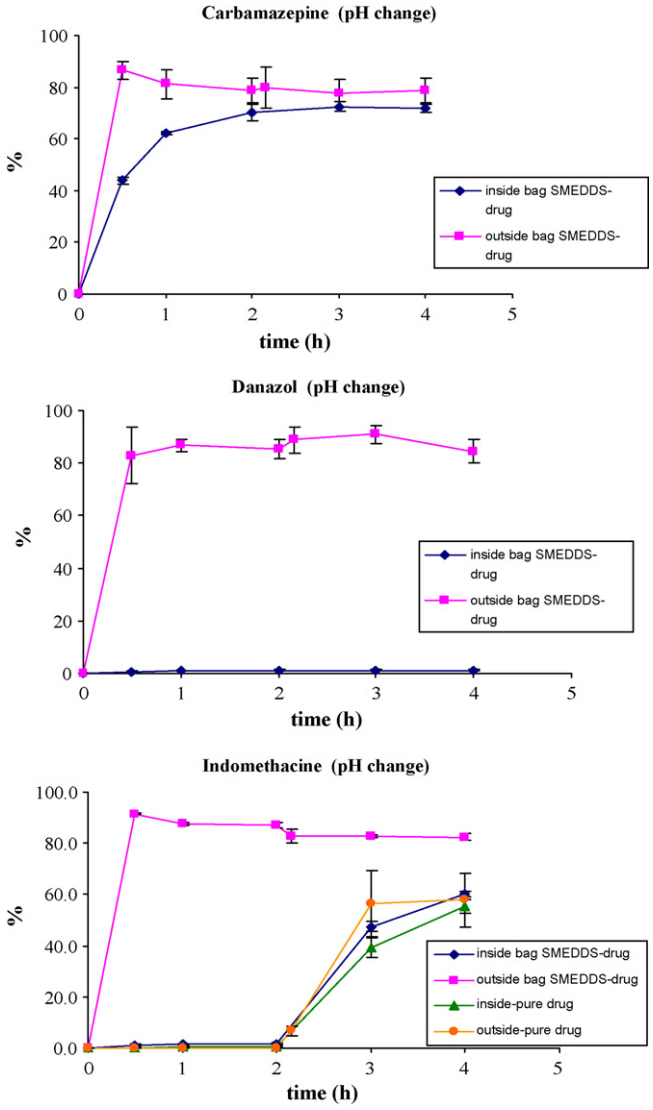

Fig. 4. *In vitro* dissolution using dialysis bag in reverse mode method ($n = 3$). Error bars indicate standard deviation.

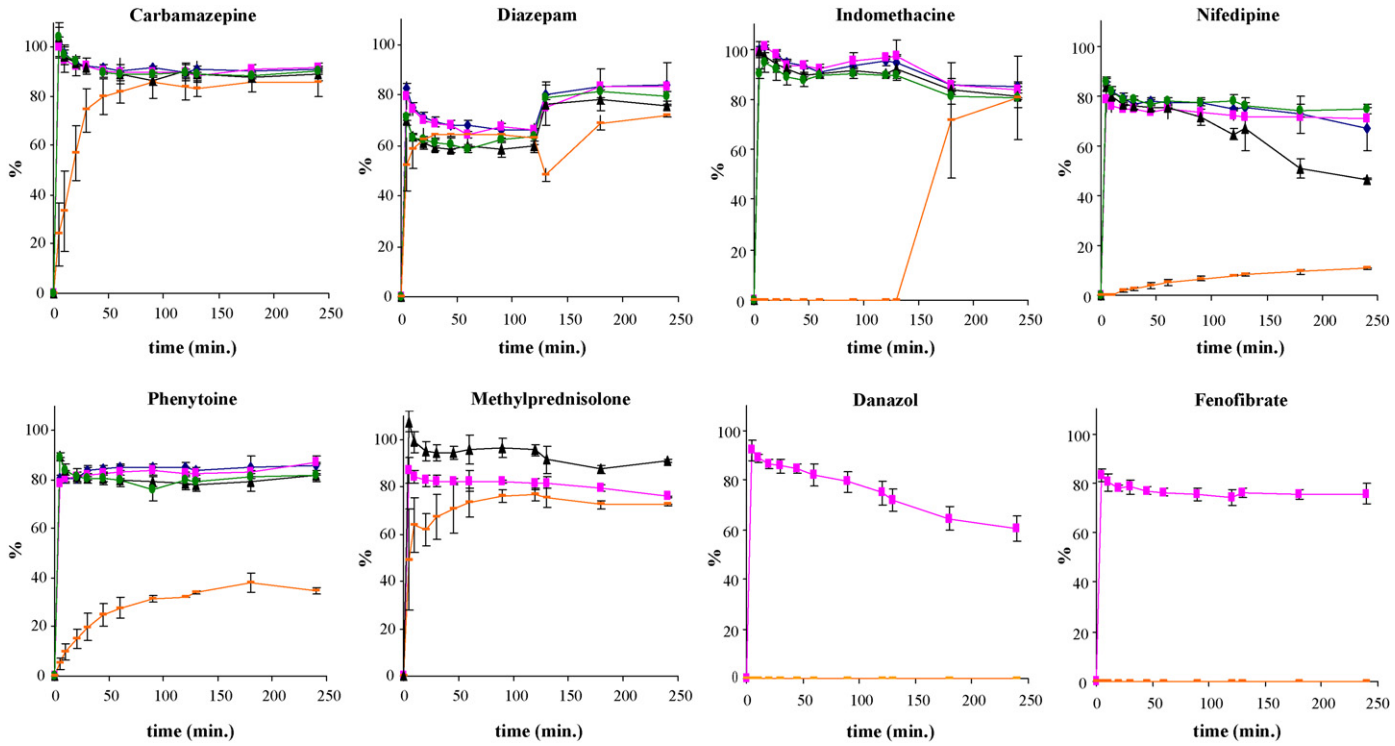

Fig. 5. Dissolution profile of compound from Tween 80–Caprol 3GO (5–1) (♦); Tween 80–Captex 200P (3–1) (■); Tween 80–Capmul MCM (5–1) (▲); Tween 80–soybean oil (7–1) (●) and pure compound (-) in SGF_{sp} for 2 h then pH was increased to 6.8 ($n = 3$). Error bars indicate standard deviation.

3.4.2. Conventional in vitro release with pH change

Although the conventional *in vitro* dissolution method has its limitations, it is still a simple method to carry out and it measures the concentration of drug, which is maintained in the dissolved state by the SMEDDS formulation. By changing the pH during the experiment, the dispersion, the solubilization ability and the drug remaining in a dissolved state were studied over time and different pHs.

SMEDDS formulations were investigated by using this conventional *in vitro* release method with pH change. The results are shown in Fig. 5. In general, all SMEDDS formulations could solubilize the drug and increased the drug concentration compared to that of free drug.

All carbamazepine, fenofibrate and phenytoine formulations maintained the compounds' dissolved state over time and different pHs. The carbamazepine concentration reached a maximum at ca. 100% after 5 min and it remained at about 90% in the experimental period for all four formulations while the pure drug only reached a maximum after 90 min. Fenofibrate has a very low solubility; during the *in vitro* release test, only traces of fenofibrate were detected. In the formulation form, fenofibrate concentration was maintained at 75%. The concentration of phenytoine in the SMEDDS formulations was held at about 80%, which was more than 2 times as much as the maximum of pure phenytoine (37%), which was reached after 3 h. All indomethacine formulations could solubilize at least 90% indomethacine during the first 2 h while only a trace of indomethacine was found in the case of pure indomethacine. However, indomethacine SMEDDS formulations displayed a slight decrease in concentration. For nifedipine formulations, Tween 80–Captex 200P and Tween 80–soybean oil formulations could maintain the dissolved state better than Tween 80–Caprol 3GO and Tween 80–Capmul MCM formulations. A decrease from 86% to 67% was observed in the Tween 80–Caprol 3GO formulation, while a higher decrease from 84% to 46% occurred in the formulation with Tween 80–Capmul MCM. Nonetheless, this low concentration (46%) was still 4 times higher than the maximum of pure nifedipine (11%). A marked decrease, from 92% at 5 min to only 60% at 4 h, was also observed for the danazol formulation. As no degradation products were observed during HPLC analysis, the concentration decrease in those formulations is probably due to the decrease of the SMEDDS solubilization ability; thus, the drug remaining in dissolved state is lower. The diazepam formulations showed a lower dissolution at low pH and a higher at higher pH. This can be explained by the high sensitivity to hydrolysis in acidic medium of the diazepinone seven-membered ring in the diazepam structure (Cabrera et al., 2005).

Although SMEDDS formulations increased the drug concentration in dissolved state, not all of them could remain the maximum concentration over time and pH change. Conventional *in vitro* release with pH change has proven to give valuable information about the ability of the SMEDDS formulation to keep the drug in a dissolved state.

4. Conclusion

In this study, only eight out of ten selected compounds could be formulated as SMEDDS. Formulate-ability in SMEDDS was found in general to be dependent on the solubility of the drugs in the excipients and the log *P* of the compounds (optimal 2–4).

The results from the dialysis bag method in reverse mode suggest that the measured concentration of the compound inside the dialysis bag corresponds to the solubility in the experimental medium. This does not agree with some previous studies of other authors, which concluded that the reversed dialysis bag method was better in simulating the *in vivo* circumstances for the drug. Our results suggest that the formation of micelles inside the dialysis bag was delayed or disturbed. Conventional *in vitro* release

methodology with pH change appears as a simple method which gives valuable information about the dispersion and the solubilization ability of the SMEDDS formulation at different pHs and over time.

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