Drug Development and Industrial Pharmacy, 35:199–208, 2009

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040802244318

informa
healthcare

A Systematic Dilution Study of Self-Microemulsifying Drug Delivery Systems in Artificial Intestinal Fluid Using Dynamic Laser Light Backscattering

Céline Ditner
Institute of Pharmaceutical Technology, University of Basel, Basel, Switzerland

Roberto Bravo
Actelion Pharmaceuticals Ltd., Preclinical Galenics, Allschwil, Switzerland

Georgios Imanidis and Martin Kuentz
Institute of Pharma Technology, University of Applied Sciences Northwestern Switzerland, Muttenz, Switzerland

Self-microemulsifying drug delivery systems provide a key technology to formulate poorly soluble drugs. The development of candidate formulations is commonly based on a screening of in vitro dilution characteristics. Because in vitro dilution is conducted in many different ways, the comparability of data is often limited and the involved factors are not properly understood. This article aims to systematically study the impact of formulation factors, temperature, dilution medium, and its amount in view of the phase behavior and particle size. Three types of self-microemulsifying delivery vehicles were formulated and diluted in artificial intestinal fluid and water. Different testing conditions were studied in the framework of statistical designs. The main response parameter was the colloidal particle size, which was measured using dynamic laser light backscattering. The tested formulations resulted in swollen micelles at a high dilution of 1:100 up to 1:1,000. At a lower dilution of 1:10, the particle size was increased depending on the system. Interactions of the dilution level with other parameters were found significant. Based on the obtained results, it seems reasonable to screen such systems first at a higher dilution in water at room temperature to facilitate experimentation. The selected systems may then, in a second step, be further studied at a different lower dilution in water or in artificial intestinal fluid at 37°C. We found that the Cremophor formulations were particularly robust with respect to producing constant small particles at different dilution levels. More research is needed to explore the biopharmaceutical relevance of these in vitro findings.

Keywords oral drug absorption; self-microemulsifying drug delivery systems; dilution test; artificial intestinal fluid; statistical design

Address correspondence to Martin Kuentz, Institute of Pharma Technology, University of Applied Sciences Northwestern Switzerland, Gründenstr. 40, CH-4132 Muttenz, Switzerland. E-mail: martin.kuentz@fhnw.ch

INTRODUCTION

A high percentage of new drugs exhibit low water solubility with respect to their therapeutic dose (Takagi et al., 2006), which often results in erratic oral drug absorption. Such compounds can have additional biopharmaceutical problems like high variability of exposure and lack of dose linearity. An important technology to overcome these absorption limitations is to dissolve the drug in lipid-based formulations (Hong, Kim, Song, Park, & Kim, 2006; Porter, Trevaskis, & Charman, 2007; Porter, Pouton, Cuine, & Charman, 2008). These systems can be categorized according to the lipid classification system (Pouton, 2000, 2006). Simple oils can be formulated as well as complex lipid mixtures. Especially the latter type bears a high potential to cope with biopharmaceutically challenging drugs. Self-emulsifying drug delivery systems (SEDDSs) can prevent a drug from precipitation upon dilution in the intestinal fluids (Gursoy & Benita, 2004). Depending on the formulation components, a microemulsion can result in the intestine so that the concentrated mixture is essentially a self-microemulsifying drug delivery system (SMEDDS) (Ciu, Zhao, Chen, & He, 2005; Cuiné, Charman, Pouton, Edwards, & Porter, 2007; Holm & Jensen, 2006; Iwanaga, Kushibiki, Miyazaki, & Kakemi, 2006; Shen & Zhong, 2006). Following oral administration, the drug should be kept in a solubilized form that can even be a supersaturated state (Gao et al., 2003). Such high drug concentration gradients are beneficial for the following permeation step (Kapitza, Michel, van Hoogevest, Leigh, & Imanidis, 2007). However, additional mechanisms are known, as to how SMEDDS may increase oral drug bioavailability. The incorporation of the active principle in micelles or small colloidal particles can reduce intestinal clearance and in some cases even hepatic first pass is avoided, when a substantial drug fraction is

transported via the intestinal lymph (Dahan & Hoffman, 2005; O'Driscoll, 2002).

The development of oral-lipid-based formulations for new drugs often starts with a solubility screening in different excipients. Phase diagrams should be conducted in parallel with study the phase behavior. Interesting candidate formulations can then be further screened by means of in vitro tests that are designed to anticipate the fate of the formulation in vivo. A simple test is the dilution of SEDDS and SMEDDS (Pouton, 1997) to detect issues of potential drug precipitation or spontaneous phase separation.

A dilution is mostly done in water or in artificial intestinal fluids (Galia et al., 1998; Vertzoni et al., 2004). Today, it is possible to further consider the influence of the formulation lipolysis to optimally mimic the in vivo situation (Brogård, Troedsson, Thuresson, & Ljusberg-Wahren, 2007; Fatouros & Müllertz, 2008; Kossena, Charman, Boyd, & Porter, 2005; Porter et al., 2004, 2007; Sek, Porter, Kaukonen, & Charman, 2002). Such a lipolysis test can reveal changes of the formulation like the digestion of an oil or surfactant (Cuiné et al., 2008). A recent study showed a rank-order correlation between the patterns of Danazol solubilization during in vitro digestion of SEDDS formulations and their performance in a dog study (Cuiné et al., 2007). It was observed that a decrease in lipid content and an increase in proportions of surfactant and co-solvent resulted in reduced Danazol bioavailability.

Certainly, any lipolysis test is more complex and time consuming than simple formulation dilutions. It is further unclear whether this test complexity is needed for all types of lipid systems with respect to their in vivo performance. One example is microemulsions, where the lipolysis can be less critical for drug absorption than using pure oils (Porter & Charman, 2001). It must further be noted that excipients can exert biological effects on drug absorption such as on the level of transporters, pre-systemic metabolism, or lipoprotein processing (Dahan & Hoffman, 2005; Kapitza et al., 2007; Kuentz, Wyttenbach, & Kuhlmann, 2007; Seeballuk, Lawless, Ashford, & O'Discoll, 2003, 2004). Accordingly, any dilution or lipolysis test has a priori limitations with respect to the in vivo prediction. However, they are still valuable for selecting formulations that are later tested in animals and ultimately in human. It seems that especially for the initial formulation screening, a simple dilution test is meaningful (Kuentz et al., 2007).

Dilution tests are performed in various ways in the industry. The different protocols differ for example regarding the dilution factor, type of medium, temperature, and testing time. A comparison of data is hardly possible and there is a lack of general knowledge about the testing parameters and formulation factors with respect to the dilution process. Such a basic physical knowledge is, however, needed to rationally design future in vitro tests of lipid drug delivery systems. This article aims to improve the understanding of the dilution process in artificial intestinal fluids by using dynamic laser light backscattering. Systematic experiments are conducted with three different pharmaceutical surfactant systems. First, the pure surfactants are diluted and then generic SMEDDS formulations are proposed to study different test parameters. Finally, selected systems are analyzed in the framework of response surface designs to quantitatively investigate the colloidal particle size.

MATERIALS AND METHODS

Materials

The middle chain triglyceride oil Miglyol® 812 as well as the medium chain partial glycerides Imwitor® 742 were excipients from the company Sasol (Witten, Germany). The surfactants Cremophor® RH40 (polyoxyl 40 hydrogenated castor oil) and Solutol® HS15 (macrogol 15 hydroxystearate) were purchased from BASF AG (Ludwigshafen, Germany), whereas the Tween® 80 (polysorbate 80 or polyoxyl 20 sorbitane monooleate) and the ethanol 96% were obtained in Ph.Eur. quality from the local vendor Hänseler AG (Herisau, Switzerland).

Each SMEDDS formulation had a total of 5 g and compounding was carried out in glass vials. The oil phase was first mixed using a Vortex® and consecutively the surfactant was added. Cremophor® RH40 and Solutol were initially melted. Finally, the co-solvent was admixed and the vials were properly closed. All formulations were monophasic systems, which were visually confirmed.

The dilution was instantaneously performed and particle size was measured after 10 min or according to the levels outlined by the statistical design. All tests at 37°C were carried out in a thermostatic water bath.

For the preparation of the artificial intestinal medium, Lipoid S100 (94% phosphatidyl choline) was used from Lipoid (Ludwigshafen, Germany) together with sodium taurocholate (≥97.0%) from Sigma-Aldrich (Buchs, Switzerland). The fasted standard simulated intestinal fluid (FaSSIF) had the composition as reported by Vertzoni et al. (2004) comprising 3 mM sodium taurocholate and 0.75 mM phosphatidyl choline. The manufacture was done by a direct solution method, where the taurocholate was first dissolved before the phospholipid was admixed.

Dynamic Laser Light Backscattering

The dynamic laser light backscattering is a technology where a time correlation function of the scattered intensity is obtained. The decrease of this function with time is correlated with the diffusion coefficient D of a particle in solution. The Stokes–Einstein equation relates this property with the hydrodynamic radius R of a particle:

$$R = \frac{kT}{6\pi\eta D}$$

where k is the Boltzmann constant, T is the absolute temperature, and η is the viscosity of the continuous phase. The dispersion technology software 5.0 (Malvern Instruments, Malvern,

UK) calculated for each measurement a *Z*-average value together with the polidispersity index (PDI).

The measurements were conducted with a Zeta Sizer Nano-ZS (Malvern Instruments) having a 4 mW He-Ne Laser with a wave length of 633 nm. The detection signal of the dynamic laser light backscattering was recorded at an angle of 173°. The measurements were conducted at room temperature (RT) or alternatively performed at 37°C using a Peltier element.

Statistical Design and Data Analysis

The program STATGRAPHICS Centurion XV Version 15.1.03 was used for all calculations. A categorical statistical design was constructed to study five independent effects including their interactions. The first factor was the type of system having three levels labeled according to the comprising surfactant: Cremophor® RH40 (Cremo), Solutol® HS15 (Solu), and Tween® 80 (Twe). A second factor was the presence or absence of ethanol in the formulation. Table 1 displays the composition of the three surfactant systems with and without the co-solvent. As a third factor, the degree of dilution was chosen with the levels 1:10, 1:100, and 1:1,000. This dilution was done in water, as well as in FaSSIF. A fifth factor was the temperature at which the dilutions and particle size measurements were carried out (RT or 37°C, respectively). The categorical design had 43 degrees of freedom for the estimation of the residual mean square error. The randomized experiments are displayed in Table 2.

The data were analyzed using a multifactor analysis of the variance (ANOVA). Consecutively, the significant factors based on the *F*-test were studied in a multiple range test. Fisher's least significant difference (LSD) procedure was followed. LSDs are confidence intervals for each pair of means at the selected confidence level using Student's *t* distribution. This procedure was introduced by Fisher, and the magnitude of the LSD limits indicates the smallest difference between any two means that can be declared to represent a statistically significant difference.

Following the initial factor study, the kinetics of the dilution process was more thoroughly studied. The surfactant systems were used in their version containing ethanol (Table 1) and the dilution medium was FaSSIF at 37°C. Response surface designs were constructed with the levels 1:10, 1:55, and 1:100 for the dilution factor and 10, 50, and 90 s for the time span at which the dilution was carried out. Twelve runs including three center points were tested for each of the response surface designs. The three ethanol-containing formulations (Table 1) were also relevant for the dilution study of surfactant alone. The dilution of the pure surfactant solutions (performed in triplicate) therefore had the same amount of surfactant (at 1:10 and 1:100) as with the same dilution in the response surface study.

RESULTS AND DISCUSSION

Dilution of the Pure Surfactants in Water and Artificial Intestinal Fluid

The dynamic light scattering values from the media FeSSIF and FaSSIF are published (Nielsen et al., 2007) with 5.8 ± 0.0 and 57.2 ± 2.2 nm, respectively. A size difference occurs based on the different media composition and pH values. Because these colloidal particles are present in artificial medium, any dilution of a formulation will result in a complex particle statistics arising from different particle populations.

This can be inferred from Table 3, in which the dilution of pure surfactant was investigated. Corresponding values for the different surfactants were higher in FaSSIF as opposed to using pure water as dilution medium. However, the difference was minor so that the concentration of the excipient micelles still dominated. Smallest size was generally observed with the polysorbate micelles and Cremophor RH exhibited in FaSSIF biggest micelles. Comparing the different dilution factors, there seemed to be a trend toward increasing size. This was particularly remarkable in water, where no particles in the medium could interfere. From a physical point of view, such changes can occur as a consequence of a change in aggregation number (Stokes & Evans, 1997). However, care is needed when interpreting these moderate differences. It was expected that such differences upon dilution would be quite different with SMEDDS formulations, where swollen micelles are formed.

TABLE 1
Composition of the Selected SMEDDS Formulations with Excipient Amounts in % (w/w)

Composition	CO (%)	CE (%)	PO (%)	PE (%)	SO (%)	SE (%)
Cremophor® RH40	40	34	—	—	—	—
Polysorbat 80	—	—	52	44.2	—	—
Solutol® HS15	—	—	—	—	30	34
Imwitor® 742	30	25.5	24	20.4	35	25.5
Miglyol® 812 (MCT)	30	25.5	24	20.4	35	25.5
Ethanol	—	15	—	15	—	15

TABLE 2
Mean Particle Size of the Statistical Categorical Design (Randomized)

System	Ethanol	Dilution	Medium	Temperature	Mean Particle Size (nm)
Cremophor	Ethanol	10	Water	RT	28.7
Tween	—	100	Water	37°C	24.4
Tween	Ethanol	10	FaSSIF	37°C	231.0
Solutol	Ethanol	100	FaSSIF	RT	34.7
Solutol	—	100	Water	RT	45.5
Solutol	—	10	FaSSIF	RT	381.0
Tween	Ethanol	10	Water	RT	35.8
Cremophor	—	1,000	Water	RT	28.4
Solutol	—	1,000	FaSSIF	RT	46.1
Cremophor	Ethanol	1,000	Water	37°C	30.2
Solutol	Ethanol	1,000	Water	RT	30.7
Solutol	Ethanol	100	Water	RT	36.2
Cremophor	Ethanol	100	Water	37°C	30.4
Tween	Ethanol	100	Water	37°C	25.7
Cremophor	—	1,000	FaSSIF	37°C	43.0
Tween	—	100	Water	RT	21.6
Tween	—	100	FaSSIF	37°C	65.4
Tween	—	1,000	FaSSIF	37°C	41.6
Cremophor	Ethanol	100	FaSSIF	37°C	33.3
Cremophor	Ethanol	100	FaSSIF	RT	37.6
Solutol	—	100	FaSSIF	37°C	39.1
Cremophor	—	100	FaSSIF	37°C	33.1
Tween	Ethanol	1,000	FaSSIF	37°C	45.4
Solutol	Ethanol	10	FaSSIF	RT	35.9
Cremophor	—	10	FaSSIF	37°C	29.6
Solutol	—	1,000	Water	RT	43.0
Cremophor	Ethanol	1,000	Water	RT	28.6
Solutol	—	100	FaSSIF	RT	123.0
Cremophor	Ethanol	10	FaSSIF	RT	33.4
Solutol	—	100	Water	37°C	158.0
Cremophor	—	100	FaSSIF	RT	34.1
Cremophor	—	100	Water	37°C	30.8
Solutol	Ethanol	1,000	FaSSIF	37°C	40.7
Cremophor	—	1,000	Water	37°C	31.1
Tween	—	10	FaSSIF	RT	60.2
Cremophor	Ethanol	100	Water	RT	28.4
Solutol	Ethanol	1,000	Water	37°C	38.6
Tween	Ethanol	1,000	Water	37°C	34.5
Cremophor	—	1,000	FaSSIF	RT	41.8
Tween	Ethanol	1,000	FaSSIF	RT	44.1
Tween	—	10	FaSSIF	37°C	204.0
Solutol	Ethanol	10	Water	RT	36.5
Cremophor	Ethanol	1,000	FaSSIF	RT	43.3
Tween	—	1,000	FaSSIF	RT	44.2
Tween	Ethanol	100	FaSSIF	RT	36.4
Solutol	Ethanol	10	FaSSIF	37°C	176.0

(*Continued*)

TABLE 2
(*Continued*)

System	Ethanol	Dilution	Medium	Temperature	Mean Particle Size (nm)
Cremophor	—	10	FaSSIF	RT	31.5
Tween	—	10	Water	37°C	107.0
Tween	Ethanol	10	FaSSIF	RT	59.7
Tween	—	100	FaSSIF	RT	34.8
Cremophor	Ethanol	1,000	FaSSIF	37°C	40.8
Solutol	Ethanol	10	Water	37°C	366.0
Cremophor	—	100	Water	RT	28.2
Solutol	Ethanol	100	FaSSIF	37°C	48.2
Tween	—	10	Water	RT	26.2
Cremophor	—	10	Water	RT	27.6
Tween	Ethanol	10	Water	37°C	98.4
Solutol	Ethanol	100	Water	37°C	38.0
Solutol	—	1,000	FaSSIF	37°C	22.9
Cremophor	—	10	Water	37°C	32.6
Cremophor	Ethanol	10	Water	37°C	42.9
Tween	Ethanol	100	FaSSIF	37°C	52.6
Tween	Ethanol	100	Water	RT	20.7
Solutol	Ethanol	1,000	FaSSIF	RT	41.5
Cremophor	Ethanol	10	FaSSIF	37°C	46.7
Solutol	—	10	Water	RT	499.0
Tween	Ethanol	1,000	Water	RT	22.6
Tween	—	1,000	Water	37°C	20.7
Tween	—	1,000	Water	RT	26.9
Solutol	—	1,000	Water	37°C	56.0

TABLE 3
Particle Size of Diluted Surfactant Solutions in Nanometer
(Measured in Triplicate)

Dilution	Cremophor RH (nm)	Solutol HS15 (nm)	Tween 80 (nm)
Water 1:10	15.17 ± 0.21	12.63 ± 0.06	9.77 ± 1.24
Water 1:100	17.53 ± 1.91	19.03 ± 3.39	10.70 ± 0.28
[a]FaSSIF 1:10	18.40 ± 1.61	18.27 ± 3.00	12.40 ± 0.46
[a]FaSSIF 1:100	42.17 ± 9.95	35.93 ± 4.94	30.03 ± 6.47

[a]Pure FaSSIF has an average particle size of 57.2 ± 2.2 nm (Nielson et al., 2007).

Generic Self-Microemulsifying Formulations and the Evaluation of Different Testing Parameters

One objective of this study was to evaluate generic formulations of SMEDDS using the three different surfactants Cremophor RH, Solutol HS15, and Tween 80. It could not be expected that quantitatively equal formulations can be found, where only the type of surfactant is different. Such well-comparable formulations can normally not result due to the individual nature of the phase behavior. However, a high similarity among the formulations was targeted. Starting points were mixtures of middle chain triglycerides with mono- and diglycerides of this oil (Constantinides & Scalart, 1997). In a next step, phase diagrams were constructed to verify the miscibility of the components (Ditner, 2007). Each phase diagram was constructed in a version with and without the addition of the co-solvent ethanol. Table 1 exhibits the selected formulations. All formulations had a 1:1 ratio of Miglyol to Imwitor, and for every surfactant mixture, there exists a formulation with and without 15% ethanol. The total amount of surfactant and oil was different for all formulations as a direct consequence of the individual phase behavior. Thus, true generic formulations having the same composition with respect to surfactant, oil, and co-solvent can hardly be obtained. The SMEDDS can therefore only be similar at best, which should be kept in mind for the interpretation of the results.

The ANOVA from the micelle size revealed significant effects for the type of system ($p \leq .001$), the excipient ethanol ($p = .016$), and the dilution ($p \leq .001$). Interestingly, the type of dilution medium had no significant effect ($p = .793$) and also the effect of the temperature had a borderline p value of .052.

Some effects were depending on the levels of others, that is, factor interactions were revealed.

The particle size effect of the system was found to depend on whether or not ethanol was in the formulation. Figure 1 shows the interaction plot of the factors system and ethanol, which essentially averages over all experiments of the design. The interaction was found to be significant ($p = .003$). In case of the Solutol mixtures, addition of ethanol decreased substantially the droplet size. However, there was no effect of ethanol observed when this comparison was with formulations of Cremophor or Tween. Because the latter formulations already showed very small droplet sizes, it can be argued that the ethanol had here a lower potential of size reduction. It was reported earlier that the effect of ethanol on droplet size is highly specific for the system (Khoo, Humberstone, Porter, Edwards, & Charman, 1998). It appears that ethanol is mostly used in SMEDDS solubilizing the drug, but the cosolvent does not necessarily lead to smaller particles upon dilution.

Another significant interaction was the effect of the dilution factor depending on the formulation ($p \leq .001$). Figure 2 shows that when the Solutol formulation is used, the particle size decreases while increasing the dilution. The main step was observed from a 1:10 dilution compared with a 1:100 ratio. At a dilution of 1:100 and 1:1,000, the confidence bands were overlapping so that there is only a trend toward smallest colloidal particles at the lowest concentration of the surfactant. Inspecting the Tween formulations revealed no difference at the lower dilution, but it was again the 1:10 dilution that produced comparatively largest particles in line with the observation from the Solutol formulations. The third system comprising Cremophor exhibited no differences in droplet size at all. The micelles were obviously formed in a way being independent of the formulation concentration. According to these findings, it seems questionable to compare different formulations at a single dilution step, because the concentration behavior was found to be formulation specific. This conclusion was also supported by an additionally observed factor interaction: the effect of ethanol in the formulation depending on the dilution factor ($p = .046$).

From Figure 3, it can be inferred that systems without ethanol show an overall tendency toward smaller colloidal particles at higher dilution. The main difference was again found between 1:10 and 1:100 dilutions. Similarly, also the formulations without ethanol had the main difference in particle size coming from the lowest dilution. However, at the level of 1:100 and 1:1,000 no distinctions could be made with respect to the particle size.

The state of dilution seemed to have an impact on the effect of the temperature with respect to particle size (Figure 4). The interaction was borderline with a p value of .077. The overall effect of the temperature on particle size also resulted in a low p value (.052) and almost separated confidence bands. There was a trend that at 37°C the colloidal particles were larger than at RT. Figure 4 reveals that at a dilution of 1:10, this difference leads to clearly separated confidence bands, whereas this effect was hardly seen comparing the 1:100 and 1:1,000 dilution. Based on these results it can be argued that tests of highly diluted systems might be conducted at RT instead of 37°C, which would facilitate the

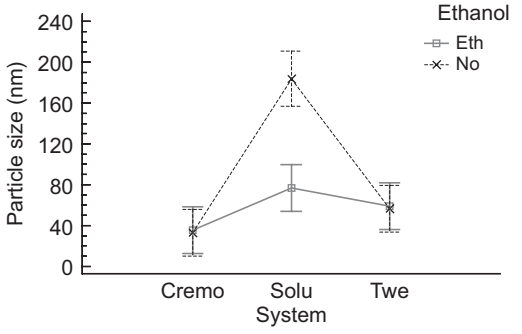

FIGURE 1. Interaction plot of the colloidal particle size for the different generic self-microemulsifying drug delivery system (SMEDDS) depending on ethanol in the formulation.

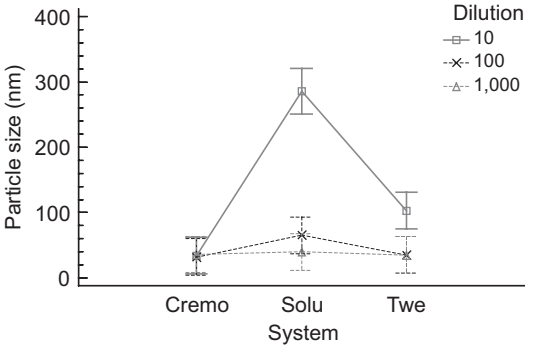

FIGURE 2. Interaction plot of the colloidal particle size for the different generic SMEDDS depending on the dilution factor.

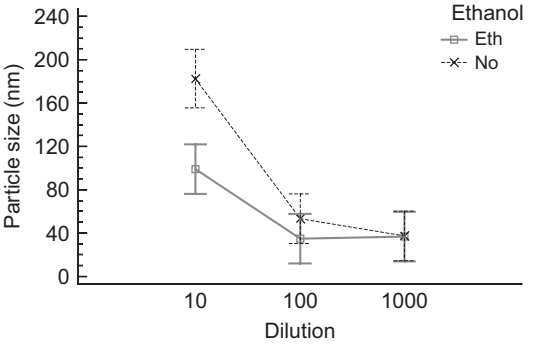

FIGURE 3. Interaction plot of the colloidal particle size for the different dilution levels depending on ethanol in the formulation.

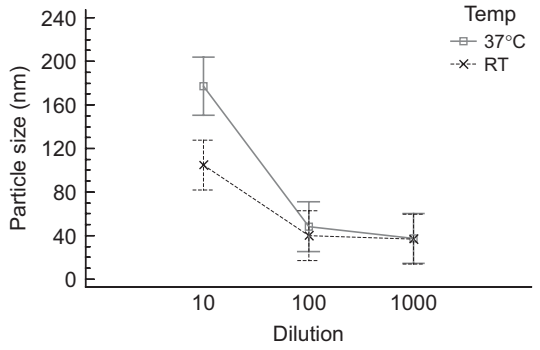

FIGURE 4. Interaction plot of the colloidal particle size for the different dilution levels depending on the temperature.

experimentation. However, care is needed with generalized conclusions extending beyond the systems investigated. Such cautiousness is also needed interpreting the lacking effect of the type of intestinal fluid. We did not find an overall significant difference for dilutions in water and FaSSIF with respect to the droplet size ($p = .793$). Because the interaction of the systems with the type of intestinal fluid had a rather low p value of .103, individual trends for a given formulation cannot be excluded.

Apart from the mean droplet size, the PDI was also investigated to assess the size distribution. Mean PDI values of the different systems ranged from 0.08 to 0.25. The narrowest particle size distributions were observed for the Cremophor systems followed by the Tween formulations. The highest PDI values were obtained using mixtures of Solutol. The PDI value was not only highly system specific ($p \leq .001$), but again depended on the level of dilution. At 1:10, the droplet distribution was significantly higher than at 1:100 or 1:1,000 ($p \leq .001$). Significant interactions were found between the system and the dilution factor ($p \leq .001$), as well as for the system and the temperature ($p = .0021$). It was again the individual phase behavior of the mixtures that affected the polidispersity of the evolving colloidal particles.

To complete the discussion of this factor influence study, an important aspect should be revisited. A dilution using water or FaSSIF mainly differs by the fact that the biorelevant medium contains particles itself. The measured size is therefore always the results of a mixed particle population. It is interesting to note at which dilution level the formulation is no longer dominating the measured particle size. The FaSSIF medium has a concentration of 0.22% (wt/wt) of combined taurocholate and phospholipid. At a dilution of 1:10 or 1:100 the particulate components of the diluted formulation is expected to dominate, whereas at 1:1,000 the particles of FaSSIF greatly influence the mean particle size. This consideration is in line with the observation that formulation differences leveled of at 1:1,000.

Response Surface Analysis of the Factors Dilution and Time with Regard to the Colloidal Particle Size

The screening of the testing factors revealed the importance of the dilution factor depending on the given formulation. A multilevel design was performed to study this effect more thoroughly and to investigate the impact of time. Figure 5 shows the response surface ($R^2 = .971$) for testing the Cremophor formulation at 37°C in FaSSIF. Interestingly, the response surface was comparatively flat. Even though the factors dilution and time were both found significant on particle size ($p \leq .001$ and $p = .001$, respectively), their influence was comparatively limited. The particle size influence of the dilution was mainly observed after longer times, e.g., 1.5 h. Mean particle size decreased here from about 80 nm to nearly 30 nm. This was quite different compared with a measurement after 10 min, where the mean micelle size was practically constant in the range of 30–40 nm. Because the entire surface showed only moderate changes, this SMEDDS was considered to be a robust formulation with regard to its kinetics of dilution. However, we should keep in mind that the dilution testing started from a level of 1:10.

It was assumed that the initial dilution occurs on a rather fast time scale in vivo, which does not exclude problems by encountering an area of separation or liquid–crystalline structures in this part of the phase diagram. The biopharmaceutical relevance of robust dilution kinetics between 1:10 and, for example, 1:1,000 still needs to be clarified.

Figure 6 shows the dilution of the ethanol-containing Solutol formulation at 37°C in FaSSIF ($R^2 = .987$). The size effect of the dilution was highly significant ($p \leq .001$). The smallest micelles were again found at the highest dilution, where a limiting size of about 20–30 nm was reached. At a lower dilution, the swollen micelles increased in size to become colloidal emulsion droplets. In this range, where the colloidal particles were rather small droplets, we found that also the PDI values were highest. It appears that in this size range, the droplets are prone to a size increase with time. Given the high polidispersity and particle size, it is possible that

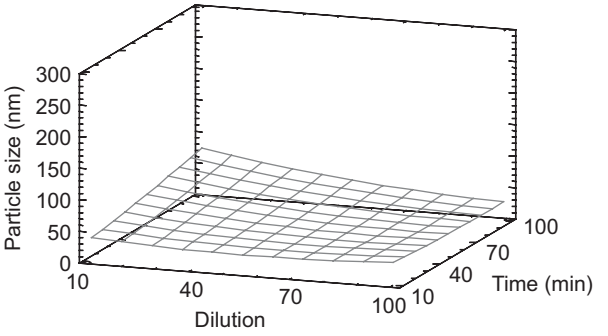

FIGURE 5. Response surface area of the colloidal particle size using the SMEDDS with Cremophor RH and ethanol (CE) diluted in FaSSIF at 37°C.

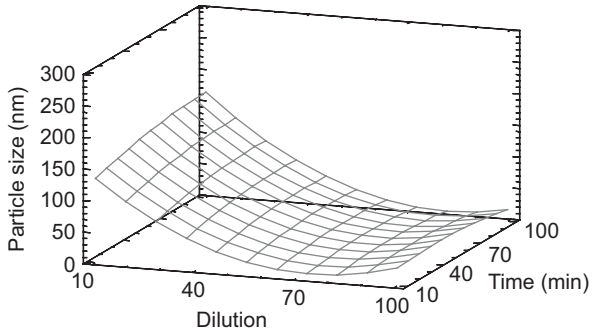

FIGURE 6. Response surface area of the colloidal particle size using the SMEDDS with Solutol HS and ethanol (SE) diluted in FaSSIF at 37°C.

coalescence occurred leading to a increased droplet size. There exists a size range of colloidal droplets, where time effects become apparent.

This time effect was also observed diluting the ethanol-containing Tween system in FaSSIF as shown by Figure 7 (R^2 = .944). In less diluted systems, the colloidal particle size almost doubled comparing a fast dilution with a rather slow dilution at 100 min. However, this was again different for the highly diluted systems, where the response surface was comparatively flat, indicating the absence of strong aging effects. The ANOVA revealed for the entire response surface that the size effects of dilution and time were both found to be significant ($p \leq .001$ and $p = .020$).

It is interesting that some of the obtained results can be theoretically predicted. The high polidispersity of particles at a low dilution and the decrease to a nearly constant micelle size upon further dilution were explained by a model of Borkovec (1989). The model was based on interfacial free energy. The extent of micelle swelling depended on the bending energy of the saturated monolayer. An increase of the splay modulus or decreasing of the spontaneous curvature favored swollen micelles of microemulsions. Thus, system-specific factors define phase behavior in general and the formation of a microemulsion in particular.

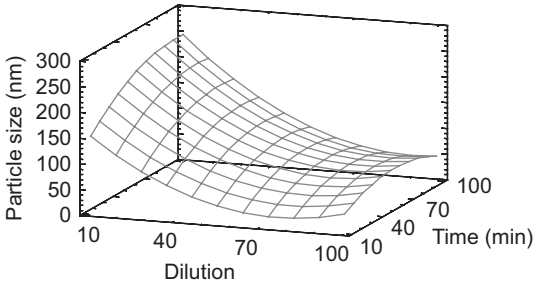

FIGURE 7. Response surface area of the colloidal particle size using the SMEDDS with Tween 80 and ethanol (PE) diluted in FaSSIF at 37°C.

CONCLUSION

When a liquid-filled capsule or soft capsule is swallowed, the fill mass is highly diluted in vivo. All dilution experiments at 1:100 to 1:1,000 resulted in particles being in a nano size range. Accordingly, the three types of systems were essentially self-microemulsifying. This classification according to particle size in the diluted state has a biological significance. Small colloidal particles can bring a drug to absorption quite independent of lipolysis (Porter & Charman, 2001). A drug can be transported in micelles or swollen micelles that can transport a drug across the unstirred water layer (Porter et al., 2007). However, it must be noted that smaller differences of particles in the colloidal domain may not necessarily influence the bioavailability of a drug (Constantinides et al., 1995).

We observed the most pronounced differences in colloidal particle size at the low dilution level of 1:10. Based on the significance of the dilution factor, it seems reasonable that any in vitro assessment of lipid formulation should not be done at a singe dilution level. A high dilution mimics best the final in vivo dilution, whereas we found at a lower dilution of 1:10 the most discriminating results. The latter condition has the additional benefit that a potential drug precipitation can be optimally observed. On this dilution level, only the tested Cremophor RH formulations provided colloidal particle of clearly below 100 nm, whereas the other generic formulations showed larger colloidal particles. This bigger particle size was accompanied by some time dependence. Unfortunately, there is little known about the time scale on which in vivo dilution takes place. It is therefore difficult to interpret the relevance of this finding. However, the kinetics of the dilution process can be crucial for the in vivo performance of the delivery system. Given the complexity of the in vivo processes, particle size can play a role on many levels. The particle size may in vivo affect physical processes like aggregation, coalescence, potential drug precipitation, and also drug trafficking or even food effects. Thus, the observed differences in the course of dilution are likely to be of biological relevance when formulating a drug. A robustness of the physical dilution process, as observed with the Cremophor mixtures, might be advantageous for the in vivo performance of a SMEDDS. Future studies could treat the variability of the obtained particle size as a factor on its own.

The current study revealed some factor interactions that are relevant to design pragmatic in vitro tests. The effects of temperature and the type of artificial intestinal fluid did not greatly influence the micelle size at a high dilution. Accordingly, a broad series of formulation candidates may be screened in two steps. A dilution of, for example, 1:100 in water at RT can be easily done with a great number of candidate formulations. Selected SMEDDS may then be further studied at a lower dilution in FaSSIF at 37°C.

These present dilution studies may also influence the manner in which SMEDDS are tested in rodents. Many companies

ad hoc dilute a SMEDDS before administration per gavage. Based on our findings, it can be expected that this instantaneous high dilution has limited relevance for in vivo dilution. Any discriminating effects of the dilution process itself may be leveled off. An undiluted SMEDDS could be alternatively given by gavage. The concentration of the excipients may not well mirror the situation in human, but the results are expected to be more discriminating.

This study addressed different formulation and testing factors in view of a dilution of self-emulsifying vehicles. Factor interactions were observed that on the one hand show the complexity of the dilution process but on the other hand also provide a rational for a pragmatic screening of formulations.

ACKNOWLEDGMENTS

The authors thank the University of Applied Sciences Northwestern Switzerland for funding the research project.

REFERENCES

Borkovec, M. (1989). From micelles to microemulsion droplets: Size distribution, shape fluctuations and interfacial tensions. *J. Phys. Chem., 91*(10), 6268–6291.

Brogård, M., Troedsson, E., Thuresson, K., & Ljusberg-Wahren, H. (2007). A new standardized lipolysis approach for the characterization of emulsions and dispersions. *J. Colloid. Interface Sci., 308*, 500–507.

Ciu, S., Zhao, C. H., Chen, D., & He, Z. (2005). Self-microemulsifying drug delivery systems (SMEDDS) for improving in vitro dissolution and oral absorption of pueraria lobata isoflavoine. *Drug Dev. Ind. Pharm., 31*, 349–356.

Constantinides, P. P., Lancaster, C. M., Marcello, J., Chiossone, D. C., Orner, D., Hidalgo, I., Smith, P. L., Sarkahian, A. B., Yiv, S. H., & Owen, A. J. (1995). Enhanced intestinal absorption of a RGD peptide from water-in-oil microemulsions of different composition and particle size. *J. Control. Release, 34*, 109–116.

Constantinides, P., & Scalart, J. P. (1997). Formulation and physical characterisation of water-in-oil microemulsions containing long-versus medium-chain glycerides. *Int. J. Pharm., 158*, 57–68.

Cuiné, J. F., Charman, W. N., Pouton, C. W., Edwards, G. A., & Porter, C. H. (2007). Increasing the proportional content of surfactants (cremophor EL) relative to lipid in self-emulsifying lipid-based formulations of danazol reduces oral bioavailability in beagle dogs. *Pharm. Res., 24*(4), 748–757.

Cuiné, J. F., Mcevoy, C. L., Charman, W. N., Pouton, C. W., Edwards, G. A., Benameur, H., & Porter, C. H. (2008). Evaluation of the impact of surfactant digestion on the bioavailability of danazol after oral administration of lipidic self-emulsifying formulations to dogs. *J. Pharm. Sci., 97*(2), 993–1010.

Dahan, A., & Hoffman, A. (2005). Evaluation of chylomicron flow blocking approach to investigate the intestinal lymphatic transport of lipophilic drugs. *Eur. J. Pharm. Sci., 24*, 381–388.

Ditner, C. (2007). Untersuchung des Verdünnungsverhaltens selbstemulgierender Systeme. Master Thesis, University of Basel, Institute of Pharmaceutical Technology.

Fatouros, D. G., & Müllertz, A. (2008). *In vitro lipid digestion models in design of drug delivery systems for enhancing oral bioavailability*. *Expert Opin. Drug Metab. Toxicol., 4*(1), 65–76.

Galia, E., Nicolaides, E., Hörter, D., Löbenberg, R., Reppas, C., & Dressman, J. B. (1998). Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs. *Pharm. Res., 15*(5), 698–705.

Gao, P., Rush, B., Pfund, W., Huang, T., Bauer, J., Morozowich, W., Ming-Shang, K., & Hageman, M. (2003). Development of a supersaturable SEDDS (S-SEDDS) formulation of paclitaxel with improved Oral Bioavailability. *J. Pharm. Sci., 92*(12), 2386–2398.

Gursoy, R., & Benita, S. (2004). Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. *Biomed. Pharmacother., 58*, 173–182.

Holm, R., & Jensen, I. H. M. (2006). Optimization of self-microemulsifying drug delivery systems (SMEDDS) using a D-optimal design and the desirability function. *Drug Dev. Ind. Pharm., 32*, 1025–1032.

Hong, J. Y., & Kim, J. K., Song, Y. K., Park, J. S., & Kim, C. K. (2006). A new self-emulsifying formulation of itraconazole with improved dissolution and oral absorption. *J. Control. Release, 110*, 332–338.

Iwanaga, K., Kushibiki, T., Miyazaki, M., & Kakemi, M. (2006). Disposition of lipid-based formulation in the intestinal tract affects the absorption of poorly water-soluble *Drugs Biol. Pharm. Bull., 29*(3), 508–512.

Kapitza, S. B., Michel, B., van Hoogevest, P., Leigh, M., & Imanidis, G. (2007). Absorption of poorly water soluble drugs subject to apical efflux using phospholipids as solubilizers in the caco-2 cell model. *Eur. J. Pharm. Biopharm., 66*, 146–158.

Khoo, S. M., Humberstone, A. J., Porter, C. H., Edwards, G., & Charman, W. (1998). Formulation design and bioavailability assessment of lipidic self-emulsifying formulations of halofantrine. *Int. J. Pharm.*, 155–164.

Kossena, G. A., Charman, W., Boyd, B. J., & Porter, C. H. (2005). Influence of the intermediate digestion phases of common formulation lipids on the absorption of a poorly water-soluble drug. *J. Pharm. Sci., 94*(3), 481–492.

Kuentz, M., Wyttenbach, N., & Kuhlmann, O. (2007). Application of a statistical method to the absorption of a new model drug from micellar and lipid formulations- evaluation of qualitative excipient effects. *Pharm. Dev. Technol., 12*, 275–283.

Nielsen, F., Gibault, E., Ljusberg-Wahren, H., Arleth, L., Pedersen, J. S., & Müllertz, A. (2007). Characterization of prototype self-nanoemulsifying formulations of lipophilic compounds. *J. Pharm. Sci., 96*(4), 876–892.

O'Driscoll, C. (2002). Lipid-based formulations for the intestinal lymphatic delivery. *Eur. J. Pharm. Sci., 15*, 405–415.

Porter, C., & Charman, W. (2001). In vitro assessment of oral lipid based formulations. *Adv. Drug Deliv. Rev., 50*, S127–S147.

Porter, C. H., Kaukonen, A. M., Taillardat-Bertschinger, A., Boyd, B. J., O'Connor, J. M., Edwards, G. A., & Charman, W. (2004). Use of in vitro lipid digestion data to explain the in vivo performance of triglyceride-based oral lipid formulations of poorly water-soluble drugs: Study with halofantrine. *J. Pharm. Sci., 93*(5), 1110–1121.

Porter, C. H., Pouton, C., Cuine, J., & Chaman, W. (2008). Enhancing intestinal drug solubilisation using lipid-based delivery systems. *Adv. Drug Deliv. Rev., 60*, 673–691.

Porter, C. H., Trevaskis, N., & Charman, W. (2007). Lipid and lipid-based formulations: Optimizing the oral delivery of lipophilic drugs. *Nat. Rev. Drug Discov., 6*(3), 231–248.

Pouton, C. (1997). Formulation of self-emulsifying drug delivery systems. *Adv. Drug Deliv. Rev., 25*, 47–58.

Pouton, C. W. (2000). Lipid formulations for oral administration of drugs: Non-emulsifying, self-emulsifying, and self-microemulsifying drug delivery systems. *Eur. J. Pharm. Sci., 11*(2), S93–S98.

Pouton, C. W. (2006). Formulation of poorly water-soluble drugs for oral administration: Physicochemical and physiological issues and the lipid formulation classification system. *Europ. J. Pharm. Sci., 29*, 278–287.

Seeballuk, F., Lawless, E., Ashford, M., & O'Discoll, C. (2003). The effect of pluronic® block copolymers and cremophor® EL on intestinal lipoprotein processing and the potential link with P-glycoprotein in caco-2 cells. *Pharm. Res., 20*(7), 1085–1092.

Seeballuk, F., Lawless, E., Ashford, M., & O'Discoll, C. (2004). Stimulation of triglyceride-rich lipoprotein secretion by polysorbate 80: In vitro and in vivo correlation using caco-2 cells and a cannulated rat intestinal lymphatic model. *Pharm. Res., 21*(12), 2320–2326.

Sek, L., Porter, C., Kaukonen, A. M., &, Charman, W. (2002). Evaluation of the in-vitro digestion profiles for long and medium chain triglycerides and

the phase behaviour of the lipolytic products. *J. Pharm. Pharmacol., 54*, 29–41.

Shen, H. R., & Zhong, M. K. (2006). Preparation and evaluation of self-emulsifying drug delivery systems (SMEDDS) containing atorvastatin. *J. Pharm. Pharmacol., 58*, 1183–1191.

Stokes, R. J., & Evans, D. F. (1997). *Can we quantitatively model micelle formation and stability in fundamentals of interfacial engineering* (pp. 219–222). Chichester: Wiley New York.

Takagi, T., Ramachandran, C. H., Bermejo, M., Yamashita, S., Yu, L., & Amidon, G. (2006). A provisional biopharmaceutical classification of top 200 oral drug products in the United States, Great Britain, Spain and Japan. *Mol. Pharm., 3*(6), 631–643.

Vertzoni, M., Fotaki, N., Kostewicz, E., Stippler, E., Leuner, C. H., Nicolaides, E., Dressman, J., & Reppas, C. H. (2004). Dissolution media simulating the intralumenal composition of the small intestine: Physiological issues and practical aspects. *J. Pharm. Pharmacol., 56*, 453–462.