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ORIGINAL ARTICLE

Critical concentrations in the dilution of oral self-microemulsifying drug delivery systems

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Abstract

Background: Self-microemulsifying drug delivery systems provide a key technology to formulate challenging drugs. These formulations are commonly screened in early development by simple in vitro dilution tests. However, there is often a lack of rationale of how these tests are performed; so this article aims to improve this situation by studying critical concentrations in the dilution of self-microemulsifying formulations. *Methods*: Dynamic laser light backscattering, conductivity measurements, and electron paramagnetic resonance spectroscopy were conducted. *Results*: All model formulations exhibited profound changes at a similar aqueous dilution, which was interpreted as a percolation threshold of the formulation in water. It marked the change of a bicontinuous microemulsion to discrete micelles. The systems exhibited at this point maximal particle dispersion with a threshold of polydispersity. A marked change was also observed in the paramagnetic resonance spectra and with the conductivity measurements. This altered microenvironment can be relevant for solubilized drugs. *Conclusions*: Future dilution tests should include a formulation-to-water ratio of roughly 1:5 (w/w), which is in the proximity of the individual threshold concentration. Additional dilutions may be tested below and clearly above this value to reflect the physiological dilution process.

Key words: *Critical concentration; dilution; drug delivery systems; electron paramagnetic resonance; percolation theory; self-microemulsifying*

Introduction

New drugs often exhibit low aqueous solubility, which can result in biopharmaceutical issues of incomplete or variable drug absorption[1]. Such biopharmaceutical challenges can be managed by adequate formulation technology such as by using oral lipid-based formulations[2–4]. These lipid-based systems can be categorized in simple oils and different types of self-emulsifying systems[5,6]. The complex mixtures are named according to their dilution performance as self-emulsifying or self-microemulsifying drug delivery systems (SEDDS or SMEDDS, respectively). The latter concentrate of an evolving microemulsion, in particular, has been subject to intensive research. Some recent applications showed increased oral bioavailability of oridonin[7], vinpocetine[8], and fenofibrate[9], whereas other authors observed the reduction of a food effect[10,11].

A pivotal mechanism of SMEDDS is that they can keep the drug solubilized during their intestinal passage[5,12]. Even a state of supersaturation may be obtained[13] and the high concentration gradients drive the subsequent permeation step[14]. Apart from this mechanism, there are certainly other reasons as to why SMEDDS can increase oral bioavailability. Thus, some drugs can profit from inclusion in micelles to circumvent a presystemic clearance and in cases for which lymphatic absorption is promoted, even a hepatic first pass can be reduced[15,16]. These different absorption processes are influenced by formulation components in a complex manner. Such confounded in vivo effects certainly limit the relevance of any in vitro tests[17,18]. Therefore, even today it is not possible to fully predict the performance of such formulations based on in vitro test, yet these tests are still pivotal for the development. In vitro tests can screen formulations with a low potential to provide adequate exposure. Thus, dilution and dispersion tests can characterize a formulation and may reveal unwanted drug precipitation. While these basic

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tests provide a first evaluation of the formulations, there are also more elaborate in vitro methods that further consider the physiological digestion. Such lipolysis tests have been reported by several authors[19–22] and it is possible to reveal changes of drug solubility during the formulation digestion. A recent study applied an in vitro lipolysis test[23] to study self-emulsifying formulations of Danazol. The test showed increased drug precipitation if the ratio of surfactant to lipid was increased and this finding agreed with the results of a dog study. Another successful application of a dynamic lipolysis test involved a neuro-fuzzy network to establish an in vitro–in vivo correlation[24]. Certainly, these lipolysis tests mimic the in vivo situation better than simple physical dilution or dispersion tests. However, the latter basic methods are still of importance for the development of oral lipid formulations. At an early stage, especially, there is a large group of formulation candidates to be screened. A simple dilution in water or physiological media is used to characterize the dispersion and helps anticipating potential drug precipitation. Unfortunately, the selected test conditions such as the type of media, the dilution level, as well as the temperature are highly variable in industry and academia so that research is needed to define the test protocol in a rational way[25]. Such a rationale also needs a better mechanistic understanding of the formulation dilution process.

The current view of SMEDDS dilution can be qualitatively described in the following way[26]. An initial L2 structure exhibits microdomains that are inverted micelles. Once a little water is added, the microdomains increasingly swell to form an water-in-oil microemulsion. Further aqueous dilution typically results in bicontinuous structures that finally change into a oil-in-water microemulsion. The latter transition is of particular interest with respect to drug precipitation, and the aim of this article is to study the structural changes by means of several experimental techniques. Thus, diluted SMEDDS are investigated using dynamic laser light backscattering, conductometry, and paramagnetic resonance spectroscopy. Finally, these research methods should not only lead to a better understanding of the process but should also result in a proposal of simple dilution tests in the future.

Materials and methods

Materials

The middle-chain triglyceride oil Miglyol®812 as well as the medium-chain partial glycerides Imwitor®742 were obtained from 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 Tween®80 (polysorbate 80 or polyoxyl 20 sorbitane monooleate) as well as the ethanol 96% were purchased from the local vendor Hänseler AG (Herisau, Switzerland).

Each SMEDDS formulation had a total of 5 g and the oily phase was mixed first before the surfactant was added. The Cremophor®RH40 and Solutol®were initially melted prior to being mixed with the other formulation components. Finally, a monophasic system was obtained for all SMEDDS that were visually controlled. The samples were obtained from instantaneous aqueous dilution, and measurements were performed after 10 minutes equilibration time at ambient temperature.

Dynamic laser light backscattering

Dynamic laser light scattering involves a time correlation function of the scattered intensity. This correlation function provided a diffusion coefficient (D) of the particles in solution as well as a measure of their polydispersity. Based on the diffusion coefficient, the Stokes–Einstein equation was used to calculate the hydrodynamic particle radius (r):

$$r = \frac{kT}{6\pi\eta D}, \tag{1}$$

where k is the Boltzmann constant, T the absolute temperature, and η is the viscosity of the continuous phase. The dispersion technology software 5.0 (Malvern Instruments Ltd., Malvern, UK) was used for the calculation of the Z-average value together with the polydispersity index (PDI).

The instrument was a Zeta Sizer®Nano ZS (Malvern Instruments Ltd.) 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°, and all measurements were conducted at room temperature.

Electron paramagnetic resonance spectroscopy and measurements of conductivity and density

Electron paramagnetic resonance (EPR) spectra were recorded with an EPR spectrometer 9.48 GHz Miniscope®MS 100 from Magnettech (Berlin, Germany). The measurements were conducted with the following parameters—modulation frequency, 100 kHz; microwave power, 7.94 mW; scan range, 4.95 mT; sweep time, 60 seconds; modulation amplitude, 0.15 mT. The spin probes tempol and tempolbenzoate were purchased from Sigma-Aldrich (Buchs, Switzerland). Figure 1 depicts an example of a typical EPR spectrum.

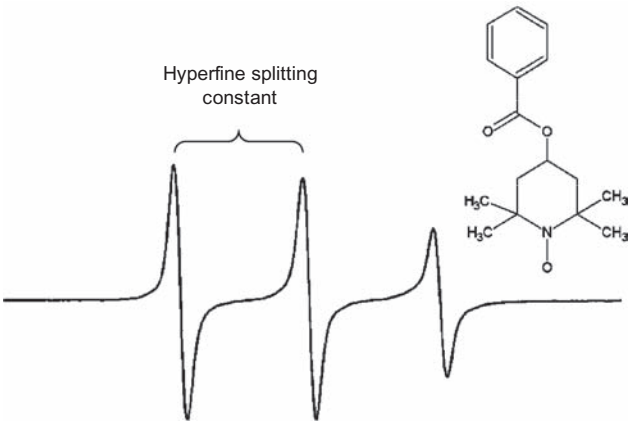

Figure 1. Example of an EPR spectrum using tempolbenzoate as spin probe.

The specific conductivity was measured using a Multiparameter laboratory meter WTW inoLab®pH/ION/Cond 750 (WTW, Weinheim, Germany) and the densities of the formulations were determined by means of an oscillatory frequency method. The latter instrument was a DA-100 M produced by Mettler-Toledo (Greifensce, Switzerland).

Statistical analysis and molecular modeling

The program STATGRAPHICS®Centurion XV Version 15.1.03 was used for the linear regression, the analysis of variance, as well as for the preparation of the graphs. Finally, the molecular properties of the different spin probes were calculated using the program Molecular Modeling Pro®V.6.2.6 (Norgwyn Montgomery Software Inc., North Wales, PA, USA). The clogP value was estimated using the Moriguchi method.

Results and discussion

Three candidate SMEDDS were obtained from previous phase behavior studies[25] and the compositions can be inferred from Table 1. The main difference in the mixtures was the nature of the surfactant providing a formulation with Cremophor®(CF), Polysorbate (PF), and Solutol®(SF). These different formulations all resulted in transparent microemulsions at the highest aqueous dilution of 1:100 (w/w). At this dilution level (room temperature), the formulations displayed similar colloidal particle sizes being 28.7 ± 0.1 nm (CF), 20.2 ± 0.0 nm (PF), and 23.8 ± 0.2 nm (SF). Interestingly, the particle sizes were already in a nano range at a dilution of 1:10, namely, 27.6 ± 0.4 nm (CF), 28.6 ± 2.1 nm (PF), and 22.6 ± 0.1 nm (SF). As a colloidal particle size was obtained in such a broad dilution range, the formulations appear to have robust dilution behavior in water.

There were also samples measured at a very low dilution level. However, especially at 1:2 (w/w) and 1:1 (w/w), the particle size distribution became highly polydisperse. Figure 2 displays the PDI as a function of the aqueous dilution level. Inspecting the heterogeneity of the particle sizes, the Polysorbate formulations were generally more polydisperse than the other SMEDDS. On the other hand, all formulations had in common that close to 1:5 (w/w), the PDI changed drastically. At a dilution of 1:2 (w/w) or 1:1 (w/w), the particle size distribution was generally multimodal and highly polydisperse. This is an interesting observation if we think of the diffusion and stranding process as a mechanism of self-microemulsification[27]. Thus, once water accesses the lipid system, particles are obtained that are highly polydisperse. A range of heterogeneity is given, where coherent oily structures exist together with some discrete particles. Accordingly, the results indicate a threshold of heterogeneity with respect to aqueous SMEDDS dilution. Further addition of water resulted far away from this threshold in a nearly monosized distribution.

To better understand the observed polydispersity threshold, it is necessary to consider the volume fraction of the formulations. For practical reasons, the dilution factor (*DF*) is defined as the mass of the

Table 1. Composition of the selected SMEDDS formulations with excipient amounts in % (w/w).

Composition	Cremophor® formulation	Polysorbate formulation	Solutol® formulation
Cremophor®RH40	34%	—	—
Polysorbate 80	—	52%	—
Solutol®HS15	—	—	50%
Imwitor®742	25.5%	24%	20%
Miglyol®812 (MCT)	25.5%	24%	20%
Ethanol	15%	—	10%

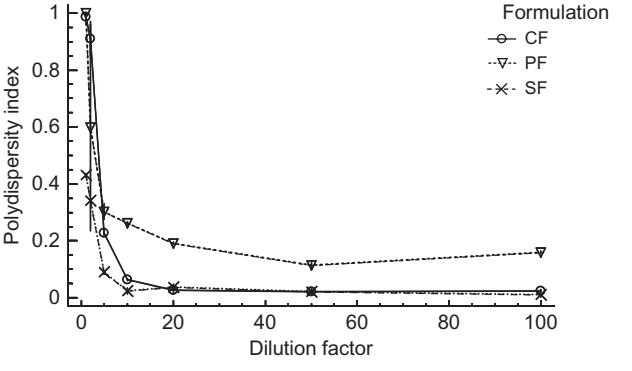

Figure 2. Polydispersity index of the different SMEDDS as a function of the dilution factor.

aqueous dilution medium (m_{Aq}) divided by the mass of the lipid formulation (m_L), $DF = m_{Aq}/m_L$. As the total volume (V_{tot}) is the sum of the aqueous and lipid volume, the volume fraction of the lipid formulation (ϕ_L) can be calculated by

$$\phi_L = \frac{V_L}{V_{Tot}} = \frac{1}{1+\rho_L/\rho_{Aq}\cdot DF}, \quad (2)$$

where ρ_{Aq} denotes the water density and ρ_L is the density of the formulation. The latter value was determined by oscillatory frequency measurements. The values are listed in Table 2. Based on Equation (2) and assuming a unity density for water, the volume fraction of the dilutions 1:2, 1:5, and 1:10 were calculated. From Table 2 can be inferred that a value of $\phi \cong 0.17$ resulted for the 1:5 (w/w) dilution, which roughly corresponded to the marked change in polydispersity (Figure 2). This critical volume fraction can be compared with results of percolation theory. The latter theory describes a geometric phase transition, which was first used by Sher and Zallen[28] to estimate a critical volume fraction for spheres being 0.15. Later the concept of percolation theory was applied to interpreting microemulsion phenomena[29]. This initial work considered the increasing conductivity in water-in-oil microemulsions and a critical volume fraction of the water was found to be slightly below 0.15, which was also supported by a more recent application using a pharmaceutical microemulsion[30]. This percolation threshold of water-in-oil can be viewed as a lower threshold with respect to aqueous dilution. Sager and Eicke[31] described the existence of another percolation threshold of oil-in-water. This threshold marks the transition from a bicontinuous structure to isolated micelles at higher water content and such a threshold must also exist in the dilution of a SMEDDS. Thus, Equation (2) can be simplified by neglecting the density ratio to estimate the critical dilution factor $DF_c \cong 1/\phi_c - 1$. Using the basic theoretical estimate of 0.15 for the critical volume fraction, we find a critical dilution factor of 5.7 and it is therefore a short step to identify the upper percolation threshold with the observed change in polydispersity of the SMEDDS.

It would be interesting to consider the concentration of particles during the dilution process, but this parameter is difficult to obtain experimentally. However, in the case of the highly diluted oil-in-water microemulsions, we can assume that a number of N_p swollen micelles include the entire lipid volume of the formulation (V_L):

$$V_L = N_P \cdot \frac{4}{3}\pi \cdot r^3. \quad (3)$$

Division of Equation (3) by the total volume (V_{tot}) leads to Equation (4), where C_P is the concentration of the particles:

$$C_P = \frac{3}{4\pi} \cdot \frac{\phi}{r^3}. \quad (4)$$

Certainly, Equation (4) holds primarily for solutions of spherical micelles. It seems less appropriate to use Equation (4) in a range of high polydispersity, as here a mean particle size has limited relevance. However, a transformation of all measured size data according to Equation (4) was still meaningful in our case to analyze the different ranges of the dilution process. The obtained values can be considered as being nominal particle concentrations. Figure 3 shows the graph in the case of the Cremophor®system. It was possible to identify two distinct ranges and the highly diluted samples with a formulation volume fraction of less than about 0.15 could be clearly differentiated from the less diluted samples. The latter range displayed high experimental scattering of the values, which reflected the existing heterogeneity. It was qualitatively observed that the particle concentration increased with a decreasing volume fraction of the formulation. The generation of particles reached a maximal value between roughly 0.1 and 0.15

Table 2. Formulation densities and volume fractions of the different systems.

	Cremophor® formulation	Polysorbate formulation	Solutol® formulation
Density ρ_L (g/mL)	0.964 ± 0.001	1.016 ± 0.001	0.993 ± 0.001
ϕ_L at 1:2	0.342	0.330	0.335
ϕ_L at 1:5	0.172	0.165	0.168
ϕ_L at 1:10	0.094	0.090	0.092

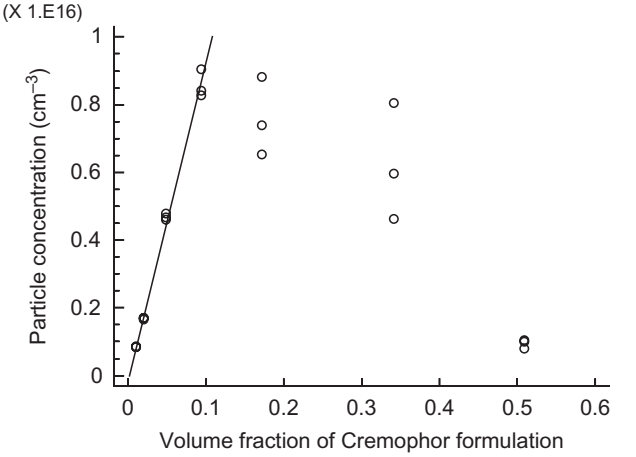

Figure 3. Calculated nominal particle concentration (cm^{-3}) of the Cremophor®system upon aqueous dilution.

of the formulation volume fraction. Above this maximal particle dispersion, a decline of the concentration was observed with higher dilution. As this decrease appeared to be linear, it can be assumed that the maximal particle number remained unchanged leading to a constant fall of the concentration with the amount of water added. Indeed a linear model of the particle concentration adequately fitted the data in this range (dilution of 1:10 (w/w) and higher) of the Cremophor®system with an R^2 (adjusted for d.f.) value of 0.995 ($P < 0.0001$).

Figure 4 displays the particle concentration of the diluted Polysorbate formulation. Two ranges were clearly differentiated. As in the previous case, the values at low dilution must be carefully interpreted, because of the existing heterogeneity. Despite the high polydispersity, Figure 4 also shows the existence of a peak concentration. This extreme value separated the two ranges again in a range of about 0.1–0.15 of the formulation volume fraction. A linear relationship was found in the highly diluted range with R^2 (adjusted for d.f.) of 0.603 ($P = 0.0018$). The comparatively low fitting adequacy reflected the experimental scatter of the concentration values, which was different from the last example of the Solutol®system. Figure 5 depicts the particle concentrations as a function of the formulation volume fraction. The two ranges were again clearly observed. Accordingly, the dividing peak concentration appeared to be close to 0.15. Beyond the 1:5 dilution, it was possible to fit the data by means of a linear regression with R^2 (adjusted for d.f.) of 0.993 ($P < 0.0001$).

To summarize, all SMEDDS exhibited ranges of increasing and decreasing particle concentration. The formulation volume fraction that divided the two ranges corresponded roughly to the polydispersity threshold. This maximal particle concentration of a SMEDDS can be interpreted as an oil-in-water percolation threshold. Samples that are less diluted contain the majority of the oil in a coherent structure, whereas clearly fewer particles exist. On the other hand, the higher diluted formulations were shown to consist of mainly isolated micelles, having a concentration that decreased linearly with the sample dilution. This fundamental change in the microstructure was also expected to provide sharp changes in other macroscopic properties such as the electrical conductivity.

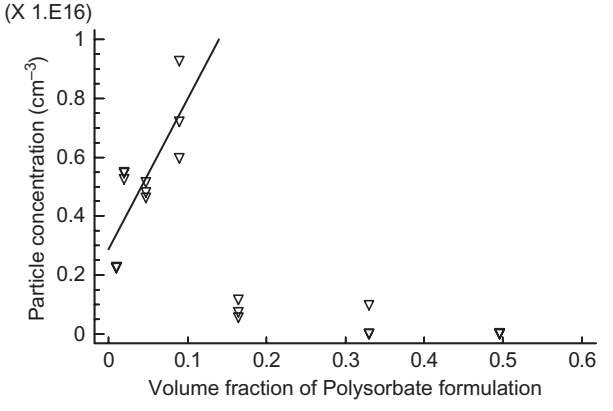

Figure 4. Calculated nominal particle concentration (cm^{-3}) of the Polysorbate system upon aqueous dilution.

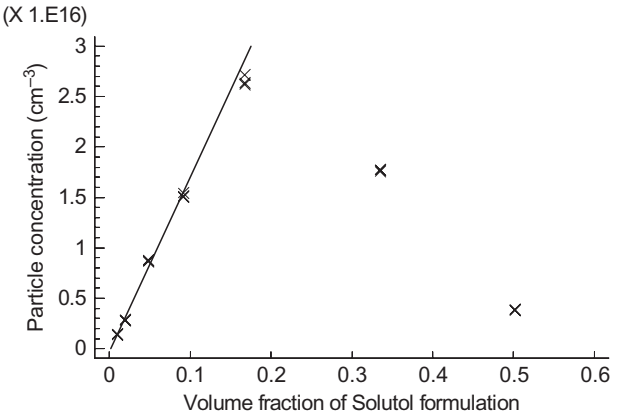

Figure 5. Calculated nominal particle concentration (cm^{-3}) of the Solutol®system upon aqueous dilution.

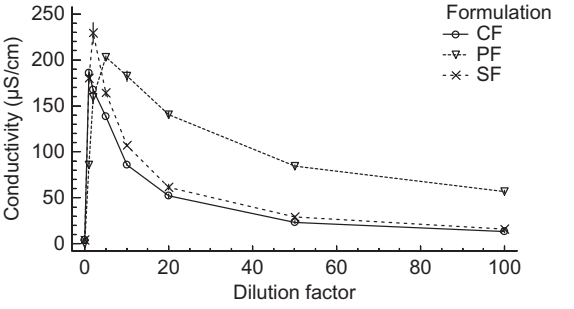

Figure 6. Specific conductivity (μS/cm) of the different SMEDDS as a function of the dilution factor.

Figure 6 depicts the change in specific conductivity of all SMEDDS formulations during the aqueous dilution. In the case of Polysorbate formulation, this maximal conductivity seemed to be slightly higher than the 1:5 (w/w) dilution, whereas for the Cremophor®and Solutol®system, the peak was reached with lower dilution. It can be assumed that the conductance in demineralized water was mainly governed by the concentration of the charge carrying particles. However, conductivity is a dynamic property and studies of aromatic and aliphatic hydrocarbon microemulsions have previously shown a range of different volume percolation thresholds[32]. This critical concentration is therefore highly dependent on the specific microemulsion properties.

As the dilution process involves critical concentrations for which the microstructure abruptly changes, it is fair to question the relevance for a dissolved drug. There may, however, not be a general answer to this question, as all drugs have their specific physioco-chemical properties. A drug precipitation depends, for example, on its concentration in the formulation and the solubility in the dilution medium. The latter value changes during the dilution process[33], and therefore it is important to study the changes of a drug's environment.

The diluted SMEDDS were studied by means of EPR using two spin probes of different polarity. Figure 7 shows the hyperfine splitting constant of the spectra obtained from tempol. It is known that this splitting constant is a measure of polarity, viscosity, and acidity in the environment of the spin probes[34]. Such microenvironment changes occurred during the dilution of the different SMEDDS as depicted by Figure 7. There was a general increase in the hyperfine splitting constant and highest values were reached with the most diluted samples. As tempol had moderate lipophilicity of clogP 1.3, it might preferentially reside in the palisade region of the micelles and a smaller fraction of the molecules could be directly dissolved. This microenvironment seemed to be quite different from that of the slightly diluted samples, in which a much lower splitting constant was obtained. A change was mainly recorded below and at close to the 1:5 (w/w) dilutions. Beyond 1:10 (w/w), nearly a constant value was reached for the evolving micelle solution. The tempol spectra supported the view of a fundamental structural change at a dilution close to 1:5 (w/w).

Further EPR measurements were conducted using the spin probe tempolbenzoate. Figure 8 shows the results for the different SMEDDS. The hyperfine splitting constant of the Polysorbate system displayed a similar behavior as with tempol. A marked increase was followed by a plateau of the splitting constant during the dilution process. This pattern was interestingly not

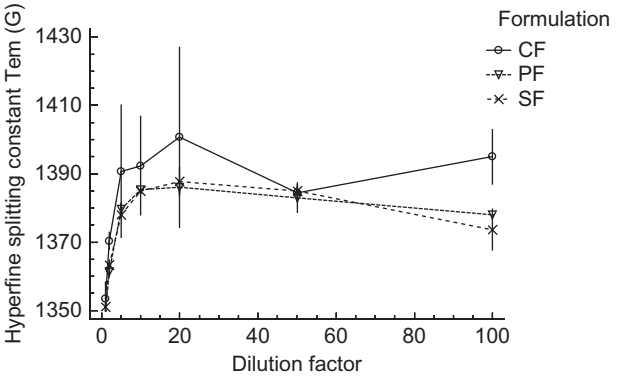

Figure 7. Hyperfine splitting constant obtained from EPR measurements using tempol as spin probe in the SMEDDS.

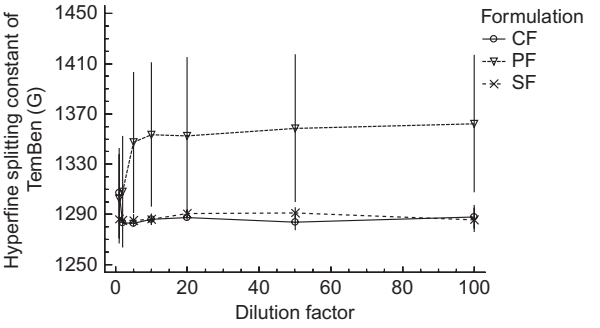

Figure 8. Hyperfine splitting constant obtained from EPR measurements using tempolbenzoate as spin probe in the SMEDDS.

shown by the other two SMEDDS, in which, overall, only moderate changes were observed. Even a slight decrease was shown by the Cremophor®formulation, which was an interesting aspect of the tempolbenzoate. The latter spin probe was more lipophilic with a clogP of 2.9 and the molecule probably stayed in the lipid microdomains of a bicontinuous structure or in the cores of micelles. This may explain the absence of marked changes in the case of the Cremophor®and Solutol®formulation. As the HLB of tempolbenzoate was 6.7, the molecule also revealed an amphiphilic nature. Specific interaction with the given surfactant systems may therefore account for the recorded changes of the EPR spectra. However, the changes took place at a low dilution, and, once the samples were at 1:10 (w/w), a plateau of the hyperfine splitting constant was observed. These results further support the view of a critical concentration close to the 1:5 (w/w) dilution and the relevance of these structural changes is dependent on the physico-chemical properties of a solubilized compound.

Conclusion

This work contributed to a better understanding of the SMEDDS dilution process. It was shown that upon dilution, a critical concentration is reached at about lipid/water of 1:5 (w/w), which is considered as a percolation threshold. This critical volume fraction generally denotes a transition from a continuous to a discrete system or vice versa. In the case of microemulsion, this refers to transition from a bicontinuous structure to a discrete solution of swollen micelles. This work applied this theoretical concept to the dilution of SMEDDS and the threshold was identified as being equal to a concentration of maximal particle generation and maximal polydispersity. The change from one structure to another can affect a dissolved compound as it was

shown with tempol using EPR spectroscopy. In light of these results, we recommend testing candidate SMEDDS at a dilution level below, close to, and clearly beyond the critical concentration. The threshold can be found close to a ratio lipid/water of 1:5 (w/w). This change in the microstructure may not only be of interest in view of potential drug precipitation. A stage of maximal particle dispersion and polydispersity may also have the highest potential to interact in vivo for example with food components. Further research is needed to study drug-containing SMEDDS and to explore the biopharmaceutical relevance of critical dilution concentrations.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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