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ESR studies on the influence of physiological dissolution and digestion media on the lipid phase characteristics of SEDDS and SEDDS pellets

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ABSTRACT

The aim of the current study is the evaluation of a recently optimized SEDDS, composed of Solutol® HS15 and medium chain glycerides, and self-emulsifying pellets by means of ESR.

Tempol-benzoate (TB)-loaded SEDDS were produced and electron spin resonance (ESR) spectroscopy was used to evaluate the diluted self-emulsifying mixtures. Moreover, ESR *in vitro* digestion experiments were carried out to have an insight on the characteristics of the different phases formed during the digestion process and to evaluate the distribution and the localization of TB in these phases. In addition, self-emulsifying pellets were produced using nitroxide-loaded SEDDS and the microenvironment within the pellets during release process was monitored in an online process using ESR spectroscopy.

After dilution of nitroxide-loaded SEDDS, the percent of TB localized in the lipophilic compartment was decreasing with increasing the surfactant fraction in the mixture. Moreover, it was found that different phases with variable viscosity and polarity were produced as a result of the enzymatic digestion of SEDDS in physiologically relevant media. This change in lipid composition has largely affected the distribution and the localization of the spin probe during the digestion process. A rapid increase in the mobility of the spin probe inside the pellets was noticed after exposure to the release media. Additionally, TB was localized within the self-emulsifying mixture environment for the time of the experiment.

ESR is considered a powerful non-invasive tool to assess the microenvironment of the diluted SEDDS and to monitor *in vitro* digestion process. Digestion induces a change in lipid composition which can affect the solubilization capacity of the administered drug. Therefore, monitoring *in vitro* digestion process using ESR spectroscopy will help in providing greater understanding of the interaction between the administered drug and the digested lipid vehicles.

1. Introduction

Up to 41% of newly discovered chemical entities fail in the drug development process due to their poor aqueous solubility (Lipinski et al., 2001). Poor solubility leads to long dissolution times and low bioavailabilities.

Several formulation approaches have been developed to achieve a rapid solubilization. Self-emulsifying drug delivery systems (SEDDS) compete with amorphous systems, nanoparticles, microemulsions and other formulation approaches to improve oral the bioavailability of poorly soluble drugs (Wakerly et al., 1986; Charman et al., 1992).

SEDDS are oral lipid dosage forms composed of mixtures of natural or synthetic oils, solid or liquid surfactants, or alternatively, one or more hydrophilic solvents and cosolvents/surfactants (Shah et al., 1994; Pouton, 1997, 2006). After contact with gastric fluids these formulations disperse freely and form oil-in-water nanodroplets which accommodate lipophilic drugs.

The rapid, self-driven dispersion leads to an improvement of the rate and the extent of drug absorption and the reproducibility of plasma profiles (Gursoy and Benita, 2004). The *in vivo* fate of SEDDS includes (i) dilution, (ii) interaction with mixed micelles and (iii) enzymatic digestion processes. Dilution or digestion of lipid-based formulation, in the presence of endogenous materials (bile salts (BS), phospholipid (PL) and cholesterol), induces a change in the lipid composition. As a result, different colloidal phases (micelles, vesicles, and liquid crystalline phases) are formed in the intestinal lumen (Patton and Carey, 1979; Carey et al., 1983; Rigler et al., 1986; Fatouros et al., 2007; Porter et al., 2007). The intestinal phase behavior of formulation-derived lipids and their digestion products play a significant role in the solubilization of co-administered

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drugs (MacGregor et al., 1997; Christensen et al., 2004). A better understanding of the interactions between a co-administered drug and the digested oily vehicles during pre-absorptive intraluminal processing may lead to a more rational selection of the lipids for incorporation into a lipid-based formulation. Unfortunately, the characterization of SEDDS in the presence of digestive enzymes is difficult due to the heterogeneity and turbidity of the samples. However, this limitation is avoided by the use of electron spin resonance (ESR) spectroscopy. Therefore we explored the possibility of ESR to characterize SEDDS under digestive conditions. ESR is considered a powerful spectroscopic technique for the non-invasive *in vitro* and *in vivo* monitoring of drug delivery systems (Mäder et al., 1994; Yamaguchi et al., 1996; Raffi et al., 2002; Petelin et al., 2004). Since the majority of drug delivery samples are diamagnetic and ESR silent, the incorporation of paramagnetic molecules (e.g. nitroxide spin probes) is necessary. The ESR spectra of nitroxide spin probes provide information about their local environment. For instance, the hyperfine splitting constant (a_N) is a sensitive parameter to the polarity of the environment (see Fig. 2 for illustration) and this property is used to monitor the partitioning and the localization of spin probes in compartments of different polarity (Jores et al., 2003). Further information about the principles and applications of ESR are described in a recent review (Lurie and Mäder, 2005).

In the current study ESR spectroscopy was used to monitor digestion induced changes of the microenvironment of self-emulsifying mixtures, which have been developed by us, composed of a mixture of medium chain mono- and di-glycerides, triglycerides of caprylic/capric acid and Solutol® HS15. ESR was used to assess the distribution and localization of the spin probe in digested SEDDS. Moreover, self-emulsifying pellets with nitroxide-loaded SEDDS were produced and evaluated by ESR to investigate whether or not SEDDS incorporation into MCC pellets will impact the fate of the SEDDS.

2. Materials and methods

2.1. Materials

Avicel PH 101 (microcrystalline cellulose (MCC)) was purchased from FMC BioPolymer (PA, USA), and was used as a pellet forming material. Solutol® HS 15 (macrogol-15-hydroxystearate) was kindly provided by BASF AG (Ludwigshafen, Germany). Captex 355 EP/NF (triglycerides of caprylic/capric acid) and Capmul MCM (medium chain mono- and di-glycerides) were kindly provided by Abitec Corporation (Janesville, WI, USA). Pancreatin (activity equal to 8× USP specification) and bile extract [used as a heterogeneous source of bile salts (BS)] were obtained from Sigma (Steinheim, Germany). Phospholipon 90G was kindly provided by Phospholipid GmbH (Cologne, Germany). Tempol-benzoate (4-benzoyloxy-2,2,6,6-tetramethylpiperidine-1-oxyl, TB) was purchased from Sigma–Aldrich (Steinheim, Germany).

2.2. Methods

2.2.1. Preparation of self-emulsifying mixtures

The compositions of the formulations tested are listed in Table 1. The components were mixed together at 50 °C and the spin probe was dissolved in the mixtures (2 mmol/kg). This was followed by cooling the mixtures to room temperature.

2.2.2. ESR spectroscopy

Nitroxide-loaded SEDDS mixtures were produced and the microenvironment within the oil droplets after dilution was evaluated by means of ESR spectroscopy. The nitroxide spin probe tempol-benzoate (TB, Fig. 1) was used as a model for poorly water-soluble drugs. One gram of TB-loaded (2 mmol/kg) SEDDS mixture was added to 200 ml of distilled water in a dissolution apparatus rotating at a rate of 70 rpm and a temperature of 37 °C. Samples were taken after 30 min and the ESR spectra were recorded by means of a 9.3–9.55 GHz X-band spectrometer (MiniScope MS200, Magnettech, Berlin) using the following parameters: sweep 10 mT, sweep time 60 s, modulation amplitude 0.1 mT. Furthermore, the ESR spectra of TB in different formulation components were recorded using the same conditions.

Simulation of the ESR spectra was performed by the use of Nitroxide spectra simulation software (Biophysical laboratory, EPR center, Josef Stefan Institute, Ljubljana, Slovenia).

Table 1
Formulations composition of the different self-emulsifying mixtures produced.

Formulation	Self-emulsifying mixture composition (%, w/w)	
	Captex® 355:Capmul® MCM	Solutol® HS15
Formulation A with 1:1 Captex to Capmul ratio		
A1	80	20
A2	75	25
A3	70	30
A4	65	35
A5	60	40
A6	55	45
A7	50	50
Formulation B with 2:1 Captex to Capmul ratio		
B1	80	20
B2	75	25
B3	70	30
B4	65	35
B5	60	40
B6	55	45
B7	50	50

2.2.3. Monitoring of in vitro lipid digestion by ESR

ESR spectroscopy experiments were carried out to monitor the *in vitro* lipid digestion of the selected self-emulsifying mixture. The lipophilic spin probe TB was mixed first with the self-emulsifying mixture to achieve a final concentration of 1 mM. 1% of the nitroxide-loaded self-emulsifying mixture B5 was mixed with 7.5 ml digestion buffer (53.4% KH_2PO_4 1/15 M, 46.6% $Na_2HPO_4{\cdot}2H_2O$ 1/15 M, 150 mM NaCl, 5 mM $CaCl_2{\cdot}2H_2O$, pH 6.8)

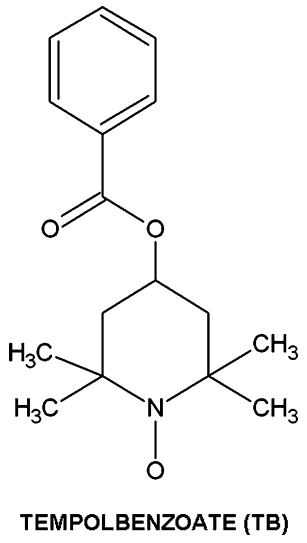

Fig. 1. Chemical structure of the ESR spin probe tempol-benzoate.

containing either a low (5 mM BS; 1.25 mM PL) or a high (20 mM BS; 5 mM PL) concentration of bile salts (BS) and phospholipids (PL) to simulate the intestinal fluids in the fasting (FaSSIF) and the fed state (FeSSIF) (Ladas et al., 1984; Hernell et al., 1990; Kaukonen et al., 2004). Experiments were started by the addition of a 65.77 mg pancreatin enzyme (450 U/ml of pancreatic lipase activity). The mixture was then incubated in an end over end apparatus rotating at a rate of 15 rpm and a temperature of 37 °C and pH was adjusted to 6.8 every 10 min. 100 μl samples were taken at fixed time intervals, filled into capillary tube and measured by X-band ESR. In addition, ESR spectra of TB in different media were recorded as reference spectra. For further control experiments, blank aqueous digestion phases were obtained by carrying out the experiment with drug free self-emulsifying mixtures for 1 h, followed by ultracentrifugation (108,000 × *g*, 30 min, 37 °C, Avanti J-301 centrifuge, Beckman Coulter Inc., CA, USA) and separation of the aqueous phase from the pellet phase.

2.2.4. Formulation of pellets

Self-emulsifying mixture B5 was selected for formulating the self-emulsifying pellets. Pellets were produced using extrusion/spheronization technique utilizing microcrystalline cellulose as a spheronization aid. The self-emulsifying mixture was mixed with MCC in a kneader for 15 min followed by addition of water until a mass suitable for extrusion is obtained. The resulting wet mass was then extruded at 40 rpm in a radial screen twin-screw extruder (Fuji-Paudal, Japan) equipped with a die of 1 mm diameter circular holes. The extrudate was then spheronized for 3 min in a 250 mm radial plate spheronizer (Fuji-Paudal, Japan) using a cross-hatch frictional plate of 3 mm × 3 mm pitch and 1.2 mm depth. The produced pellets were then dried in a desiccator over silica gel at room temperature. Composition of the studied pellets is shown in Table 2.

2.2.5. ESR flow through cell experiment

ESR flow through experiment was carried out to assess the microenvironment within the pellets during the release process (Besheer et al., 2006; Abdalla and Mäder, 2007). 200 mg of TB-loaded pellets (2 mmol/Kg) were placed in a flow cell and phosphate buffer (pH 6.8) was pumped into the cell at a rate of 4 ml/min. The ESR spectra were recorded continuously by means of a 1.3 GHz L-band spectrometer (Magnettech Gmbh, Berlin, Germany) equipped with a reentrant resonator. The ESR parameters used were as follow: field center 49 mT, scan range 12 mT, scan time 60 s and modulation amplitude of 0.14 mT.

Table 2
Composition of the investigated pellets.

Ingredients	Pellets composition (%, w/w)
MCC PH 101	60
Solutol® HS15	16
Captex® 355	16
Capmul® MCM	8
Tempol-benzoate	2 mmol/kg

3. Results and discussion

3.1. Preparation and in vitro assessment of the self-emulsifying mixtures

The polarity of the emulsion droplet is one of the important factors for the performance of SEDDS as it determines the affinity of the drug compound to oil and/or water which consequently affect the incorporation rate (Shah et al., 1994; Gursoy and Benita, 2004). ESR experiments were carried out to assess the microenvironment and to monitor partitioning and localization of the spin probe TB in different environments within the produced emulsions. In addition, ESR spectra of TB in different formulation components were recorded and simulated to serve as references. Fig. 2 (right diagram) shows the sensitivity of the line shape to the viscosity of the different media. In a low viscosity medium, like water, the rotation (tumbling) of the spin probe is very fast. The anisotropy of the hyperfine coupling is therefore averaged and three sharp lines of similar amplitude are observed. As the viscosity increases (from Captex® 355 to Caprol® MPGO), the mobility is decreased and the anisotropy is only partially averaged. This leads to broadening of the line widths. The degree of broadening is not the same for all lines and the third line is the most sensitive one.

In Captex® 355, the mobility of the spin probe is very fast and it is higher than the mobility in the other formulation ingredients (Solutol® HS15 and Capmul® MCM). Even though Solutol® HS15 is a semisolid material, the ESR spectrum indicates a high mobility of

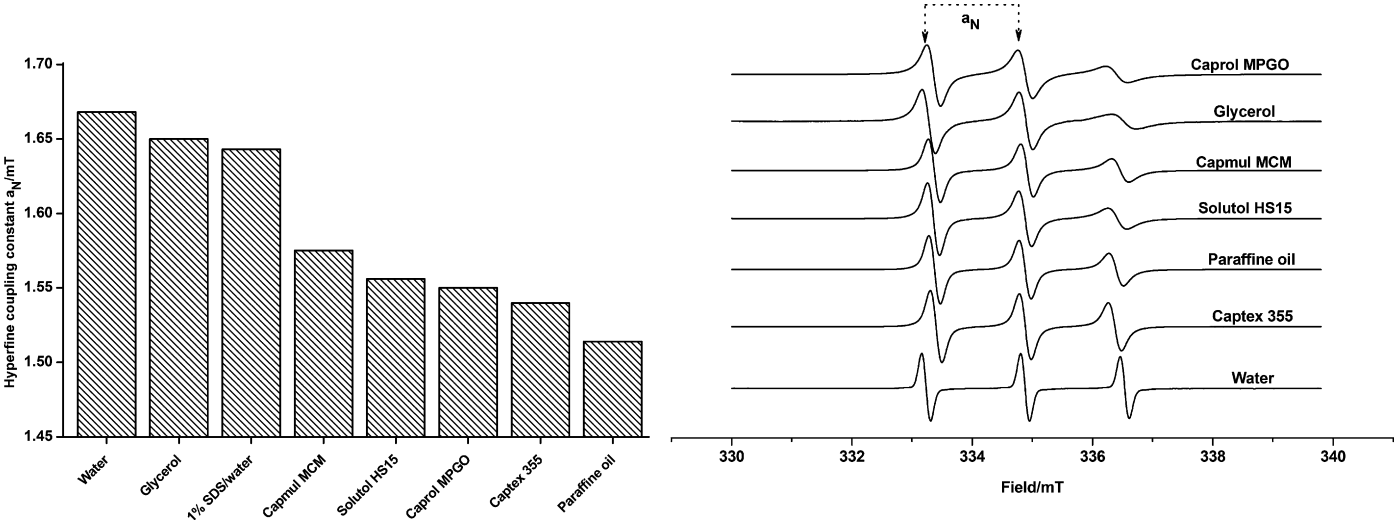

Fig. 2. ESR spectra of TB in environments with different polarity and viscosity. The hyperfine coupling constant a_N is sensitive to the polarity of the environment (left diagram) which is used to monitor the partitioning of spin probes between compartments with different polarity. Please note the effect of increasing the viscosity on the broadening of the line width (right diagram).

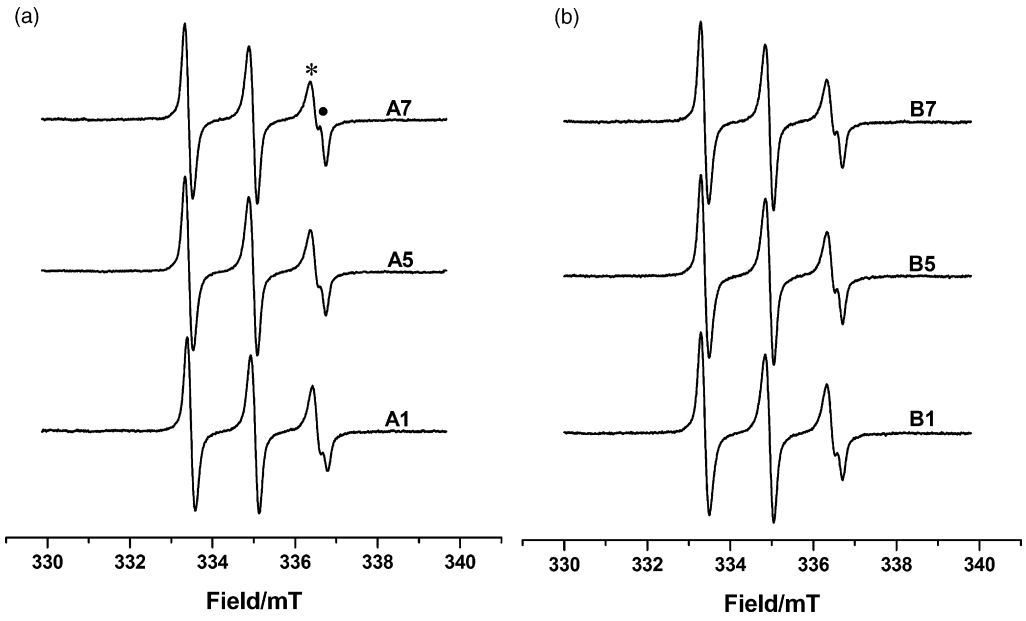

Fig. 3. ESR spectra of TB in the emulsion resulted from the dispersion of self-emulsifying mixtures (a) from formulation A and (b) from formulation B in phosphate buffer pH 6.8. ((*) Refers to the lipophilic while (●) refers to the hydrophilic species).

the spin probe. The left diagram in Fig. 2 is showing the sensitivity of the hyperfine coupling constant a_N to the polarity of the environment. This property is used to monitor the partitioning and the localization of spin probes between compartments with different polarity. As the polarity decreases from water toward paraffin oil, the hyperfine coupling constant decreases. As expected, the polarity of Captex® 355, composed of triglycerides of caprylic/capric (C_8/C_{10}) acid, is lower than the polarity of the corresponding mono- and di-glycerides mixture (Capmul® MCM).

TB was used as a model for moderately lipophilic drug with a log *P* of 2.46 (Rübe, 2006). The general pattern of the ESR spectra of TB in diluted SEDDS was very similar for formulation A (Fig. 3a) and formulation B (Fig. 3b). The splitting of the third line indicates very clearly the distribution of the spin probe in two environments with different polarity (Fig. 3). The experimental spectra of the diluted SEDDS were successfully simulated with a superposition of two ESR spectra (Fig. 4a). Fig. 4b shows the simulated ESR spectrum of the lipophilic component with a lower polarity and higher viscosity for formulation A and for formulation B. The polarity of the lipophilic compartment was slightly higher in system A (a_N = 1.53 mT), with 1:1 Captex® 355 to Capmul® MCM ratio, compared to system B (1.52 mT), with a 2:1 Captex® 355 to Capmul® MCM ratio. This finding reflects the different polarity of the more polar medium chain mono- and di-glycerides and the less polar medium chain triglycerides. The simulated ESR spectrum of the hydrophilic component with a higher polarity (a_N = 1.67 mT) and lower viscosity is displayed in Fig. 4c. Fig. 5 shows an illustration of the results of the ESR experiments.

The percent of TB localized in the lipophilic compartment is decreasing with increasing the surfactant fraction in the self-emulsifying mixture (Fig. 6). As the oil to Solutol® HS15 ratio decreases the percent of the spin probe localized in the lipophilic environment decreases. Moreover, it was found that formulation A provides more attraction to TB in all oil to Solutol® HS15 ratios (Fig. 6). In addition, it has been found that the micropolarity, in both formulations, inside the emulsion droplet (lipophilic compartment) was constant regardless of the varying oil and surfactant ratios.

3.2. Monitoring of in vitro lipid digestion by ESR

ESR *in vitro* digestion experiments were performed to characterize digestion induced changes of the TB localization and the polarity and viscosity of the lipid nanodroplets. The ESR measurement of the digestion mixture was carried out at different time intervals without the need for centrifugation or phase separation. Values of rotational correlation time (a measure of microviscosity), and the hyperfine splitting constant (a measure of micropolarity) were obtained from the simulation of the recorded spectra. The results obtained from the control experiment of TB in different media are shown in Fig. 7. There were no differences in the ESR spectra of TB between water and phosphate buffer. In both solutions, the ESR spectra could be fitted with one species with a very small rotational correlation time (<0.03 ns), and a high hyperfine coupling

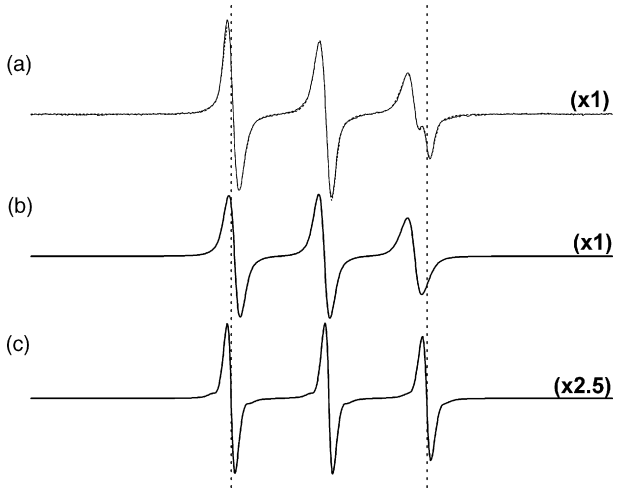

Fig. 4. ESR simulation spectrum of TB in the emulsion resulted from the dispersion of self-emulsifying mixture B5 in phosphate buffer. (a) Experimental spectrum (bold line) and simulated spectrum (dotted line), (b) spectrum of the lipophilic species and (c) hydrophilic species.

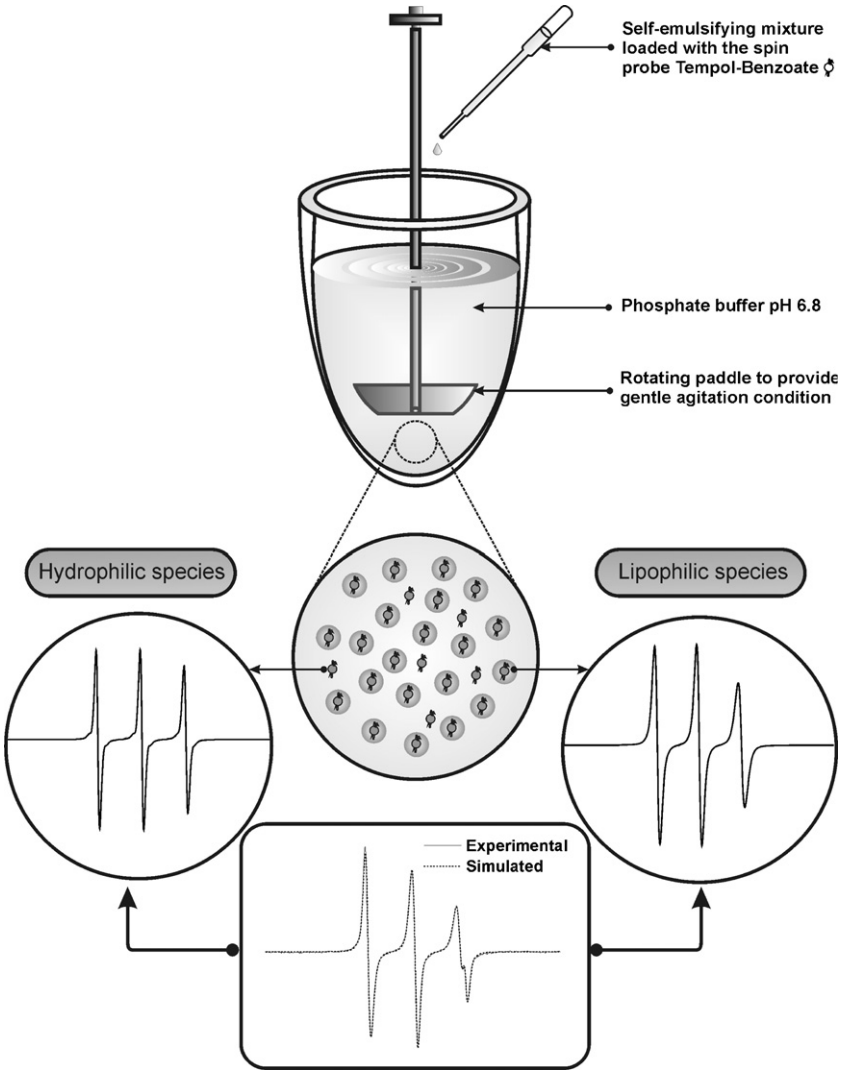

Fig. 5. Schematic presentation of the *in vitro* ESR experiment. After dilution of the TB-loaded self-emulsifying mixtures, the spin probe was localized in two environments with different polarity. The spectrum obtained was a superposition of two spectra, one corresponding to the hydrophilic species and the other to the lipophilic species.

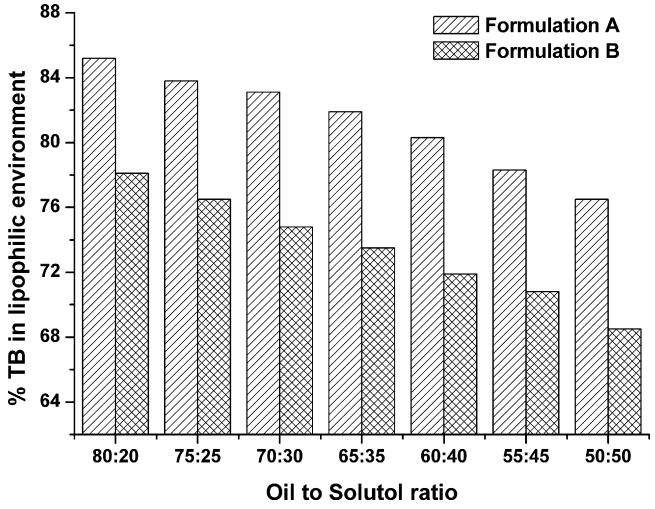

Fig. 6. Effect of changing oil to solutol ration on the distribution of TB in the lipophilic phase of the dispersed self-emulsifying mixtures.

constant (1.67 mT) (Fig. 7a and b). The ESR spectra of TB-loaded mixed micelles and aqueous digestion media could only be simulated with two species. This indicates that TB distributes between a hydrophilic and a lipophilic environment. Compared to water and phosphate buffer, the hydrophilic species showed higher rotational correlation times (0.06–0.3 ns) and slightly lower hyperfine coupling constants. This shows that the polar environment of TB is not water, but a polar lipid (micellar) phase. Based on the higher rotation correlation times (Fig. 7a) it can also be concluded that the hydrophilic TB environment is more viscous in FeSSIF compared to FaSSIF.

The second (lipophilic) species of the TB spectra in mixed micelles and digestion phases has a much lower polarity and a higher viscosity (correlation time 0.6–0.9 ns) compared to the first (hydrophilic) species. The lipophilic aqueous digestion phases are less viscous and more polar compared to the mixed micellar phases (Fig. 7a and b).

The detailed and quantitative analysis of the TB ESR spectra in different media (Fig. 7) was necessary to prepare the *in vitro* digestion studies. Also the ESR spectra obtained from the *in vitro* digestion experiment have been successfully simulated with two species, which reflect the localization of the spin probe in two different environments (Fig. 8). In the spectra of the hydrophilic

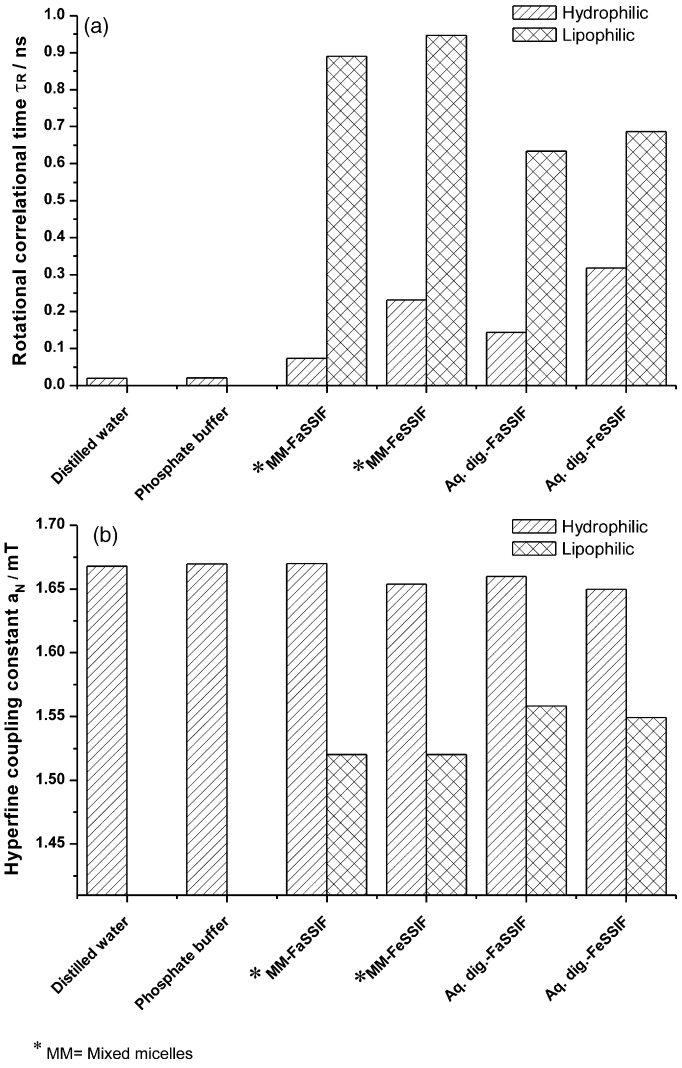

Fig. 7. Values of (a) rotational correlation time and (b) hyperfine coupling constant, for TB in different control media.

species, it was noticed that the third line has 50% lower amplitude than the middle line which indicates some restriction in mobility compared to that obtained for the spin probe in phosphate buffer. After mixing the self-emulsifying mixture with the digestion buffer, in both FaSSIF and FeSSIF conditions, TB was localized in two environments with different polarities, one hydrophilic (a_N = 1.64 mT) and one lipophilic (a_N = 1.53 mT) environment. As digestion starts in FaSSIF, the polarity of the lipophilic species increases significantly (a_N = 1.55 mT), where the polarity of the hydrophilic species decreases slightly (a_N = 1.624 mT). As digestion proceeds, the polarity of both environments remains constant until the end of the experiment. Under FeSSIF conditions, the hyperfine splitting of the lipophilic environment increases gradually to a value of 1.56 mT after 5 min and to 1.58 mT after 10 min, which indicates an increase in polarity. The polarity of the lipid phase remains constant after 10 min until the end of the experiment. The hyperfine splitting constant of the hydrophilic environment decreases to a value of 1.63 mT where it remain unchanged until the end of the digestion experiment which indicate a decreased polarity most likely due to the formation of mixed micelles. Therefore, the ESR measurements show that under FeSSIF digestion conditions the oil phase becomes more polar and the hydrophilic compartment becomes more lipophilic.

In a further step, the quantitative distribution of the spin probe in different environments was calculated. As shown in Fig. 9, the percent of TB localized in the hydrophilic environment increases significantly within the first 5 min under both FaSSIF and FeSSIF digestion conditions and it remains constant for the remaining time. This change in localization of the spin probe is caused by the enzymatic-induced hydrolysis of the apolar triglycerides, which leads to the formation of the more polar fatty acids and monoglycerides. As a result, new mixed micellar and liquid crystalline phases are formed on the expense of the digested lipophilic oil phase.

The rotational correlation time, was monitored throughout the digestion of the formulation. For the lipophilic species, both under FeSSIF and FaSSIF conditions, this parameter increases significantly within the first 5 min of the digestion process. In contrast, the viscosity of the hydrophilic species remains almost unchanged for the entire experiment time (Fig. 10a and b).

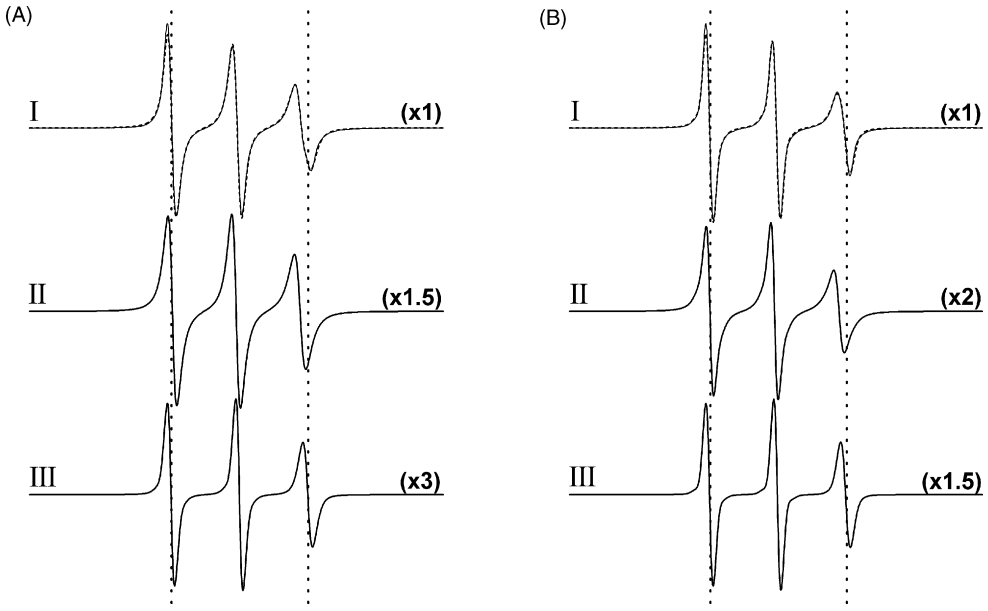

Fig. 8. ESR simulation spectra of TB after (a) 0 min and (b) 30 min of the digestion of 1% (w/v) of self-emulsifying mixture B5 in the digestion buffer under FaSSIF conditions. (I) Experimental spectrum (bold line) and simulated spectrum (dotted line), (II) spectrum of the lipophilic species and (III) spectrum of the hydrophilic species.

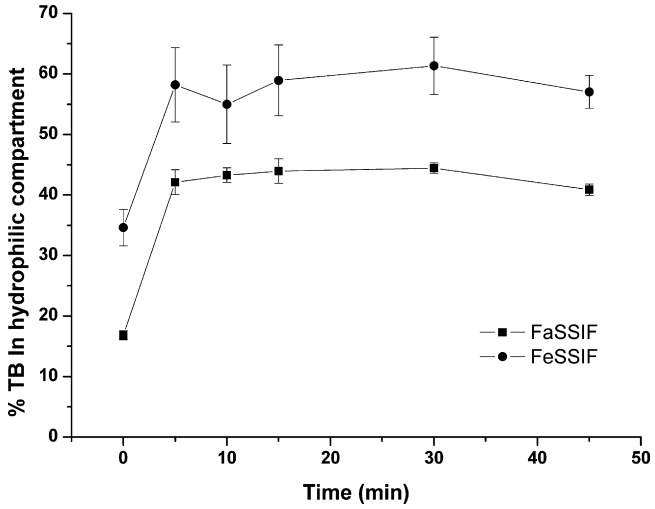

Fig. 9. Distribution of TB in the hydrophilic compartment as a function of time, after the digestion of 1% (w/v) of the self-emulsifying mixture B5 in the digestion buffer under FaSSIF (■) and FeSSIF (●) conditions.

3.3. ESR flow through experiment

The incorporation of the self-emulsifying mixture into MCC pellets might change the physicochemical properties of the lipid phase and also the release behavior. Therefore, the microenvironment within the pellets during the release process was monitored continuously and non-invasively by means of a flow through experiment using low frequency ESR spectroscopy. Additionally, the ESR spectra of TB in different formulation components were recorded as references (Fig. 11). The spectral line shape changes progressively when the nitroxide mobility changes from a free rotation, with a rotational correlation time (τ_R) of the order of 0.01 ns, to that of a restricted mobility with τ_R more than 1 μs. The results of the control experiment show that the mobility of the spin probe was higher in Captex® 355 compared to that in Capmul® MCM and Solutol® HS15. Moreover, TB mobility in the self-emulsifying mixture was

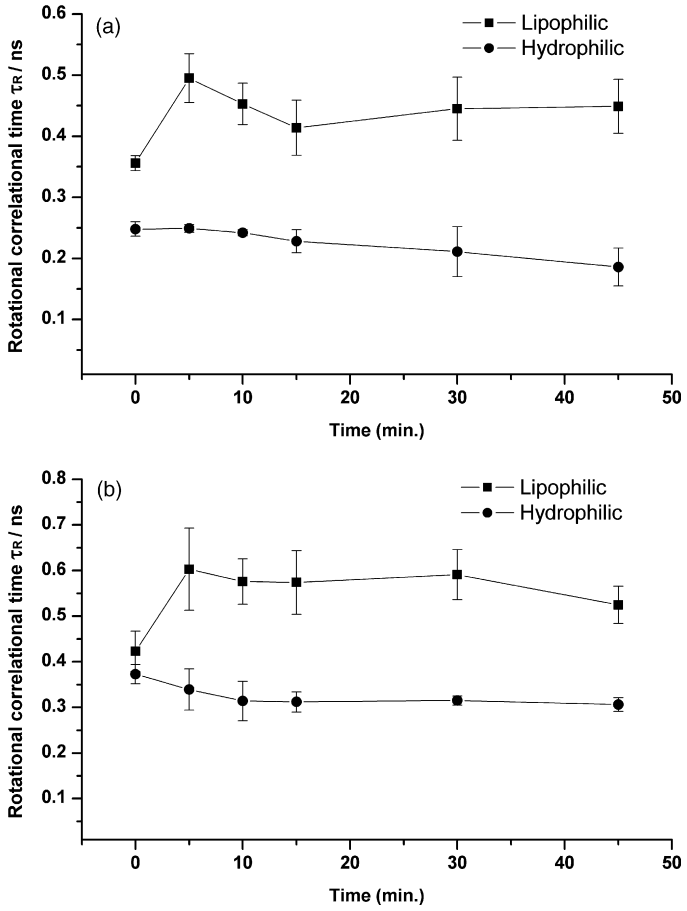

Fig. 10. Change of rotational correlation time, for lipophilic and hydrophilic species, as a function of time after the digestion of 1% (w/v) of the self-emulsifying mixture B5 in the digestion buffer under (a) FaSSIF and (b) FeSSIF conditions.

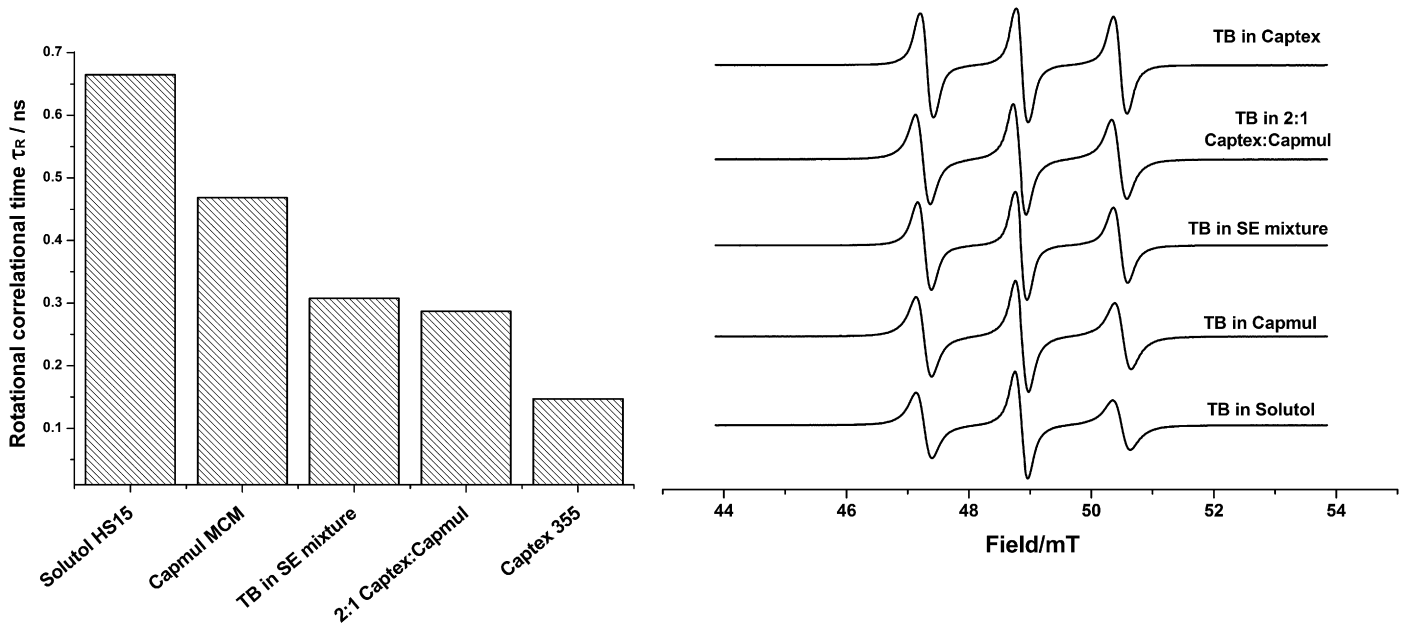

Fig. 11. ESR spectra of TB in different formulation components and mixtures, reflecting the sensitivity of the line shape (right) and rotational correlation time (left) to the viscosity of different media.

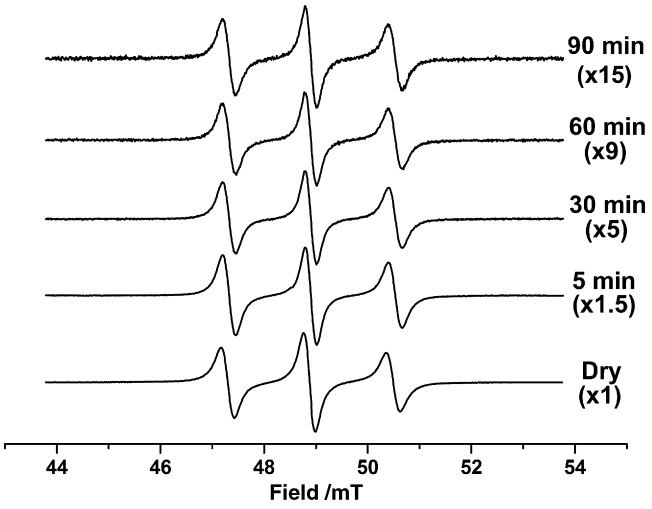

Fig. 12. ESR spectra of TB-loaded pellets before, 5, 30, 60 and 90 min after buffer exposure in the flow cell system.

higher than that in self-emulsifying pellets due to the partial immobilization of the self-emulsifying mixture within MCC. Immediately after pellets exposure to the dissolution media inside the flow cell, the mobility of the spin probe changed rapidly within the first 5 min and was comparable to that in the free self-emulsifying mixture (Fig. 12). The results also show that there was no change in the hyperfine splitting constant (a_N = 1.61 mT) for the whole period of the experiment which indicates that the spin probe was localized preferentially within the self-emulsifying mixture for the entire time of TB release from the pellets. As the flow of the media continues, the signal intensity decreases rapidly as a result of nitroxide release and only a weak signal was recorded after 90 min.

4. Conclusion

The results have shown that ESR spectroscopy provides useful information about lipid drug delivery systems at the molecular level during release and enzymatic digestion. The viscosity and polarity of different phases can be measured continuously and non-invasively even in turbid and non-transparent samples. Furthermore, the distribution of nitroxides as model drugs between the different environments can be assessed. The nitroxide TB was used in this study as one model of a poorly soluble drug. It has a log *P* of 2.46 and contains an aromatic benzyl ring. The results of the current study cannot be transferred to all poorly soluble drugs, because many parameters contribute to the behavior of drugs in lipid dispersions. The use of additional spin probes (with different Mw, log *P*, aromatic and alkyl structures, different bulkiness, etc.) is required to get a broader knowledge.

A change in the localization of the spin probe was noticed as the digestion process starts. This can be explained by the enzymatic hydrolysis of the tri- and diglycerides resulting in the formation of fatty acids and monoglycerides. The lipid digestion products form mixed micelles with endogenous materials (like bile salts, phospholipids and cholesterol), The spin probe TB was localized either in polar colloidal structures like mixed micelles or in less polar structures like vesicles or liquid crystalline phases. The change in the lipid composition associated with digestion of lipid-based formulations could affect the solubilization capacity of the administered drug. Therefore, applying *in vitro* digestion experiments for lipid formulations is important to enable prediction of the possible fate of the co-administered drug.

Results of the ESR flow through experiment show that the mobility of the spin probe in the pellets increased rapidly from partial immobilization within the dry pellets to high mobility, compared to that in the free self-emulsifying mixture, after the exposure to the buffer. It was also shown that the spin probe was localized within the self-emulsifying mixture during the release process from the pellets.

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