Development and characterization of self-emulsifying pellets by extrusion/spheronization

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This work is dedicated to my parents

my wife Marwa and my son Khaled

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Abbreviations

BCS	Biopharmaceutical classification system
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
BS	Bile salts
CMC	Carboxymethylethyl cellulose
CsA	Cyclosporin A
CW	Continuous wave
DLS	Dynamic light scattering
EPR	Electron paramagnetic resonance
ES	Extrusion/Spheronization
ESEM	Environmental scanning electron microscopy
ESR	Electron spin resonance
FaSSIF	Fasted state simulated intestinal fluid
FeSSIF	Fed state simulated intestinal fluid
GMS	Glyceryl monostearate
GRAS	Generally regarded as safe
GSE	Gas secondary electron
Hfs	Hyperfine splitting
HLB	Hydrophilic lipohilic balance
HPC	Hydroxypropyl cellulose
HPLC	High performance liquid chromatography
HPMC	Hydroxypropyl methyl cellulose
HPMCP	Hydroxypropyl methyl cellulose phthalate
LC	Liquid crystalline
LFCS	Lipid formulation classification system
Log P	Log octanol/water partition coefficient
MCC	Microcrystalline Cellulose
NMR	Nuclear magnetic resonance
PCS	Photon correlation spectroscopy
PEG	Polyethylene glycol
PG	Propylene glycol
PL	Phospholipids

PVP	Polyvinyl pyrrolidone
SE	Self-emulsifying
SEDDS	Self-emulsifying drug delivery systems
SEM	Scanning electron microscopy
SMEDDS	Self-microemulsifying drug delivery systems
TB	Tempolbenzoate
TL	Tempol

1 Introduction

1.1 Formulation approaches for poorly soluble drugs

Solubility is one of the important factors affecting oral bioavailability of a drug. Hörter and Dressman have defined a drug as 'poorly soluble' when its dissolution rate is so slow that dissolution takes longer than the transit time of the drug in the absorption site, resulting in incomplete bioavailability [1-3]. Up to 41% of newly discovered chemical entities fail in the drug development process due to their poor aqueous solubility despite having potential pharmacodynamic activity [4]. On the other hand, marketed poorly soluble drugs are administered in higher doses than actually needed to achieve the desired plasma level which consequently leads to toxicity problems. Dissolution is often the rate limiting step if the drug has reasonable membrane permeability [5-8]. Such characteristics are typical to compounds that belong to class II in the biopharmaceutical classification system (BCS) [9]. Formulation design can be a useful approach to improve the absorption and thus the oral bioavailability for such drug candidates. Drugs exhibiting poor solubility as well as low membrane permeability are categorized as BCS class IV drugs. Formulation approaches may improve the bioavailability of this class but they are likely to be compromised by their poor membrane permeability [6]. These drugs are not preferred for oral administration except in cases where they are potent enough to be effective at low plasma levels [10]. Improvement of the bioavailability of class IV drugs could be better achieved through returning to the lead optimization phases and select a chemical entity with more appropriate biopharmaceutical properties. Generally, for drugs with an aqueous solubility of less than 100 µg/ml bioavailability problems can be expected [2]. Several formulation approaches are presently employed to tackle the formulation challenges of poorly soluble drugs. The selection of a particular formulation approach depends on the optimum bioavailability that can be achieved.

According to Pouton, strategies for formulation of poorly soluble drugs include crystalline solid formulations, amorphous formulations and lipid formulations [6]. In the crystalline solid formulations, improvement of the dissolution rate is obtained through reducing the particle size and therefore increasing the surface area of the drug exposed to gastrointestinal (GI) fluids. Moreover, the crystalline drug will remain

in a physically stable state throughout the dissolution phase in the gut lumen. Strategies involved for such formulations include micronization [11-13], using different milling techniques that results in particle size range of 0.1-25 μm, and Nanosuspensions [14-22], produced by means of precipitation, pearl milling [e.g. NanoCrystals® (particle size range obtained is 100-250 nm)], high-pressure homogenization (e.g. DissoCubes®), and supercritical CO_2 technology [10]. Modification of the crystal habit of a drug could be also utilized to improve the dissolution and therefore the bioavailability as different polymorphs of a compound can exhibit different physicochemical as well as biological activity [23-25].

Amorphous formulations, such as solid dispersions, offer many advantages over other formulation techniques including dispersion of the drug in a carrier at a molecular level, absence of crystal structure of drug in the formulation, and improvement of wettability and/or solubilization effect [26-29]. Solid dispersions can be formulated by solvent-based methods, such as casting and spray drying, or melt extrusion which provide solvent-free process and therefore overcoming the problem of residual solvents [30]. The most commonly used carriers included in the formulation of solid dispersions are polyethylene glycol (PEG), polyvinyl pyrrolidone (PVP), hydroxy-propyl methyl cellulose (HPMC), hydroxypropyl cellulose (HPC), carboxymethylethyl-cellulose (CMC),hydroxypropyl methyl cellulose phthalate (HPMCP), polyacrylates and polymethyl acrylates, urea, sugars, polyols, emulsifiers, and organic acid derivatives [29]. Examples of drugs formulated as solid dispersions are griseofulvin in PVP and in succinic acid [31, 32], acetaminophen in a urea system [33], and tolazamide in PEG 8000 [34]. Complexation is also one of the approaches used to improve the solubility of poorly soluble drugs. Complexing agents like cyclodextrines and its derivatives and nicotinamide are used to enclose poorly soluble drugs within its hydrophobic core environment resulting in increased solubility [35-39].

The use of lipid based formulations has generated considerable interest as a potential formulation approach to improve the oral bioavailability of poorly soluble drugs [40-42]. Practically, lipid formulations can be described as a diverse group of formulations with a wide variety of properties result from blending of up to five classes of excipients ranging from triglyceride oils, through mixed glycerides, lipophilic surfactants, hydrophilic surfactants, and water soluble surfactants [43]. The appropriate formulation of a poorly soluble drug can decrease the intrinsic limitations

of slow and incomplete dissolution of such drugs by facilitating the formation of solubilized phases from which absorption takes place. The achievement of such phases will not essentially take place from the formulation itself, but alternatively from taking the advantage of the intraluminal processing to which lipids are subjected [44]. Porter *et al* have described in a recent review three possible mechanisms by which lipids and lipophilic excipients affect absorption, bioavailability and disposition of the co-administered drug. These are: (i) enhancing drug solubilization in the intestinal milieu by changing composition and character of the colloidal phases formed within the intestinal lumen such as vesicles and mixed micelles, (ii) by interacting with enterocyte-based transport process and therefore potentially changing drug uptake, efflux, disposition and formation of metabolites within enterocytes, (iii) by changing the pathway of drug transport to the systemic circulation from the normal portal vein pathway to the intestinal lymphatic system pathway which consequently reduce first pass drug metabolism as it travels directly to the systemic circulation without passing through the liver [45]. Lipid formulations include simple solutions, self-emulsifying drug delivery systems (SEDDS), and systems with very little oil that disperse to form micellar solutions [43]. Pouton has introduced the lipid formulation classification system (LFCS) in which he classified lipid-based formulations into four groups according to the polarity of the components used [6, 43]. Formulations with the drug dissolved in triglyceride oils [mainly vegetable oil or medium chain triglycerides classed as GRAS (generally regarded as safe) by regulatory agencies] and/or mixed glycerides are classified as type I. Since solvent capacity of triglycerides are low, oil solutions may be a realistic option for potent drugs or for compounds with log P>4. For less hydrophobic drugs solvent capacity could be improved by mixing triglycerides with other excipients such as mixed mono- and di-glycerides. The solvent capacity can be improved more by the inclusion of a lipophilic surfactant (HLB< 12) which also promotes efficient self-emulsification. This type of formulation is known as SEDDS and referred to as type II systems. Formulations which include water-soluble components, surfactant with HLB higher than 12, are referred to as type III systems in the LFCS and are called self-microemulsifying systems due to the optical clarity which can be obtained with these systems. Type III formulations could be further split into type IIIA and type IIIB with the later containing higher proportion of hydrophilic components. A recent modification has been introduced to the LFCS through the inclusion of type IV which represents the recent trend towards

formulations containing predominantly a mixture of hydrophilic surfactants and cosolvents (table 1.1). This type of formulations offer the advantages of better solvent capacity on dilution than cosolvent alone and reducing variability and irritancy caused by high local concentrations of surfactants as the cosolvent facilitate its dispersion in the GIT [6, 46].

Table 1.1 Lipid formulation classification system (LFCS) showing typical composition of various types of lipid formulations according to Pouton [6]

Excipients in the formulation	Content of formulation (%, w/w)				
	Type I	Type II	Type IIIA	Type IIIB	Type IV
Oils: triglycerides or mixed mono and diglycerides	100	40- 80	40- 80	<20	–
Water-insoluble surfactant (HLB<12)	–	20- 60	–	–	0- 20
Water-soluble surfactant (HLB>12)	–	–	20- 40	20- 50	30- 80
Hydrophilic cosolvents (e.g. PEG, Propylene glycol, transcutol)	–	–	0- 40	20- 50	0- 50

Characteristic features, advantages and disadvantages of various types of lipid formulation included in the LFCS are shown in table 2.1. The in vivo performance of lipid-based formulations and the fate of the co-administered drug in the GIT depend on the physical changes that take place upon the dilution of the formulation and the influence of digestion on drug solubilization. As stated before, the main advantage of lipid formulations is their ability to maintain the drug in a solubilized state throughout its residence time in the GIT. Care should be taken in the development of lipid based formulations to prevent loosing such advantage and prevent precipitation of the drug at any stage, which is more common to occur with systems with high concentration of hydrophilic excipients. Using LFCS combined with appropriate in vitro tests such as bile salt mixed micelle solubility testing, formulation dispersion/drug precipitation investigations and in vitro digestion tests could be used to predict the likelihood of drug precipitation and to optimize the choice of lipid formulation for a particular drug [6, 47-51].

Table 2.1 Characteristic features, advantages and disadvantages of the various types of lipid formulations according to Pouton [6]

LFCS	Characteristics	Advantages	Disadvantages
Type I	Non-dispersing; requires digestion	GRAS status; simple; excellent capsule compatibility	Formulation has poor solvent capacity unless drug is highly lipophilic
Type II	SEDDS without water-soluble components	Unlikely to lose solvent capacity on dispersion	Turbid o/w dispersion (particle size 0.25-2 μm)
Type IIIA	SEDDS/SMEDDS with water-soluble components	Clear or almost clear dispersion; drug absorption without digestion	Possible loss of solvent capacity on dispersion; less easily digested
Type IIIB	SMEDDS with water-soluble components and low oil content	Clear dispersion; drug absorption without digestion	Likely loss of solvent capacity on dispersion
Type IV	Oil-free formulation based on surfactants and cosolvents	Good solvent capacity for many drugs; disperses to micellar solution	Loss of solvent capacity on dilution: may not be digestible

1.2 Self-emulsifying drug delivery systems

1.2.1 General

Self-emulsifying drug delivery systems (SEDDS) are considered one of the promising approaches for tackling formulation problems associated with drugs with poor aqueous solubility. SEDDS is an oral lipid dosage form composed of a mixture of natural or synthetic oils, solid or liquid surfactants, or alternatively, one or more hydrophilic solvents and cosolvents/surfactants [52-55]. These formulations disperse freely when they come to contact with gastric fluids and form an oil-in-water emulsion or microemulsion utilizing mild agitation conditions provided by gastric motility. In such system, the lipophilic drug is presented in solution, in small droplets of oil, leading to the elimination of the dissolution step which can be the rate limiting step in the absorption of poorly soluble drugs and therefore improving the rate and extent of drug absorption and the reproducibility of the plasma profile [56]. Fine oil droplets are expected to be rapidly emptied from the stomach and promote a better distribution of the drug throughout the GIT. This can minimize irritation caused by extended contact between drug substance and the gut wall. Compared to conventional oily solutions, SEDDS provide a large interfacial area which enhances drug absorption through increasing the rate of diffusion from oil to the aqueous media of the GIT [53]. The extent of drug absorption from lipid vehicles is significantly affected by the dispersability of the administered lipid. Additionally, because of the inherent physical instability, the large volume of the two phase emulsion and the poor precision of the dose the use of conventional emulsions are problematic. The use of either microemulsions or SEDDS helps overcoming these restrictive problems. Other advantages offered by SEEDS include ease of formulation, enhanced solvent capacity and high stability [57].

1.2.2 Formulation of SEDDS

Self-emulsification has been shown to be specific to the nature of the oil/surfactant pair, the surfactant concentration, oil to surfactant ratio, and to temperature at which self-emulsification takes place [52, 58]. In light of these informations, it has also been demonstrated that only very specific combinations of

pharmaceutical excipients could result in efficient self-emulsifying systems [52, 59, 60]. A large variety of liquid or waxy excipients, ranging from oils through biological lipids, hydrophilic and hydrophobic surfactants, to water soluble cosolvents, are available for SEDDS formulation. The earliest reports of Self-emulsifying systems using pharmaceutical materials were of pastes, utilizing waxy alcohol ethoxylates [61]. The selection of appropriate excipients for SEDDS formulation should take in consideration their acceptability due to potential toxicity [62, 63]. As a general rule it is reasonable to use the simplest most effective mixtures, limiting the number of excipients used to a minimum [64]. Table 3.1 summarizes the most commonly used excipients used to formulate SEDDS. Generally, the following classes of excipients are currently employed in the formulation of SEDDS.

1.2.2.1 Oils

Oil is one of the most important component in the formulation of SEDDS because of its ability to solubilize marked amounts of lipophilic drugs, to facilitate self-emulsification as well as increasing the fraction of lipophilic drug transported via the intestinal lymphatic system and consequently increasing drug absorption from the GIT which depends on the molecular nature of the triglyceride oil [65-69]. Both long and medium chain triglycerides with different degrees of saturation have been used for the design of self-emulsifying formulations. Medium chain glycerides derived from coconut oil present a more attractive choice than other glycerides in formulating SEDDS since, a) they are more stable against oxidation, b) Self-microemulsifying drug delivery systems incorporating these glycerides can be formulated at ambient temperature over a wide range of compositions, and c) they are reported to improve the intestinal absorption of co-administered drugs [63, 70-76]. Edible oils could represent a logical choice for the development of SEDDS but their use could be limited due to the poor ability to dissolve large amounts of many lipophilic drugs. Therefore, edible oils are mixed with more polar lipids such as partial glycerides and PEGylated lipids to improve its solvent properties. Alternatively, modified or hydrolyzed vegetable oils have been used since they have better emulsification and drug solubilization properties [59, 60, 77]. Recently polyglycolyzed glycerides with varying fatty acid and polyethylene glycol (PEG) chain lengths have shown their ability to promote self-emulsification of oil in water and efficiently improve the oral absorption and hence the bioavailability of lipophilic drug [54, 78]. These products are derived from the reaction between selected, high purity food grade vegetable oils

and pharmaceutical grade PEG and therefore expected to be well tolerated by the body [77].

1.2.2.3 Surfactants

Surface active agents, or surfactants, are molecules characterized by having two regions in their chemical structure, one hydrophobic, and the other is hydrophilic. The hydrophobic region would consist of a hydrocarbon chain while the hydrophilic region can be either an ionisable, or neutral water soluble group [79]. Several compounds exhibiting surfactant properties may be used for the formulation of self-emulsifying mixtures. The most widely used surfactants in pharmaceutical applications are non-ionic or zwitterionic surfactants since they are less toxic and less affected by pH [63, 80] and ionic strength changes [77] but they may cause moderate reversible changes in intestinal wall permeability [63]. This group of surfactants are characterized by having a water soluble hydrophilic region which is not ionic and are based on molecules such as polyoxyethylene, and include alkyl-polyoxyethylene ethers, such as polyoxyethylene monohexadecyl ether [79]. Non-ionic surfactants have a hydrophilic-lipophilic balance (HLB) range of 1-20. HLB is an empirical formula used to select the suitable surfactant for emulsification of a given oil [81, 82]. Water-in-oil emulsions (w/o) are formed using emulsifiers with HLB range of 3 to 8 while oil-in-water (o/w) emulsions are formed with a range of 8-18. In most cases, it is the right blend of a low and high HLB surfactant that leads to the formation of a stable emulsion even in the absence of a co-surfactant [77, 80, 83].

Commonly used emulsifiers for formulating SEDDS are various solid, semisolid or liquid ethoxylated polyglycolyzed glycerides and polyoxyethylene 20 oleate [56]. Generally, emulsifiers of natural origin are favored as they are less toxic than synthetic ones, but they have a limited self-emulsification capacity [56, 60].

In order to achieve good self-emulsification properties surfactant concentration in a range of 30-60 % w/w should by employed but care should be taken since large concentration of surfactants may cause gastro-intestinal irritation. Moreover, at higher surfactant concentration, often higher than 65% depending on the surfactant used, the progress of emulsification is compromised by the formation of a viscous liquid crystalline gel which is formed at the oil-water interface [43]. Another factor that contributes to the selection of the right emulsifier and its concentration is the solubilization capacity of the formulation for the drug, as the drug should be kept in a

solubilized form for a long time at the absorption site and precipitation of the drug compound should be prevented [84]. Formulations with higher surfactant and hydrophilic cosolvent to oil ratios can be referred to as self-microemulsifying drug delivery systems (SMEDDS) as they disperse upon gentle agitation condition to form an optically clear microemulsion [43]. This approach was used for the formulation of cyclosporin A as Neoral ® [85-88].

It was shown in several research articles that there is a strong relation between surfactant concentration and the droplet diameter of the diluted self-emulsifying mixtures. This effect could be attributable to the localization of the surfactant molecules at the oil-water interface leading to the dispersion and solubilization of the oil droplets [89]. Conversely, the mean droplet diameter may also increase with increasing surfactant concentration [90]. This phenomenon could be caused by the interfacial disruption elicited by enhanced water penetration into the oil droplets mediated by the increased surfactant concentration and leading to ejection of oil droplets into the aqueous phase [56].

1.2.2.4 Co-solvents

The incorporation of a hydrophilic cosolvent in the formulation of SEDDS enables the dissolution of large quantities of the hydrophilic surfactant in the lipid base [56]. Additionally, the motivation for using water-soluble cosolvents may also be to increase the solvent capacity of the formulation for drugs with intermediate log *P* ($2<\log P<4$) [43]. Systems utilizing a water soluble cosolvent in addition to the hydrophilic surfactant (HLB>11) are classified as type III in the LFCS (see table 1.1). For these mixtures the water soluble components will tend to part from the oil during emulsification, and become dissolved in the aqueous phase. As a result of this phase separation, which may be in fact the driving force for emulsification by diffusion and stranding, is loss of solvent capacity with a possibility of partial precipitation of the drug [43].

Organic solvents such as ethanol, propylene glycol (PG), and polyethylene glycol (PEG), suitable for oral delivery, are examples of commonly used cosolvents. A limitation of the use of volatile cosolvents is the possible interaction with the shell of soft or hard gelatine capsules and evaporation from the matrix of the self-emulsifying mixture which could result in precipitation of the drug. Therefore, alcohol free

formulations have been designed but their lipophilic drug dissolution ability may be limited [56].

Table 3.1 Commonly used excipients to formulate SEDDS

Excipient	HLB	Chemical definition
Arlacel® 80	4.3	Sorbitan oleate
Arlacel® 186	2.8	Monoolein: Propylene glycol (90:10)
Capmul® MCM	5.5–6	C_8/C_{10} mono-/diglycerides from coconut oil
Captex® 200	oil	C_8/C_{10} diesters of propylene glycol from coconut oil
Captex® 355	oil	C_8/C_{10} triglycerides from coconut oil
Centrophase® 31	4	Liquid lecithin
Cremophor® EL	13.5	Polyoxyethylene glycerol triricinoleate 35 DAC
Labrafac® CM 10	10	C_8/C_{10} polyglycolyzed glycerides from coconut oil
Labrafil® M 1944 CSD	3–4	Primarily oleic acid ($C_{18:1}$) polyglycolyzed glycerides from apricot kernel oil
Labrafil® M 2125 CS	3–4	Primarily linoleic acid ($C_{18:2}$) polyglycolyzed glycerides from corn oil
Labrasol®	14	C_8/C_{10} polyglycolyzed glycerides from coconut oil
Miglyol® 812	oil	C_8/C_{10} triglycerides from coconut oil
Myvacet®	oil	Distilled acetylated monoglycerides
Myverol® 18-92	3.7	Distilled sunflower oil monoglycerides (90 % glyceryl linoleate)
Soybean oil	oil	Primarily oleic (25%) and linoleic (54%) triglycerides
Tagat® TO	11.3	Polyoxyethylene (25) glycerol trioleate
Tween® 80	15	Polyoxyethylene (20) sorbitan oleate
Solutol® HS15	14-16	Polyoxyethylene esters of 12-hydroxystearic acid

1.2.2.5 Other excipients

The addition of a polymeric precipitation inhibitor may stabilize a temporarily supersaturated state of the drug after dispersion of SEDDS in the GIT. Such an approach has led to the development of supersaturable SEDDS (S-SEDDS) which incorporate Hydroxypropyl methyl cellulose (HPMC) as a precipitation inhibitor. The addition of HPMC permits also to lower surfactant concentration in an attempt to reduce surfactant side-effects [91, 92]. The S-SEDDS formulation, with a HPMC load of 5%, led to a marked improvement in paclitaxel bioavailability. Furthermore, the incorporation of CsA as an inhibitor of P-gp and CYP 3A4 enzyme into the S-SEDDS further enhances the systemic exposure of paclitaxel [92].

The charge of the oil droplet in conventional SEDDS is negative due to the existence of free fatty acids [56]. The incorporation of a cationic lipid such as oleylamine at a concentration range of 1.0-3%, will result in SEDDS with a positive ζ-potential with a value of about 34-45 mV [93-95]. Positively charged emulsion droplets interact with the negative charge surface component of the gastro intestinal lumen [94]. It was found in a bioavailability study performed on perfused rats that administration of CsA in a positively charged SEDDS led to higher plasma levels compared to the corresponding negatively charged formulation [66]. Additionally, comparative oral bioavailability studies in young female rats using several different liquid dosage forms of progesterone indicated that only the positively charged SEDDS could be considered a potential effective dosage form for oral administration of progesterone since it elicited the highest and most satisfactory absorption profile [93].

Excipients such as antioxidants may be necessarily incorporated in SEEDS formulation when the oil phase is susceptible to oxidation. Antioxidants as tocopherol, ascorbyl palmitate, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate may be used [96].

1.2.3 Mechanism of self-emulsification

The mechanism by which self-emulsification take place is not yet well understood. It has been suggested that the process of self-emulsification occurs when the entropy change that favours dispersion is greater than the energy required to increase the surface area of the dispersion [97]. Moreover, the free energy of a conventional emulsion formation is a direct function of the energy required to create a new surface between oil and water phases and can be described by equation 1 [97].

$$\Delta G = \sum N \ 2\pi r^2 \sigma \qquad (1)$$

Where Δ*G* is the free energy associated with the process of emulsification, *N* is the number of droplets of radius *r*, and σ represents the interfacial energy. The two phases of the emulsion tend to separate by time in order to reduce the interfacial tension and consequently the free energy of the system. Therefore, surfactants are used in oil-water systems to decrease the interfacial energy between the two liquids and thus promote the ease of dispersion. Surfactants were found to form a monolayer around the emulsion droplet and hence reducing the interfacial energy as well as providing a barrier to coalescence. Further strengthening of the interface could be achieved by improving the viscous or elastic properties, or by providing steric or electrostatic stabilization [98].

In the case of self-emulsifying mixtures, spontaneous emulsification takes place because the free energy required to form the emulsion is either very low and positive or negative [77]. In order for emulsification to occur, it is necessary for the interfacial structure to have no resistance against surface shearing [99].

It has been suggested that the ease of emulsification is associated with the ease of water penetration into various liquid crystalline (LC) or gel phases formed on the surface of the oil droplet [100-102]. The interface between the oil and the aqueous continuous phase is formed upon addition of a binary mixture, usually composed of oil and a non-ionic surfactant, to water. This is followed by the solubilization of water within the oil phase, subsequent to gentle agitation of SEDDS, as a result of aqueous penetration through the interface which will continue until the solubilization limit, close to the interface, is reached. Further penetration will result in the formation of a dispersed LC phase. The actual amount of the formed LC phase depends on the surfactant concentration in the binary mixture. As a consequence for the LC phase

formation around the oil droplets, SEDDS become more stable to coalescence. Wakerly et al. have extensively studied the involvement of LC phases in the spontaneous emulsification process of SEDDS [52, 90]. Additionally, Craig et al. used the combination of particle size analysis and low frequency dielectric spectroscopy to evaluate self-emulsification properties of a series of SEDDS comprising Imwitor 742 and Tween 80 [55, 103]. This study showed evidence of the formation of liquid crystalline phases at concentrations corresponding to those shown to be efficient self-emulsifying systems and it was therefore suggested that self-emulsification may be associated with liquid crystal formation, although the relation was clearly complex. Moreover, it was also shown that the incorporation of a drug may change emulsion characteristics, possibly by interacting with the LC phase.

1.2.4 Improvement of drug absorption by SEDDS

Drug release from SEDDS occurs upon partitioning into the aqueous environment within the intestinal lumen after dispersion and emulsification. Many reports have outlined the improvement of bioavailability associated with lipophilic drug administration as an o/w emulsion [104-111]. On the other hand, because of the inherent physical instability the large volume of the two phase emulsion, and the poor precision of the dose, the use of conventional emulsions is problematic. Hence, SEDDS may be a promising approach to avoid such restrictive problems associated with the use of conventional emulsions.

Cyclosporin A (CsA) is a famous example of poorly soluble drugs which was successfully formulated and marketed as a SMEDDS [88]. The first marketed product for cyclosporin was Sandimmune® formulated in an olive oil based solution that also contains ethanol and Labrafil® M 1944 CS (see table 3.1) and a second soft gelatine formulation that contains corn oil, gelatine, dehydrated ethanol and Labrafil® M 2125 Cs (see table 3.1). However, the rate of the drug absorption from these formulations varies widely with oral bioavailability being in the range of 7-90 % and t_{max} between 1.5 to 22 hours [112]. Therefore, there has been a need for the development of more effective formulation for cyclosporin with more consistent absorption characteristics. A new formulation of cyclosporin has been introduced into the market with the name Sandimmune Neoral® [85]. Due to differences in composition in the oil and the surfactant between Sandimmune® and the new Sandimmune Neoral®, the later

formulation disperses to form an o/w microemulsion where the former formulation disperses into a crude emulsion [77]. Clinical, comparative bioavailability, and pharmacokinetic studies have shown that the latter formulation of cyclosporin offers substantial advantages over the older one with respect to the consistency and extent of absorption with a consequent improvement in the oral bioavailability which was shown to be less affected by the presence of food [85]. Moreover, it was also found that a large amount of dissolved CsA from Sandimmune was in the undigested lipid phase while a mixed micellar phase was formed as a result of the in vitro digestion of the oily solution following the administration of Sandimmune Neoral [113].

Surfactants exhibit a major role in absorption enhancement associated with SEDDS. They partition into the cell membrane and disrupt the structural organization of the lipid bilayer leading to permeation enhancement [63]. Additionally, it has been shown that due to micellar solubilization of lipophilic drugs in surfactants the amount of free drug and the extent of absorption was largely affected [114, 115]. On the other hand, in vitro permeability studies demonstrated a significant decrease in permeability of CsA in Caco-2 cells associated with the presence of surfactants such as Cremophor EL (table 3.1) at concentrations above 0.02% w/v via micellar solubilization which outweighed the increase in permeability mediated by P-glycoprotein (P-gp) efflux inhibition [115].

Lipid content in the formulation exerts a major impact on the oral bioavailability of poorly soluble drugs. Many mechanisms have been suggested to explain such effect including increased dissolution rate and solubility of the drug in the intestinal fluids, protection of the drug molecule inside oil droplets from chemical as well as enzymatic degradation, and the formation of lipoproteins promoting the lymphatic transport of highly lipophilic drugs [67, 110, 116-118]. After absorption into the enterocytes, lipid digestion products either enter the portal vein through direct diffusion across the cell ,leading to access to the systemic circulation through the liver, or are trafficked intracellularly to the endoplasmic reticulum (ER) system where they are re-esterified to triglycerides and then incorporated into chylomicrons (the main lipoprotein secreted by the enterocytes, [119]). Chylomicrons fuse with basolateral cell membrane of the enterocytes before release into the interstitial space and as a result of the impermeability of the vascular endothelium to large colloidal particles and the large inter-endothelial gaps present in the lymphatic endothelium, preferential direction of chylomicrons and other lipoproteins toward selective uptake by the

intestinal lymphatic system, rather than the blood capillaries, takes place [45]. Generally, short and medium chain fatty acids are transported to the systemic circulation through the portal blood and are not incorporated to a great extent in chylomicrons [120]. Conversely, long chain fatty acids and monoglycerides are re-esterified to triglycerides in the intestinal wall, incorporated into chylomicrons and secreted into the lymph vessels by exocytosis [121]. Therefore, improvement of the oral bioavailability of highly lipophilic drugs could be achieved through the manipulation of the absorption profile of the co-administered drug in a SEDDS formulation by changing the medium and the long chain triglycerides content in the formulation.

Generally, possible mechanisms for improving drug absorption from SEDDS include:

1- Increase in membrane fluidity facilitating transcellular absorption.

2- Larger surface area provided by the fine emulsion droplets and subsequent lipolysis and enhancement of drug solubilization through changing composition and character of the colloidal environment formed (for example, vesicles and mixed micelles).

3- Opening of the tight cellular junction to allow paracellular transport which is mainly relevant for ionized drugs or hydrophilic macro-molecules.

4- Inhibition of P-gp drug efflux (Increased intestinal expression of P-glycoprotein can reduce the absorption of drugs that are substrates for P-glycoprotein. Thus, there is a reduced bioavailability, therapeutic plasma concentrations are not attained) with subsequent enhancement of drug uptake from GIT [118, 122-124].

5- Stimulation of lipoproteins (mainly chylomicrons) formation which promotes the lymphatic transport of highly lipophilic drugs and offers many potential advantages including avoidance of first pass effect, specific targeting of drugs to the lymphatic system, and providing a controlled rate for entrance of drugs to the systemic circulation [96].

1.3 Lipid digestion and its effect on drug solubilization

Oral administration of lipids stimulates the secretion of gastric lipase from the chief cells lining the gastric mucosa [125-127], with subsequent secretion of pancreatic lipase and co-lipase from the pancreas [128]. Lipid digestion is initiated in the stomach by the action of gastric lipase on triglycerides (many other non-ionic esters, such as mixed glycerides and surfactants present also possible substrates for lipase) which leads to partial digestion into di-glycerides and fatty acids [129-131]. Although gastric lipolysis is known to be a minor contributor to the overall lipolysis process, some studies have suggested that it is responsible for about 25% of acyl chain hydrolysis [132, 133]. Lipids are crudely emulsified in the stomach and subsequently transferred to the small intestine where pancreatic lipase-colipase exerts its action on the interface (this enzyme attains its full catalytic activity in the presence of o/w emulsions because it is activated by the interfacial binding [134]) and catalyses the hydrolysis of tri- and di-glycerides in the lumen of the small intestine [135, 136]. This results in the formation of 2-monoglycerides and fatty acids as the major products of lipid lipolysis. Although 2-monoglycerides may undergo slow and non-enzymatic isomerization at alkaline pHs to yield 1-monoglycerides, which are then available for hydrolysis by pancreatic lipase, this process is generally believed to be limited in vivo [137]. The presence of lipids and lipolysis products stimulates the secretion of bile (composed mainly of water, bile salts, cholesterol, phospholipids, bicarbonate and bile pigments [138]) into the small intestine from the gall bladder. The components of bile constitute a vehicle for solubilization of poorly soluble fatty acids, monoglycerides and di-glycerides products of the lipid lipolysis process. These substances are incorporated into various colloidal phases formed in the intestinal lumen such as mixed micelles and vesicles (Figure 1.1).

The colloidal structures formed during in vivo lipid digestion provide a good vehicle within which lipophilic drugs may reside during gastro intestinal transit and therefore preventing precipitation and enhancing drug absorption [139]. The intestinal phase behavior of formulation-derived lipids and their digestion products are expected to play a significant role in the solubilization of administered drugs [140, 141]. Greater understanding of the interaction between the administered drug and the digestible oily vehicles during pre-absorptive intraluminal processing may lead to a more guided selection of the ideal lipids for incorporation into a lipid-based formulation [142].

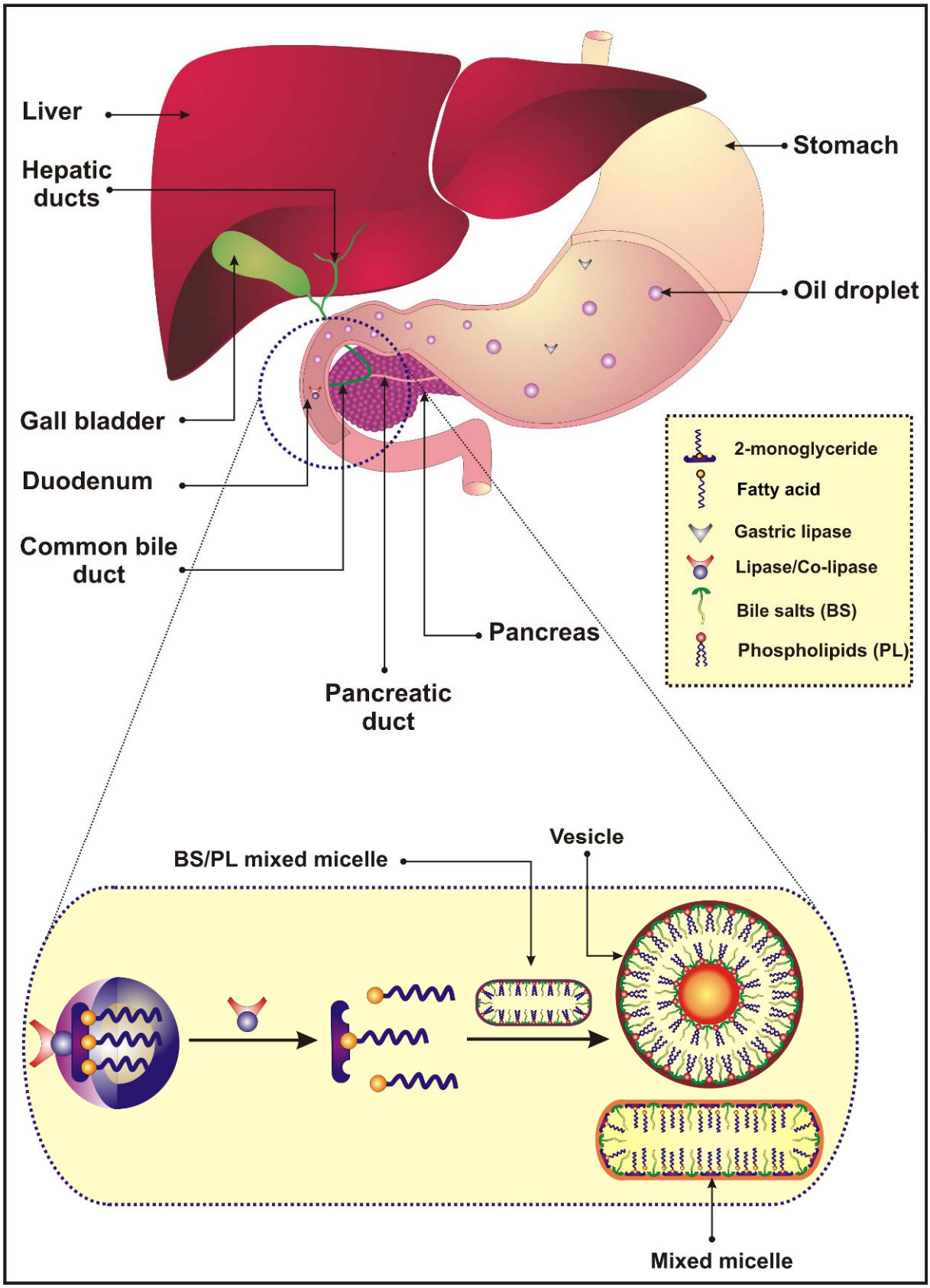

Figure 1.1 Schematic presentation of the lipid digestion process in the GIT.

1.4 Pellets

Pellets are spherical, free-flowing multi-particulate drug delivery system which range in size between 0.5-1.5 mm depending on the processing technologies employed [143]. Applications of pellets are not only found in pharmaceutical industry but also in agriculture and in polymer industry. Pellets are of great interest to the pharmaceutical industry as they have many therapeutic as well as technological advantages over conventional solid dosage forms. Advantages of using pellets include:

1) Flexibility in designing and developing the dosage form, as they could be filled into hard gelatine capsules, compressed into tablets and/or coated with varying materials and polymers to achieve certain release profile. Moreover, pellets composed of different drug entities and chemically incompatible bioactive agents can be formulated in a single dosage form [143, 144].
2) Pellets disperse freely in the gastro-intestinal tract and therefore drug absorption is maximized with a subsequent reduction in peak plasma fluctuations and minimization of potential side effects without lowering drug bioavailability [145].
3) Reduction in the variation in gastric emptying rates and overall transit time and consequently reducing intra- and inter-subject variability of plasma profile associated usually with single unit dosage forms and leads to deleterious effects on the reproducibility of the therapeutic effect of drugs [146, 147].
4) Decreasing the problem of high local concentration of drugs and thus avoiding irritation that may be caused by certain active constituents.
5) Pellets are less susceptible to dose dumping, when formulated as modified release dosage forms, than the reservoir type single unit formulations [143].
6) Good flow properties, narrow particle size distribution, less friable dosage form and uniform packaging are also among the advantages of using pellets [148, 149].
7) Low surface area to volume ratio and therefore pellets provide an ideal shape for the application of film coatings [143].
8) Pellets can also be made attractive through various shades and colors that can be easily imparted to them during the manufacturing process [143].

1.4.1 Extrusion/Spheronization

The most commonly used techniques for pellets production in the pharmaceutical industry are extrusion/spheronization (ES), solution/suspension layering, and powder layering [143]. Extrusion is the process of applying a pressure to a semisolid mass and forcing it against an orifice or a die to form an extrudate of defined geometry [150]. Extruders used in pharmaceutical industry are classified into: screw, sieve and basket, roll, and ram extruders. Screw extruders are the only continuous extrusion equipment as it can afford a continuous and smooth exit of the extruded mass through the uniform openings (Figure 2.1) [151].

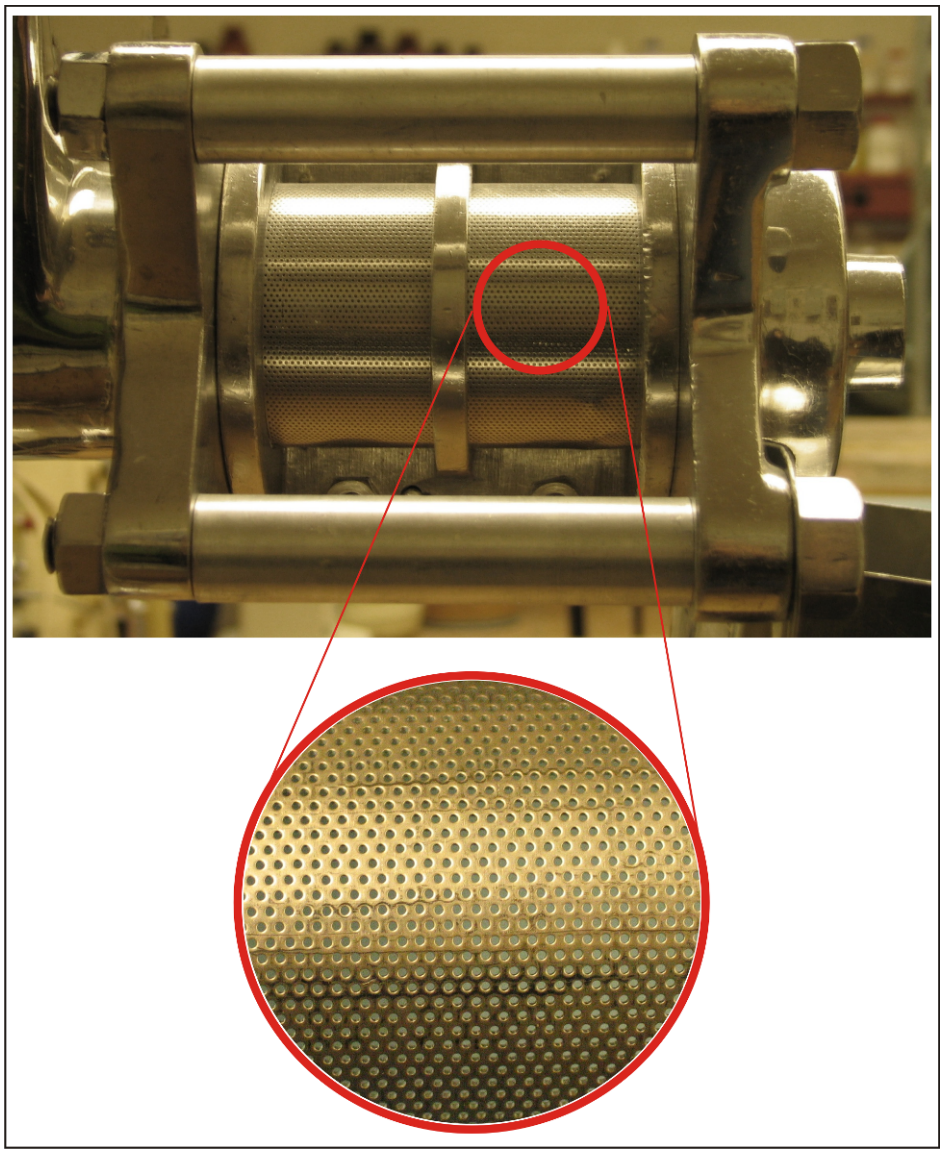

Figure 2.1 Photograph of the twin screw radial discharge extruder.

Spheronization describes the process of gradually transforming cylindrically shaped extrudates into spherically shaped pellets by contact with a rotating disk (frictional plate), by collision between particles, and by collision with the wall [152]. A spheronizer is a device consisting of a vertical hollow cylinder equipped with a horizontal rotating frictional plate (Figure 3.1). The process of spheronization was originally invented in 1966 by Nakahara, who described a method and apparatus for making spherical granules that involves extrusion of a wetted mass to provide cylindrical segments (extrudates) followed by spheronization of the extrudate in the Marumerizer [153]. The process was widely overlooked in the pharmaceutical industry until the early 1970s when several research articles have described the steps and equipments involved in the process and its direct application in pharmaceutical industry [149, 154-157]. Since then, the process of ES has become the method of choice in the preparation of pellet-based dosage forms and become a subject of intensive research.

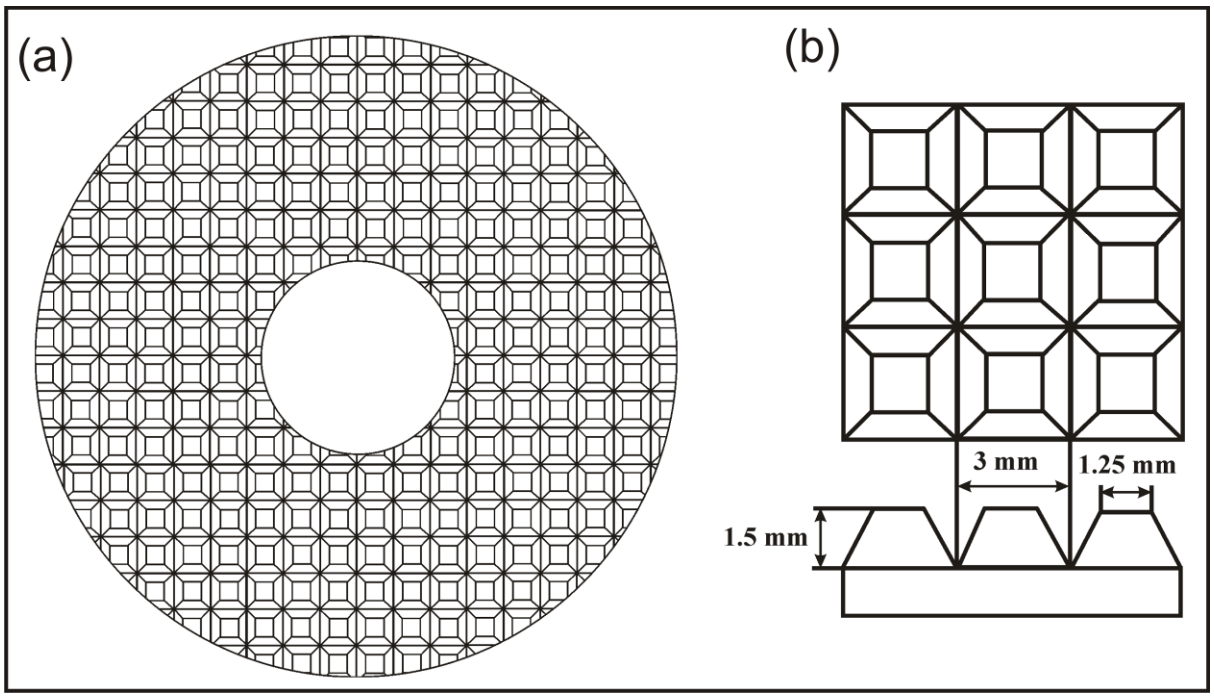

Figure 3.1 Schematic presentation of the frictional plate used in the radial plate spheronizer showing (a) typical grid pattern (the cross hatched design), and (b) details of grid pattern and a cut away view of the plate with the dimensions of plate design.

Advantages of using ES include:

- Ease of operation.
- High throughput with low wastage.
- Narrow particle-size distribution.
- Production of pellets with low friability.
- Production of pellets that are suited for film coating.
- Ease of coating.
- Good flow.
- Low dusting.

The process of ES is a multi-step process that involves the following steps:

1) Granulation through mixing of the dry ingredients with the granulation liquid.
2) Extrusion of the wet mass into cylindrical extrudates.
3) Spheronization of the extrudates into spherical pellets.
4) Drying of the produced pellets.

According to the shape of particles, different stages can be distinguished in the spheonization process. The edges of the extrudates cylinders are transformed into a more rounded form then to dumb-bells and elliptical particles and eventually to perfect spheres [152]. Baret and Remon suggested the possible coexistence of another pellets forming mechanism [158]. In this mechanism the rounded edge cylinder is subjected to twisting until it breaks into two distinct parts with both parts having a round and a flat side. Due to the rotational and the frictional forces the edges of the flat side collapse together like a flower forming the cavity observed in certain pellets. Figure 4.1 illustrates both pellet-forming mechanisms. Different process variables and parameters that can affect quality of extrudates and pellets and consequently the release properties of drugs from pellets have been discussed intensively in several research articles [159-163].

The most commonly used excipients in pellet formulations include binders, fillers, lubricants, disintegrants, separating agents, spheronization enhancers, glidants and release modifiers [164]. Microcrystalline Cellulose (MCC) is a spheronization enhancer that has been used extensively for pellets production since it provides a balance between rigidity, as it has binding properties, and plasticity which is necessary for a successful production of spherical pellets by ES technique.

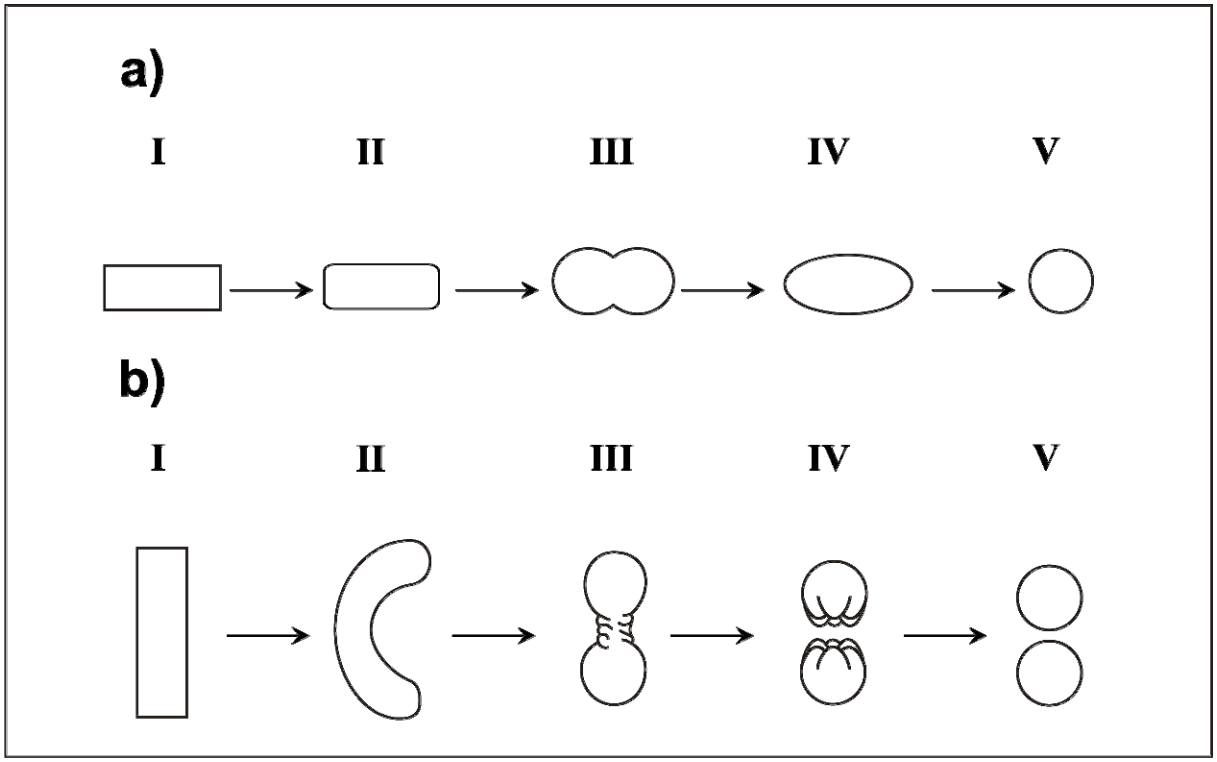

Figure 4.1 Pellets formation mechanism according to a) Rowe [152]- I. Cylinder, II. Cylinder with rounded edges, III. Dumb-bell; IV, Ellipse, V Sphere. (b) Baert et al [158]- I. Cylinder, II. Rope, III. Dumb-bell, IV. Sphere with a cavity outside, V. Sphere. (from reference [165]).

MCC is manufactured by mineral chemical acid hydrolysis of alpha cellulose (which acts on the amorphous regions of cellulose increasing the percent of crystalline bundles) and subsequent spray drying [166]. The first model to elucidate the possible role of MCC in ES process was introduced by Fielden *et al* [167]. The model described MCC as a sponge with a high ability to hold water. During extrusion, the sponges are compressed until water is squeezed out and lubricate the particles flowing through the extruder. After extrusion, the volume of the sponges will increase and the extrudate will be apparently dry and brittle, allowing it to be chopped into short lengths during spheronization. The crystallite-gel-model was introduced by Kleinebudde as an alternative model to elucidate the unique role of MCC in the process of ES [168]. This model proposed that MCC are broken to smaller particles and possibly to single crystallites during granulation and extrusion process in the presence of a liquid. The small particles produced are capable of forming a crystallite-gel and immobilize the liquid.

1.5 Electron spin resonance (ESR) spectroscopy

Electron spin resonance (ESR) spectroscopy (also known as electron paramagnetic resonance (EPR) spectroscopy) is a magnetic resonance method principally related to the more popular nuclear magnetic resonance (NMR) spectroscopy. ESR is used to detect and characterize paramagnetic materials such as free radicals and transition metals such as copper and manganese. Since the majority of drug delivery samples are diamagnetic and ESR silent, the incorporation of a paramagnetic molecule or group as spin probes is therefore necessary. The most widely used spin probes are stable nitroxide radicals. A very broad variety of low and high molecular weight nitroxides with different physicochemical properties are available. Alternatively, covalent coupling of paramagnetic species to the molecules of interest, a technique called spin labeling, has expanded the application of ESR spectroscopy [169]. Furthermore, Gamma irradiation of crystalline drugs or crystalline polymers might lead to the formation of stable radicals which can be used as endogenous paramagnetic species [170].

ESR is considered a powerful spectroscopic technique for in vitro and in vivo non-invasive monitoring of drug delivery systems [171-174]. The following section will explain the basics and applications of this technique.

1.5.1 Basics

1.5.1.1 General

Generally, atoms and molecules gain or lose energy when transferring from one energy level to the other. Spectroscopy deals with the measurement and interpretation of those transitions and therefore it can provide valuable information about the structure and dynamics of the investigated samples.

In all spectroscopic methods, electromagnetic waves are used as the energy source necessary to induce the transition from the ground to the excited level. According to Plank's law, the energy necessary for the transition from the lower to the higher energy level (ΔE) is proportional to the frequency of the electromagnetic waves.

$$\Delta E = h\nu \qquad (2)$$

Where h is Plank's constant and ν is the frequency.

ESR is a form of absorption spectroscopy that utilizes microwave radiation applied at a well defined and predictable frequency, in the GHz region, to induce the transition of electron spins from the ground to the excited level where a resonance condition is reached.

1.5.1.2 Zeeman effect and resonance condition

A single electron, being a charged particle that rotates around its own axis, creates a magnetic field called the spin magnetic moment which leads it to behave like a tiny magnet with a north and south pole. In the absence of external magnetic field, electron spins are oriented randomly and their energies do not actually differ from each other. When the electron spins is placed in an external magnetic field, and since the electron spin is quantized, i.e. it can take only one of two possible values, the spin vector of the electron aligns itself either with (and thus being in the low energy state) or against (and thus being in the high energy state) the direction of the external field. This phenomenon is known as Zeeman effect (Figure 6.1).

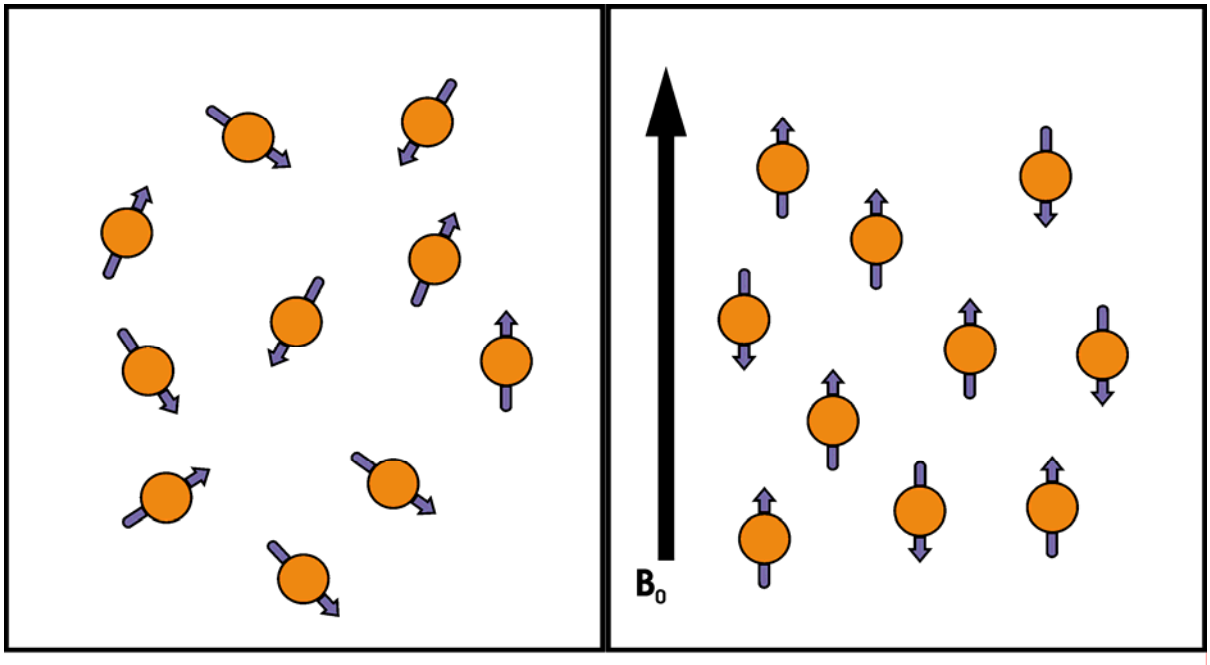

Figure 6.1 A schematic diagram illustrating the Zeeman effect. Left, random orientation of the electron spins. Right, alignment of the electron spins with or against the external magnetic field B_0 (modified from Ref. [169]).

Transition between the two energy states is induced by the absorption of a photon. An Electron in the lower energy state absorbs a photon and ends up in the upper energy state. The energy of this photon ($h\nu$) must exactly match the energy difference between the two states ($\Delta E = h\nu$). This is the so called resonance condition where 180° flipping of the electron spin takes place.

1.5.1.3 Mathematical description

The energy of a magnetic dipole in an external magnetic field is given by:

$$E = \mu B_0 \tag{4}$$

Where μ is the magnetic dipole and B_0 is the strength of the external magnetic field. The magnetic dipole of an electron is generated by its spin S,

$$\mu = g\beta S \tag{5}$$

Where β is the Bohr magneton (the intrinsic value of an electron's magnetic moment), g is the spectroscopic splitting factor, or simply the g-factor, denoting the contribution of spin and orbital motion of the electron to its total magnetic momentum. For nitroxides, the orbital momentum plays a minor role and g-factor value is quite close to that of a free electron [169].

The electron spin S is quantized with a value of either +1/2 or -1/2 (Figure 7.1). Thus after substituting the value of μ in equation 4 with that in equation 5, the energy levels are equal to $\boldsymbol{E = +1/2g\,\beta B_0}$ and $\boldsymbol{-1/2g\,\beta B_0}$ and the difference

$$\Delta E = g\beta B_0 \tag{7}$$

And therefore

$$h\nu = g\beta B_0 \tag{8}$$

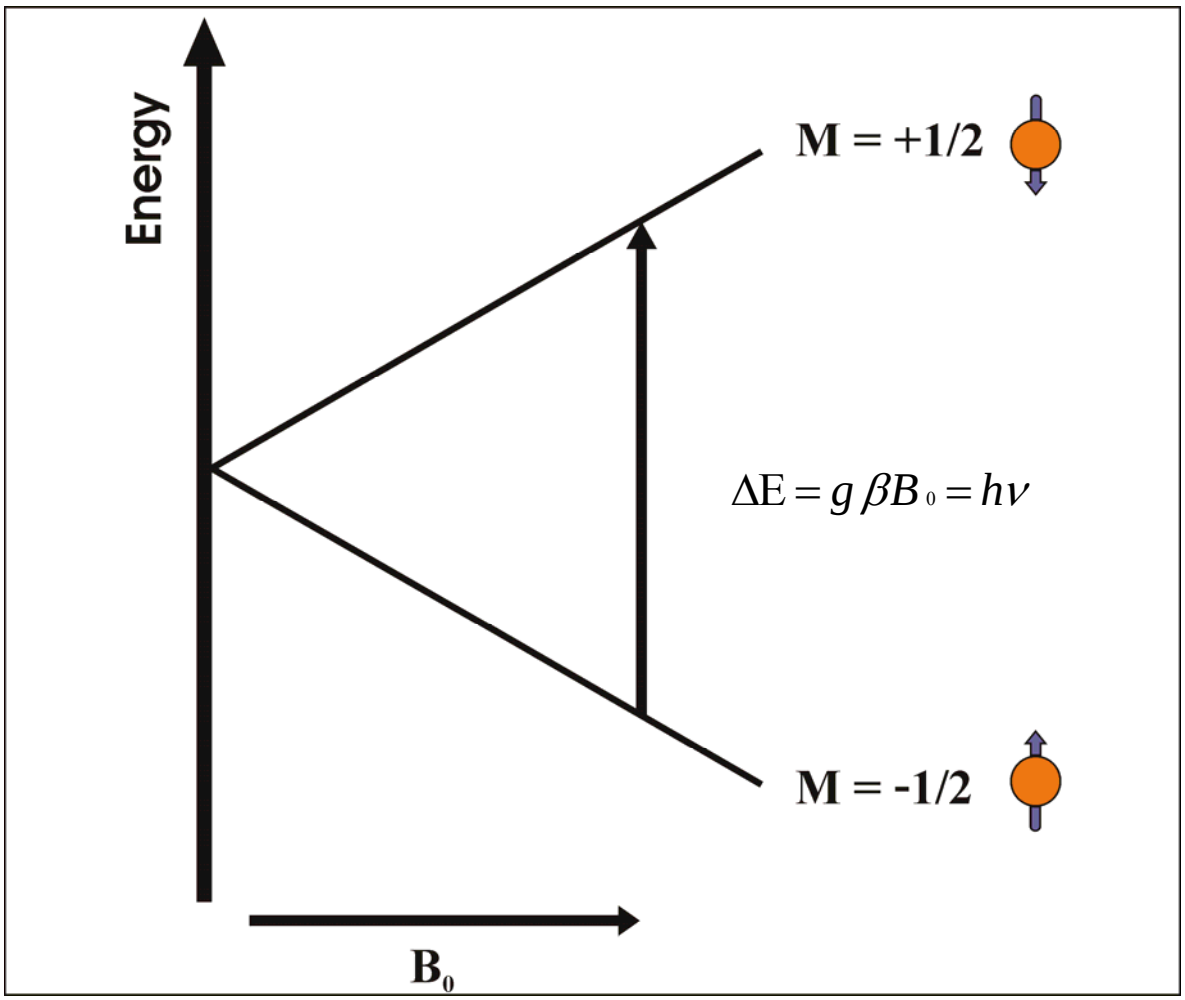

Figure 7.1 Energy level diagram showing the two possible states of an electron (S = ½) in an applied magnetic field, The lower energy level corresponds to the electron spin being parallel to the direction of the magnetic field, while its orientation is reversed in the upper energy level, as indicated by the arrows on the right side (modified from Ref. [169]).

1.5.1.4 Hyperfine Interaction

If the unpaired electron is found alone in space, a single absorption (resonance) peak would have been observed. However, due to the presence of atomic nuclei, with magnetic moment, in the same molecule multiple resonances occur in the vast majority of free radicals. If the magnetic field of the nucleus is in the direction of the external magnetic field, the unpaired electron will experience a higher local magnetic field, and vice versa. The nuclear spin is also quantized as it can attain specific orientation or states and according to the number of those possible orientations, the unpaired electron experiences the same number of different local magnetic fields. In fact, the total number (multiplicity) of possible nuclear spin states is 2I+1 possibilities

or states, where I is the nuclear spin quantum number [175]. Therefore, instead of having a single absorption peak or line, 2I+1 lines are obtained.

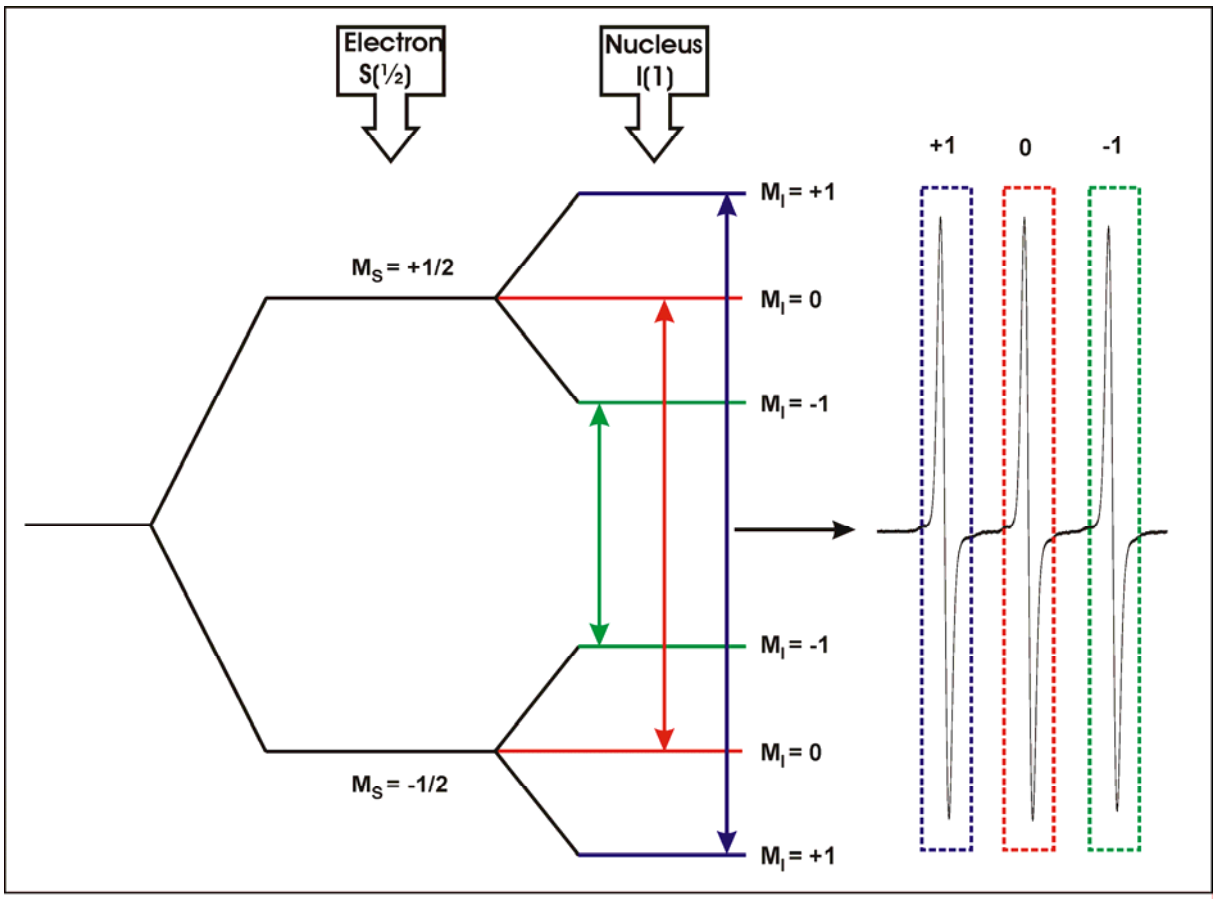

Figure 8.1 Energy levels and transitions for a nitroxide spin probe (^{14}N) with electron spin (S) = ½ and nuclear spin (I) = 1 (adapted from Ref. [176]).

For the nitrogen atom of the nitroxide radicals, ^{14}N, the nuclear spin quantum number is 1 and therefore microwave energy can be absorbed at three different levels instead of one leading to an ESR spectrum with 3 characteristic lines (Figure 8.1). For the less abundant isotope, ^{15}N, the nuclear spin quantum number is ½, and so only 2 lines are seen. The interaction of the unpaired electron spins with the magnetic nuclei is known as “hyperfine interaction” and it give rise to “hyperfine splitting”.

1.5.1.5 Relaxation

Electrons in the excited state will tend to return back to the ground state, i.e. to relax. During relaxation process energy is released either to the environment, a process called spin-lattice relaxation T_1, or by interacting with other paramagnetic species, spin-spin relaxation T_2. The resonance peak line width is inversely proportional to the relaxation rate.

1.5.2 Instrumentation

Two main categories of ESR spectrometers are available. The first and most commonly used is the continuous wave ESR (CW-ESR), and the second is pulsed or time domain ESR spectrometers. In pulsed ESR experiment a short burst of microwave radiation is applied to the sample, after which the emitted radiation is detected forming the ESR signal. However, pulsed ESR is rather difficult to implement, especially with biological or living samples, and instead CW-ESR is most widely used. In CW-ESR a constant microwave radiation with a fixed frequency is continuously used as the energy source, and the magnetic field is swept in order to achieve resonance [177]. As an alternative technique to achieve resonance, microwave radiation is swept at a constant magnetic field, but as this is technically complicated the first technique is more implemented.

The sample is placed in the resonator cavity which is tuned to a chosen frequency (Figure 9.1). Microwave radiation is applied from the microwave bridge into the resonator. In the meantime, the magnetic field is swept continuously and magnetic field modulation takes place to improve the sensitivity, where the amplitude of the external field oscillates at 100 KHz as the field is swept. During magnetic field sweep the unpaired electron spins in the sample will reach resonance condition. Under this resonance condition the sample absorbs a quantity of energy which is detected as a loss of energy by a phase-sensitive modulated diode and represents the ESR signal from the sample. The spectrometer is adjusted to detect only signals where the change of the amplitude of the microwave absorption signal has the same frequency and is in phase with the applied change of the magnetic field. That's why the output of the ESR spectrometer is presented as a first derivative, i.e. the changes of the absorption signal relative to the change of the magnetic field.

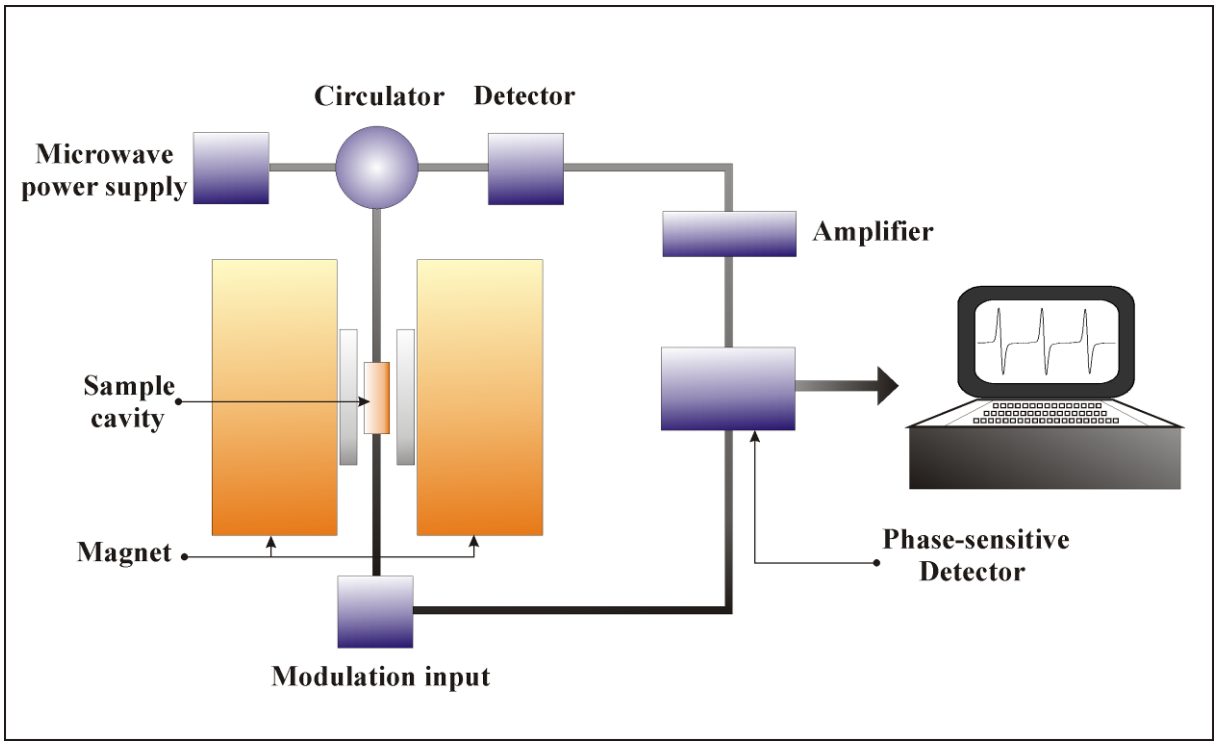

Figure 9.1 Schematic presentation of the main components of an ESR spectrometer.

CW-ESR spectrometers are classified according to the microwave frequency used into: Q-band (ν = 35 GHz), X-band (ν = 9.5 GHz) and L-band (ν = 1.2 GHz). The sensitivity increases with the increase in frequency but the increase in frequency is limited by a) the fact that as the frequency increases, the size of the resonant cavity decreases (this is necessary to create the standing waves), and b) water, a strong dielectric liquid, absorbs microwave radiation, and the absorption increase with the increase of frequency from X-band to Q-band. Accordingly, for biological studies only X-band and L-band are of interest, c) the penetration depth for in vivo studies decreases with the increase of frequency.

1.5.3 Decoding ESR spectra

Nitroxide spin probes and labels are very sensitive to their environment, and their spectra can be quite informative. The most important parameters in an ESR spectrum are the line width, hyperfine splitting (Hfs) constant and peak amplitude (Figure 10.1).

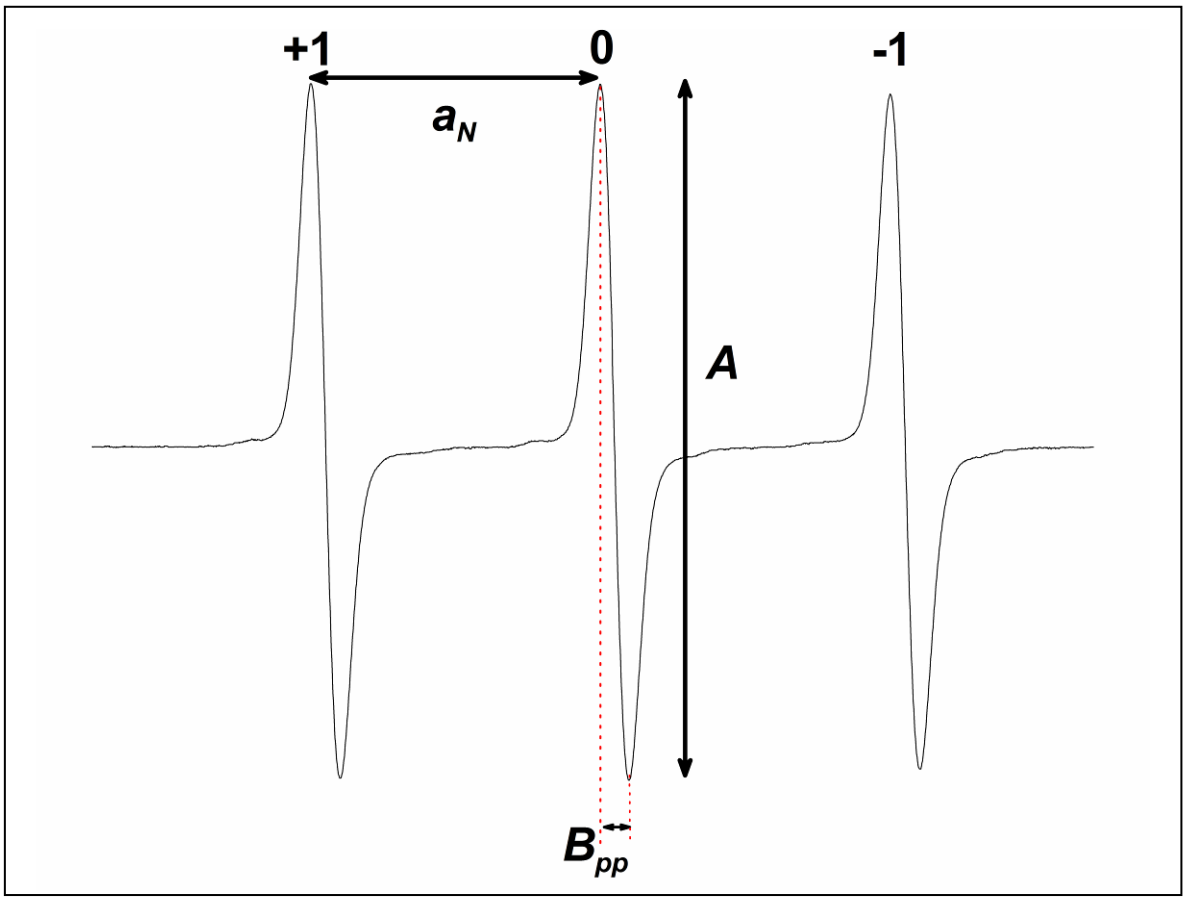

Figure 10.1 A typical nitroxide spectrum showing the important spectral parameters. B_{pp} is the peak to peak line width, a_N is the hfs constant, and A is the peak amplitude.

Incorporation of spin probes into drug delivery samples can provide a wide scope of information of their microenvironment including, but not limited to, molecular mobility and microviscosity, micropolarity, microacidity, and concentration of the oxygen. The following sections will discus some of these parameters.

1.5.3.1 Mobility and microviscosity

The unpaired electron on the nitroxide spin probe is shared between the N and O atoms. In the N atom, it occupies the Z direction of the P orbital. Accordingly, if a single crystal of the nitroxide molecule is placed in the magnetic field, the spectrum produced will depend on the orientation of the crystal, with the highest splitting of the peaks when the Z orbital is parallel to the external magnetic field. This leads to the anisotropy of the hyperfine splitting constant, i.e. it has different values in different directions.

This anisotropy is the reason for the sensitivity of ESR to the nitroxide mobility. When the probe is rotating very fast in a low viscosity medium, the rotations around the x, y and z axes cannot be distinguished and averaging of their individual contributions is seen as 3 sharp lines with an average hyperfine splitting, called isotropic hfs a_{iso} = 1/3 (a_x+a_y+a_z) and an average g-factor g_{iso} = 1/3 (g_x+g_y+g_z). But as the viscosity increases, the rotation around the three axes is no longer the same and it is slowed down around one or more axes leading to a distortion of the line shape (incomplete averaging), manifested as broadening of the lines. Upon complete immobilization, a superposition of all the possible orientations is observed and this is known as powder spectra (Figure 11.1).

The rotational correlation time (τ_R) could be calculated from the line width of the ESR spectra. τ_R is related to the viscosity through the Debye-Stokes equation [178].

$$\tau_R = \eta V / RT \qquad (9)$$

With η denoting the viscosity, V denoting the hydrodynamic volume, R is the gas constant and T is the temperature in Kelvin.

For mathematical calculations, the mobility of the nitroxide is divided into two regimes, the fast motion regime (τ_R = 10^{-11}- 10^{-9} sec) with almost complete averaging of the anisotropy, and slow motion regime (τ_R = 10^{-9}- 10^{-7} sec) with incomplete averaging and line shape broadening and distortion. In the fast motional region, τ_R can be easily calculated from the spectra using a simple empirical equation [178].

$$\tau_R = 6.5 \times 10^{-10} \Delta B_0 \left[\left(h_0 / h_{+1} \right)^{1/2} - 1 \right] \qquad (10)$$

Where ΔB_0 is the width of the central line, h_0 and h_{+1} are the heights of the central and high field lines respectively.

In the slow motional regime, an empirical relation can be used to determine the rotational correlation time.

$$\tau_R = a \left[1 - A'_z / A_z\right]^b \quad (11)$$

Where A_z is the experimental hfs constant and A'_z is the hfs at complete immobilization.

One must note that for nitroxides, the broadening of the 3 peaks is not the same. The high field peak is the most affected followed by the low field peak and then the central one.

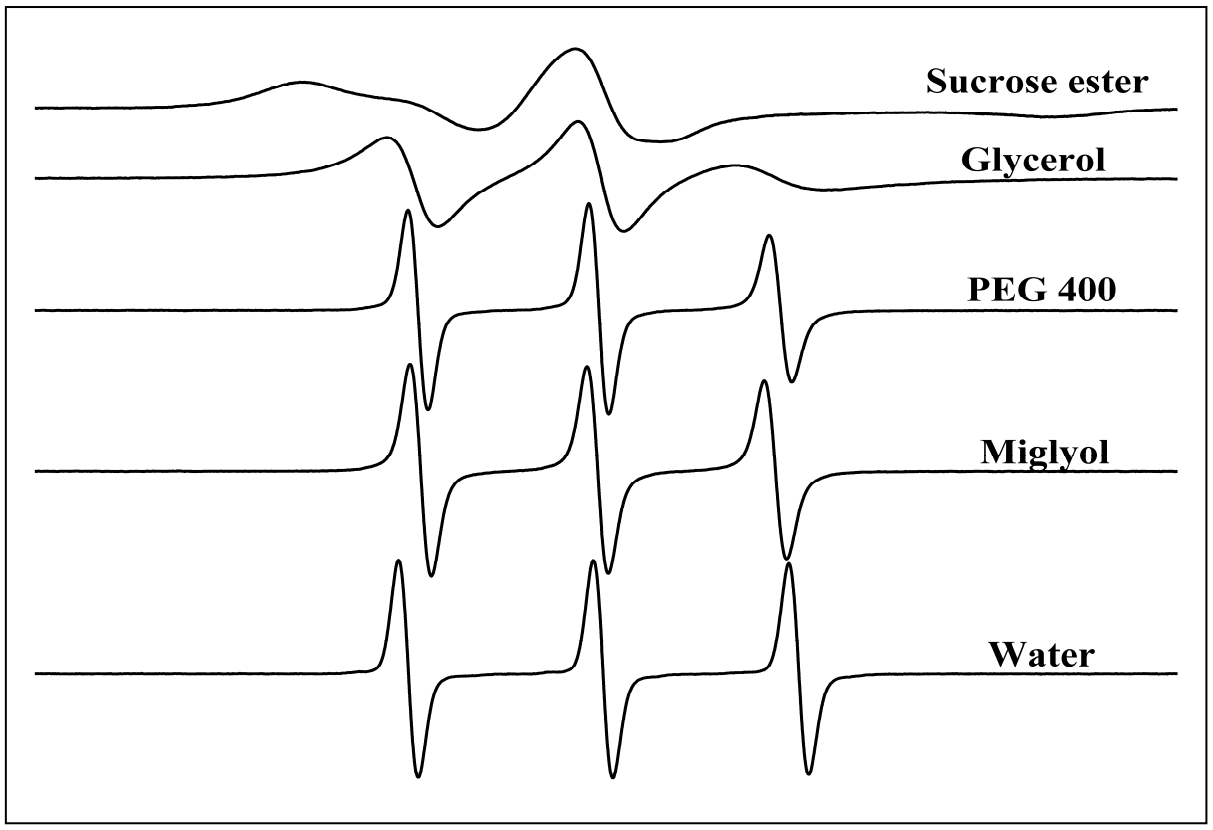

Figure 11.1 ESR spectra of TEMPOL in different environments indicating the sensitivity of the line shape to the viscosity of the media (from reference [177]).

1.5.3.2 Micropolarity

In polar solvents, the oxygen atom of the nitroxide groups attracts the partially positive parts of the solvent molecule, pushing the unpaired electron to the nitrogen atom which is the atom that causes the hyperfine splitting (hfs). This leads to an increase in the hfs. Therefore, hfs is a sensitive parameter to the polarity of the environment. This property is used to monitor localization and portioning of spin

probes or spin labeled compounds between compartments of different polarity [179, 180].

1.5.3.3 pH

Some specially designed spin probes are sensitive to the pH of their surrounding medium. In instance, imidazolidine derived nitroxides are used as pH-sensitive spin probes. The underlying principle is similar to that of the solvent polarity. Protonation of the imidazoline ring in acid media leads to a decreased spin density at the nitroxyl nitrogen, which is reflected by decreased hfs, and the opposite occurs upon deprotonation in alkaline media. This property was successfully used to measure the pH non-invasively inside tablets [181, 182], microspheres [183, 184], as well as in rat and human skin [185].

2. Preparation and characterization of a self-emulsifying pellet formulation containing Solutol® and a mixture of C18 mono- and di-glycerides

2.1 Introduction

SEDDS have shown a reasonable success in improving oral bioavailability of poorly-water soluble and lipophilic drugs [186, 187]. SEDDS are composed of a mixture of oil and a surfactant and they are capable of forming an O/W emulsion upon gentle agitation condition provided by gastro-intestinal motion [43]. In such system, the lipophilic drug is presented in emulsion, with small droplets of oil, leading to the elimination of dissolution which can be the rate limiting step in absorption of poorly-water soluble drugs. SEDDS are usually formulated in a liquid form which has some disadvantages, especially in the manufacturing process, leading to high production costs. Furthermore, incompatibility problems with the capsule shell are common. The incorporation of the self-emulsifying mixture into a solid dosage form is desirable, but challenging, because self-emulsifying properties are harder to achieve with solid materials. However, the potential advantages of solid self-emulsifying dosage forms have been described by several authors [188-190].

As discussed in the introduction section, pellets have many advantages over conventional solid dosage forms, making them of great interest to pharmaceutical industry. Flexibility in designing and developing the dosage form, and improving the safety and efficacy of bioactive agents are among these advantages. Due to the fact that pellets disperse freely in the gastrointestinal tract, drug absorption is maximized with a subsequent reduction in peak plasma fluctuations and hence minimizing potential side effects without lowering drug bioavailability. Pellets also reduce variations in gastric emptying rates and overall transit time and therefore a reduction of intra- and inter-subject variability of plasma profiles is achieved. In addition, pellets reduce the problem of high local concentration of drugs and thus avoiding irritation that may be caused by certain active constituents [143].

It is therefore very attractive to combine the advantages of self-emulsifying delivery systems with pellets. However, the development of self-emulsifying pellets is challenging, because high lipid loads often impair pellet formation. Using extrusion-

spheronization technique, mixtures of mono- and di-stearate, Solutol® HS15 (Figure 1.2) and MCC were investigated. Pellets were characterized for their size, shape, friability and dissolution. In addition, nitroxide loaded pellets were produced and the microenvironment within the pellets during the release process was monitored in an online process by the use of electron spin resonance (ESR) spectroscopy, since ESR is considered a powerful spectroscopic technique to monitor drug release processes non-invasively and continuously [191].

2.2 Materials

Avicel PH 101 (Microcrystalline cellulose (MCC)) was purchased from FMC BioPolymer (PA, USA), and was used as the pellet forming material. Solutol® HS 15 (Macrogol-15-Hydroxystearate) was kindly provided by BASF AG, Ludwigshafen, Germany. Cithrol GMS® (C18 mono-and di-glycerides) was kindly provided by Croda GmbH, Nettetal, Germany. Tempolbenzoate (4-Benzoyloxy-2,2,6,6-tetramethyl-piperidine-1-oxyl, TB) and Tempol (2,2,6,6-tetramethyl-4-hydroxy-piperidin-1-oxyl, TL), were purchased from Aldrich Chem. Co., USA. Sudan ® -red 7B dye was purchased from Riedel-de Haën AG, Germany. Diazepam was purchased from Fagron GmbH, Barsbüttel, Germany.

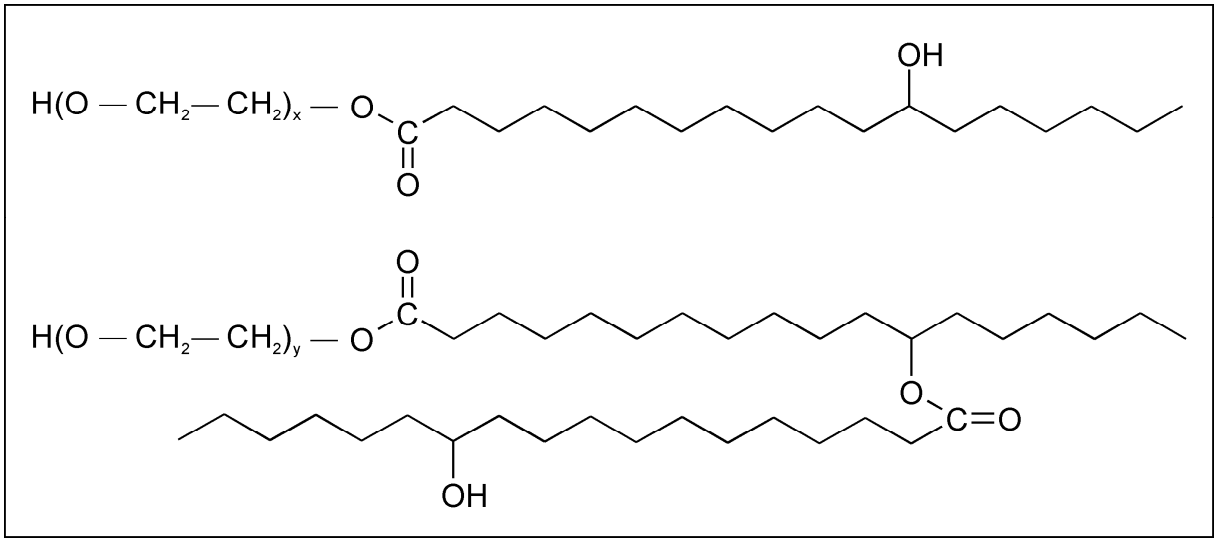

Figure 1.2 Chemical structures of the main components of the lipophilic part of Solutol® HS15 which consists of polyglycol mono- and di-esters of 12-hydroxystearic acid. A small part of the 12-hydroxy group can be etherified with polyethylene glycol.

2.3 Methods

2.3.1 Preparation of pellets

Details of the composition of the dry ingredients of the formulations prepared are given in table 1.2. The reference pellets were prepared by the same method used for the preparation of the self-emulsifying pellets.

Table 1.2 Composition of the investigated pellets (% w/w)

Ingredients	Diazepam pellets		Sudan red pellets		Pellets for ESR	
	Self-emulsifying	Reference	Self-emulsifying	Reference	Self-emulsifying	Reference
MCC PH 101	49.5	49.5	49,9	49,9	50	50
C18 mono- & di-glycerides	25	50	25	50	25	50
Solutol® HS15	25	-	25	-	25	-
Diazepam	0.5	0,5	-	-	-	-
Sudan Red	-	-	0,1	0,1	-	-
Nitroxide (TL or TB)	-	-	-	-	2 mmol/kg	2 mmol/kg

2.3.1.1 Preparation of the self-emulsifying mixture

The preparation of the self-emulsifying mixture involved the following steps:

- Melting of GMS and Solutol® HS15 at 70°C.
- Dissolving the model drug, the dye or the spin probe in the molten blend.
- Addition of water to the molten lipid blend until a creamy mass is produced.
- Cooling to room temperature.
- Addition of the dry MCC and mixing in a kneader for 15 minutes.
- Further addition of water until a mass suitable for extrusion is obtained.

2.3.1.2 Extrusion/Spheronization

The wet mass was extruded at 40 rpm in a radial screen twin-screw extruder (Fuji-Paudal, Japan) equipped with a die of 1 mm diameter circular openings and 1mm thickness. The extrudate was then spheronized for 5 min in a 250 mm radial plate

spheronizer (Fuji-Paudal, Japan) using a cross-hatch frictional plate of 3x3 mm^2 pitch and 1.2 mm depth (see figure 1.1 for illustration). The resulting pellets were dried in an oven at 50 ºC until a constant weight has been reached.

2.3.2 Pellets size analysis

Size analysis was performed using a set of standard sieves (Retsch, Hann, Germany) of a $\sqrt{2}$ progression ranging from 500 to 2800, with 100 g of pellets, agitated on a sieve shaker (Retsch, Hann, Germany) for 20 min. The modal size fraction and the interquartile range (IQR) were determined from the cumulative percent undersize curve. The geometrical mean diameter (D_g) and the geometrical standard deviation (σ_g) were determined from the log-normal distribution curve [192].

2.3.3 Pellets shape analysis

Shape analysis was preformed by the use of a stereomicroscope (SZX9, Olympus, Germany), a digital camera (DIG 1300C, Micromotion, Germany) connected to a personal computer with an Image analysis software Image C (Imtronic, Germany). 1000 pellets were used and for each pellet, 36 Feret diameters were measured and used to calculate the mean Feret diameter. The maximum Feret diameter and Feret diameter 90º to the maximum Feret diameter were obtained and the aspect ratio was calculated as the ratio between the maximum Feret diameter and the Feret diameter 90º [193].

2.3.4 Assessment of self-emulsification

For the preliminary assessment of the self-emulsifying properties of the formulation, 0.1 % Sudan Red was incorporated. 1g pellets were then gently agitated in 50 ml distilled water. Agitation was provided by gentle shaking on a shaking water bath at 50 oscillations per min and a temperature of 37ºC. Samples were taken after 30 min for microscopic examination using a light microscope (Axiolab re, Carl Zeiss, Germany) with an optical zoom of 50 x /0.70 and an eye piece of 10 x /20.

2.3.5 Friability testing of pellets

Friability testing was conducted using a friability tester (Arzneimittelwerk, Dresden, Germany). A 10 g pellet sample was placed into the drum together with 10 g glass spheres of 5 mm diameter, and rotated for 10 min at 25 rpm [194]. Pellets were then weighed and friability was calculated according to:

$$\text{Friability } \% = \frac{m_b - m_a}{m_b} \times 100$$

Where, m_b and m_a are the masses of pellets before and after testing and the result is the mean of three runs.

2.3.6 Disintegration testing of pellets

Disintegration time of pellets was measured by the use of a disintegration tester (Erweka ZT2, Heusenstamm, Germany), modified by the installation of 500μm mesh at the bottom of the tubes. Six pellets were tested in distilled water at 37 ºC and the end point was taken at the point at which no particles were present on the sieve.

2.3.7 Electron Microscopy

Surface characteristics of pellets were investigated by means of environmental scanning electron microscopy (ESEM, Philips XL 30 FEG, Philips electron optics). SEM micrographs were obtained by means of a special gas secondary electron (GSE) detector using the wet-mode method with a pressure of 1.3 mbar and acceleration voltage of 12 kV. Moreover, pure MCC pellets, prepared with the same method, were also investigated.

2.3.8 ESR flow through cell experiment

Samples of 200 mg Tempol-benzoate (TB) or Tempol (TL) (Figure 2.2) loaded pellets (2mmol/kg) were placed in a flow through cell. Phosphate buffer (pH 6.8) was pumped into the cell, by the mean of peristaltic pump, at a rate of 4 ml/min.

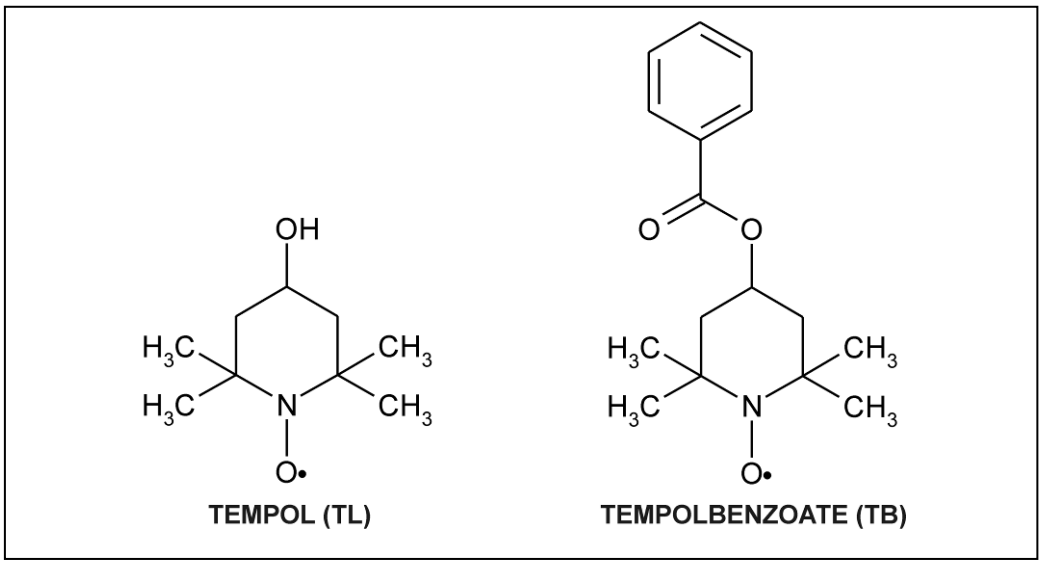

Figure 2.2 Chemical structures of the ESR spin probes Tempol and Tempolbenzoate.

Figure 3.2 is showing a schematic illustration of the ESR flow through system. The ESR spectra were recorded continuously using a 1.3 GHz L-band spectrometer (Magnettech Gmbh, Berlin, Germany) equipped with a re-entrant resonator. The ESR parameters used were as follow: field center 49 mT, scan range 12 mT, scan time 60 sec, and modulation amplitude of 0.14 mT.

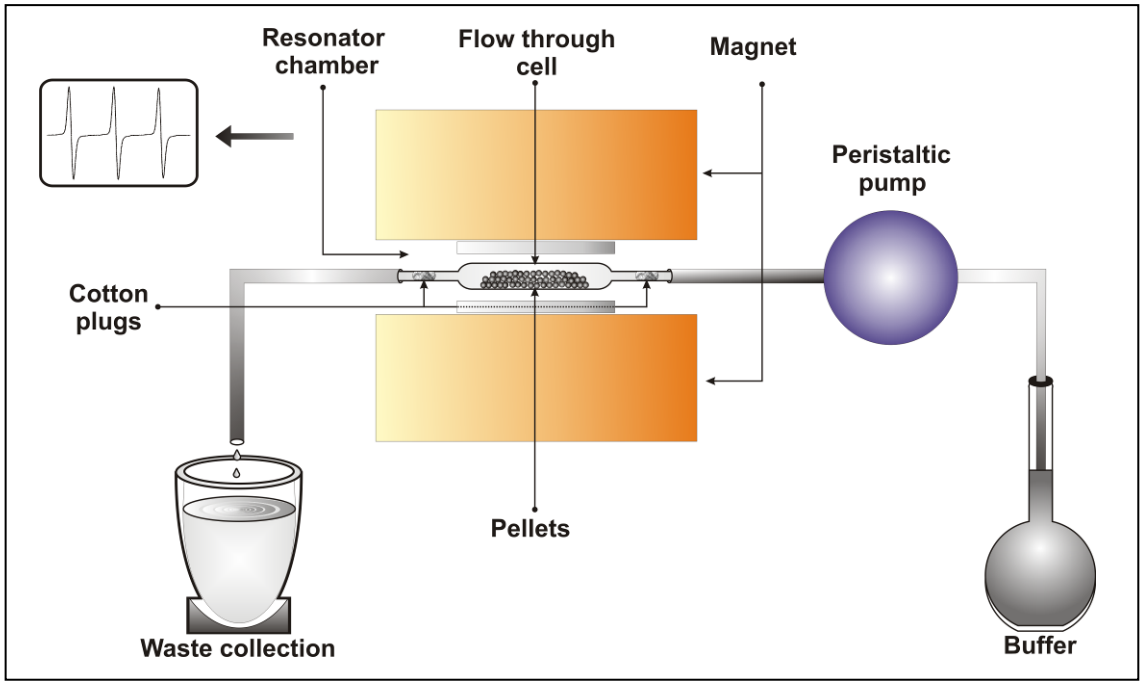

Figure 3.2. Schematic diagram showing the setup of the ESR flow through system. A flow through cell is placed inside the measurement (resonator) chamber between the magnets. The cell is connected from one end to a peristaltic pump and the other end to the waste collection.

2.3.9 Dissolution testing

The USP 24 rotating paddle apparatus (Pharma Test PTW II, Hainburg, Germany), rotating at a rate of 70 rpm and a temperature of 37 °C, was used to asses the release of diazepam from the pellets in two different conditions. In the first set of experiments, 900 ml phosphate buffer (pH 6.8) was used as the dissolution media and samples of pellets with a diazepam content of 2.5 mg were assessed. In the second set of experiments, the volume of the dissolution media was decreased to be 500 ml and pellets with diazepam content of 27.5 mg were used. 5ml samples were withdrawn at regular time intervals filtered and then assayed spectrophtometrically (Spectronic 60, Milton Ray, Ivy- land, USA) at 241 nm.

2.4 Results and discussion

2.4.1 Physical characterization of the pellets

The amount of self-emulsifying mixture required to formulate pellets with good physical properties and without any apparent agglomeration was assessed, and the formula stated in this study was the best (Figure 4.2).

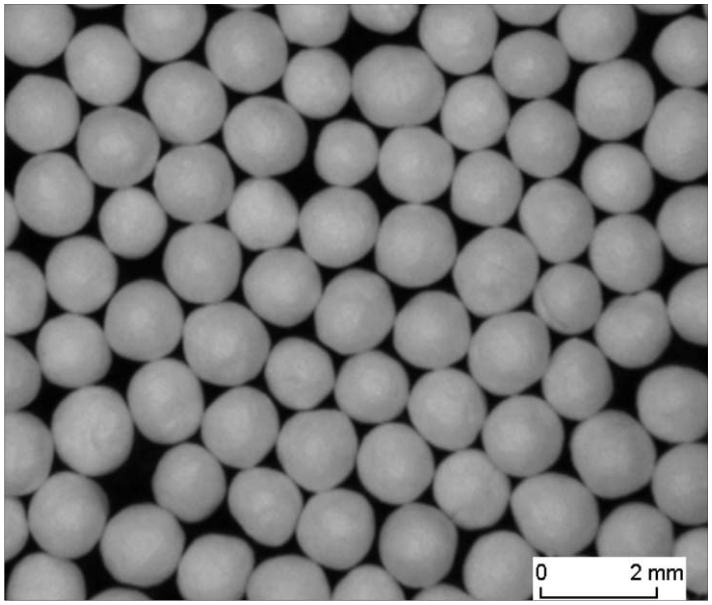

Figure 4.2 Light microscopic picture of the produced pellets.

Aspect ratios were found to be 1.091 which confirms to the limit (≤1.2) set by Chopra et al. for optimum pellet shape and flow properties [195]. The results of various physical measurements are shown in tables 2.2 and 3.2.

Table 2.2 Results of the size and shape analysis of the self-emulsifying pellets	
Sieve analysis (100g)	
D_g (µm)	1327
σ_g	1.26
Interquartile range (IQR, µm)	355
Image analysis (n=1000)	
$D_{feret.mean}$.(µm)	1307
Sd	125.69
Aspect Ratio	1.091
Sd	0.0396

The increase of Solutol® HS15 content above 35% led to the production of pellets with poor physical characteristics. On the other hand, a loss of the self-emulsifying properties was observed when the content of Solutol® HS15 was decreased below 25%. Furthermore, it was observed that the order of mixing is very critical for a successful extrusion / spheronization process. As an example, small rods rather than pellets were formed if the MCC was mixed with the molten glycerides prior to the addition of water.

Table 3.2 Friability % and Disintegration time for self-emulsifying pellets in the fraction1000-1400 µm

Friability testing (n=3)	
Friability (%)	**1.2**
Sd	**0.45**
Disintegration Testing (n=6)	
Time (min.)	**22.32**
Sd	**1.26**

ESEM Micrographs with the lowest magnification show that the produced pellets were spherical in shape, which confirms with the aspect ratio measurements (Figure 5.2). More details about the surface characteristics of pellets were obtained with increasing the magnification. Figure 5.2a shows typical features of the surface of MCC pellets with a highly rough and porous surface with apparent cellulose fibers network. After incorporation of 50 % of the self-emulsifying mixture the surface becomes smoother and less porous (Figure 5.2b). A noticeable incorporation of the SE mixture within the cellulose fibers network was observed with a subsequent decrease in the pellet porosity.

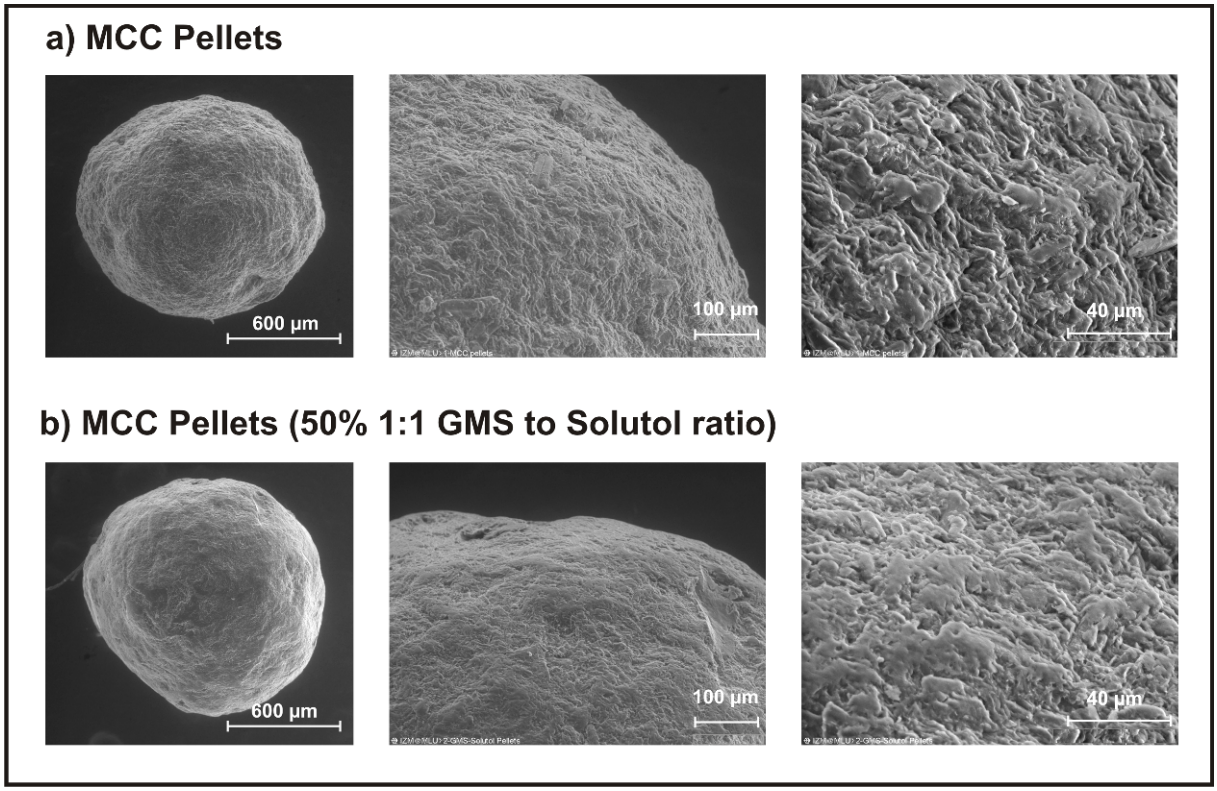

Figure 5.2 ESEM Pictures of the control MCC pellets and the self-emulsifying pellets.

2.4.2 Assessment of self-emulsification

The self-emulsifying formulation was able to transfer Sudan Red into water within the first few minutes following gentle agitation. The reference pellets, composed of MCC and GMS were not able to deliver the lipophilic dye into the media (Figure 6.2 a and b). Microscopic examination of the release media of the self-emulsifying pellets showed lipid droplets, incorporating the dye (Figure 6.2 c). On the contrary, microscopic examination of the release media of the reference pellets did not show any droplets (Figure 6.2 d).

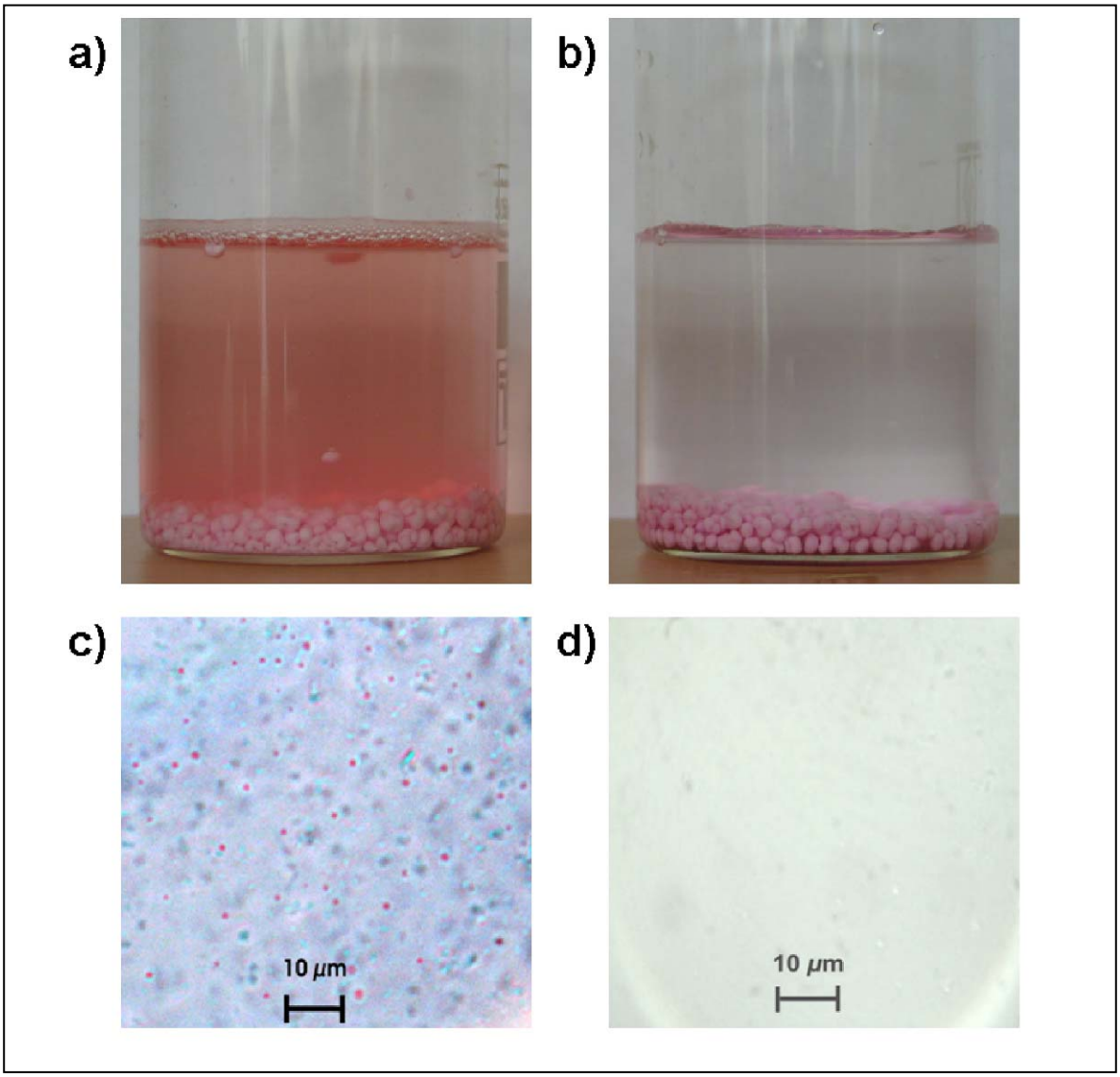

Figure 6.2 Photograph showing the release media of (a) self-emulsifying pellet formulation. (b) Reference pellets, containing Sudan red dye, after 30 min of dissolution in distilled water at a temperature of 37 °C. And microscopic picture of the release media of (c) self-emulsifying pellets (d) reference pellets.

2.4.3 ESR flow through cell experiment

The release kinetics and the microenvironment of the formulation during the release process were assessed by low frequency ESR spectroscopy. The ESR spectra of the nitroxides in Solutol, GMS and GMS-Solutol mixtures were recorded (Figure 7.2a and b). The mobility of the nitroxides is reflected in the line width. Both spin probes are more mobile in Solutol compared to GMS. The mobility in 1:1 GMS-Solutol® HS15 mixtures is comparable to pure Solutol® HS15 and higher compared to the mobility in dry pellets which contain MCC. This observation indicates that MCC causes a decreased mobility. However, the nitroxides still re-orientate quite fast in the solid material (within the nanosecond range).

After exposure to the dissolution medium, the ESR signal intensity decreased rapidly both for the hydrophilic TL and the lipophilic TB molecules due to the release of the nitroxides (Figure 7.2 c and d). Only small signals were detected after 30 minutes.

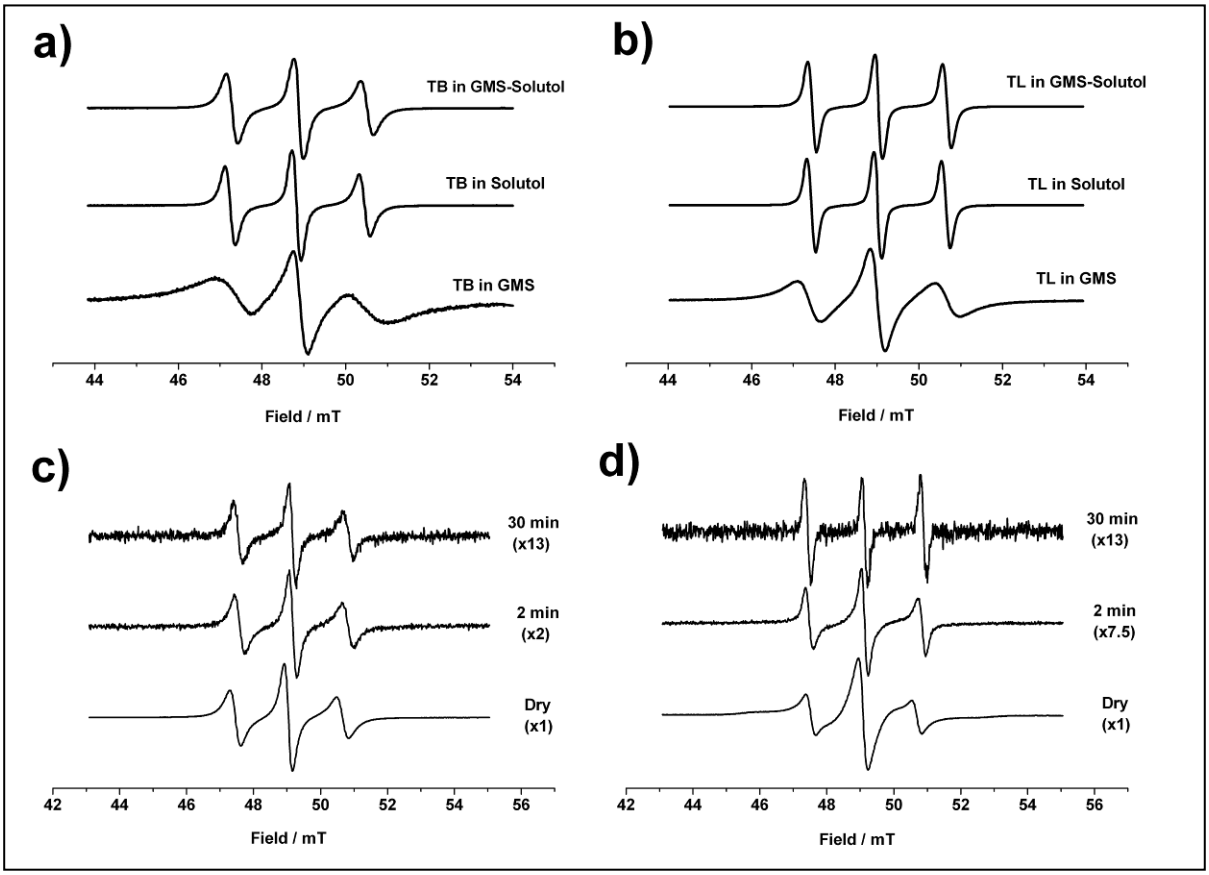

Figure 7.2 ESR spectra of TB (a) and TL (b) loaded formulation ingredients and mixtures. ESR spectra of TB (c) and TL (d) loaded pellets before, 2 and 30 min after buffer exposure in the flow cell system.

As the release media passes through the flow-cell, the shape of the TL spectra has changed significantly and rapidly into a sharp isotropic spectrum with a higher hyperfine splitting constant (a_N=1.72 mT; Figure 7.2 d), indicating the presence of the spin probe in an aqueous environment. In contrast, the ESR spectra of the more lipophilic probe TB show no significant change of the spectral shape and the hyperfine splitting constant during the release process. This indicates the preferential localization of the TB spin probe in the lipid environment all over the study period (Figure 7.2c).

2.4.4 Dissolution testing

The complete release of diazepam (Figure 8.2) from the non self-emulsifying GMS/MCC pellets has taken 3-folds the duration of that from the self-emulsifying pellets (Figure 9.2a).

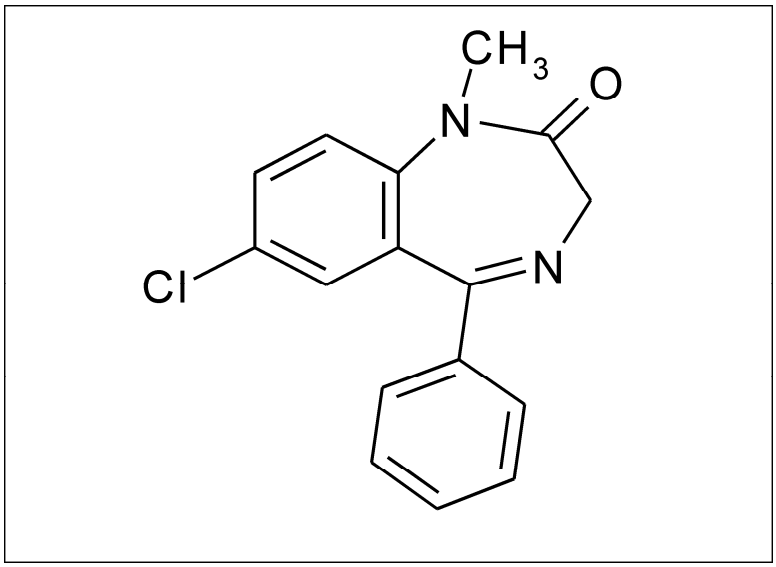

Figure 8.2 Chemical structure of diazepam.

Nearly 90% of the drug was released after 1 h, while only 55% was released from the GMS/MCC pellets after the same time. In the second experiment, with higher load of diazepam and lower volume of the dissolution media, the self-emulsifying pellets were capable of releasing diazepam into the media and a state of supersaturation was generated. Supersaturation was observed for several hours and there was no evidence of diazepam crystallization throughout the time of the experiment. On the other hand, pellets composed of MCC/GMS were only capable of releasing diazepam until the saturation solubility was reached (Figure 9.2b).

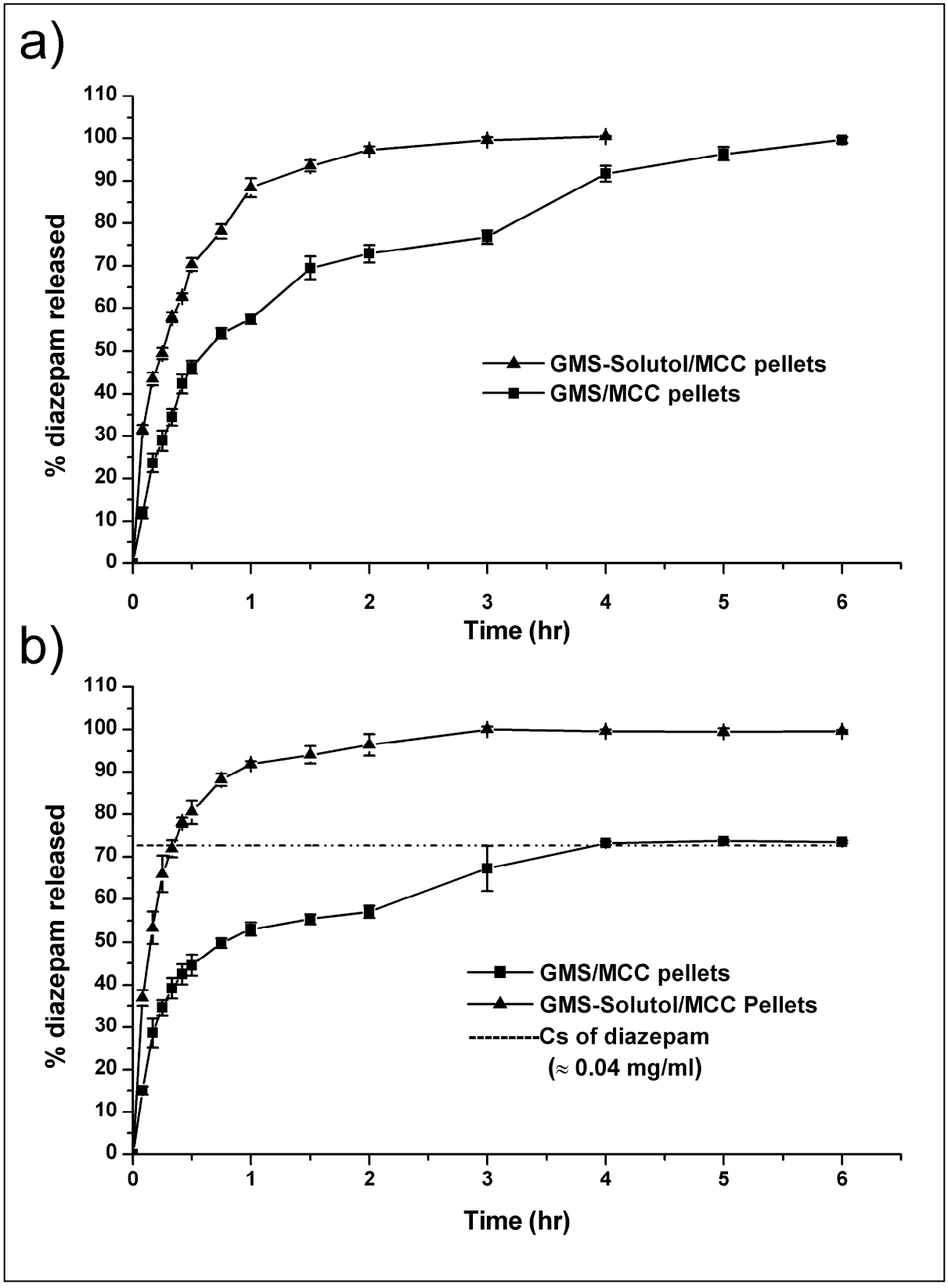

Figure 9.2 Mean (±SEM, n=3) percentage of a) Diazepam released as a function of time (h) in 900 ml phosphate buffer pH 6.8, and diazepam load of 2.5 mg from GMS/MCC pellets (■), and GMS-Solutol/MCC pellets (▲) b) Diazepam release as a function of time (h) in 500 ml phosphate buffer pH 6.8, and diazepam load of 27.5 mg from GMS/MCC pellets (■), and GMS-Solutol/MCC pellets (▲).

2.5 Conclusion

The current results demonstrate the possibility of using extrusion/spheronization to develop a self-emulsifying pellet formulation with 50% of the self-emulsifying mixture. The pellets have a spherical shape, small size distribution, and low friability. In contrast to MCC-GMS pellets, they were able to transfer lipophilic dyes or drugs into the aqueous phase of the dissolution media. Therefore, a decreased food dependency (bile acid concentration, digestion rate) can be expected.

Pellet formation is strongly dependent on the pellet composition and the order of processing. MCC is essential as a spheronization aid in pellet production, therefore a compromise between the least amount of MCC that can produce pellets with good physical characteristics and the amount of lipid, drug containing phase, should be achieved. Since GMS alone is not self-emulsifying, Solutol was used to aid the self-emulsification process.

The ESR results indicate that the lipophilic spin probe (TB) mainly localizes in the lipid environment inside the pellets until it was completely released into the media. The release data showed a noticeable improvement in the in vitro dissolution of diazepam when compared to the release from the non-emulsifying formulation. Moreover, the results from the second release study, with higher load of diazepam and lower volume of the dissolution media, showed that the formulation was able to create and maintain a state of supersaturation for the poorly water soluble diazepam.

3. Preparation and characterization of a self-emulsifying pellet formulation containing Solutol® HS15, a mixture of medium chain mono- and di-glycerides and tri-glycerides of caprylic/capric acid.

3.1 Introduction

In the previous section, the possibility of incorporating a semisolid self-emulsifying system into pellets and the production of pellets by means of extrusion/ spheronization was shown. The purpose of the current study is to develop a new SEDDS using a mixture of medium chain mono- and di-glycerides, triglycerides of caprylic/capric acid and Solutol® HS15. These systems were then assessed for self-emulsification and for droplet size diameter after dispersion.

The significance of emulsion droplet size in the in vivo performance of the formulation is not yet clear. Tarr and Yalkowsky have demonstrated enhancement of the rate of intestinal absorption of cyclosporine through the reduction of emulsion droplet size [196]. One possible explanation of the enhanced absorption observed with small particle size is the larger surface area available for partitioning of the drug and for lipase activity. Conversely, Pouton stated, in a recent review, that the role of droplet size is less important than it was assumed by some authors and he ascribed this to the fact that digestion will take place directly after the dispersion leaves the stomach and at this stage particle size will have no or little effect [6]. In this study, the droplet diameter, expressed as Z-average (the intensity weighted mean hydrodynamic size of the ensemble collection of particles), of the emulsion resulted from the dispersion of different SE mixtures was determined by means of photon correlation spectroscopy (PCS), which is a dynamic light scattering (DLS) technique.

Emulsion droplet polarity is one of the important factors for the performance of SEDDS [54]. Polarity describes the affinity of the drug compound to oil and/or water which consequently affects the release of the drug into the aqueous phase [56]. As described before in the introduction section, ESR spectra of nitroxide spin probes can provide information about their environment. For instance, the hyperfine splitting constant (a_N) is a sensitive parameter to the polarity of the environment (see figure

1.3 for illustration) and this property is used to monitor partitioning and localization of spin probes in compartments of different polarity [180]. In the current study, Electron spin resonance (ESR) spectroscopy was used to monitor the microenvironment and to follow up partitioning and localization of the spin probe in different environments within emulsions resulted from the dispersion of different self-emulsifying mixtures in water. Since the majority of drug delivery samples are diamagnetic and ESR silent, the incorporation of the nitroxide spin probe TB, as a model for poorly soluble drugs, was necessary for the ESR experiment.

Digestion of lipid based formulations, in the presence of endogenous materials (bile salts (BS), phospholipids (PL) and cholesterol), induces a change in lipid composition and results in the formation of different colloidal phases (micelles, vesicles, and liquid crystalline phases) in the intestinal lumen [197-201]. The change in lipid composition, induced by digestion, plays a major role in the solubilization process and consequently the absorption of the co-administered drug [45, 202, 203]. Therefore, development of an *in vitro* digestion model was a subject of interest for many research groups [141, 204-208]. *In vitro* lipid digestion experiment was carried out to asses the change in the solubilization capacity of progesterone in different SE mixtures after dilution and digestion in simulated intestinal fluids. Moreover, in vitro digestion was monitored by ESR spectroscopy to have an insight on the characteristics of the phases formed during the digestion process and to asses the distribution and localization of the spin probe in such phases [209].

The system that shows a compromise between good self-emulsifying properties, acceptable solubilization of the model drug and optimum surfactant concentration was selected to be incorporated into pellets by means of extrusion/spheronization.

Pellets were then characterized for size and shape. Additionally, one aim of the study was to explore the possibility of using a benchtop NMR instrument to monitor T_2 distribution in order to evaluate the state of the lipid after the incorporation of different percentages of the SE mixture into MCC pellets. Moreover, nitroxide loaded pellets were produced and an ESR spectroscopy experiment was carried out to assess the microenvironment within the pellets during the release process. A dissolution experiment was designed to asses the release of progesterone from the pellets in different media.

3.2 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). Progesterone (Figure 1.3) was purchased from Sigma-Aldrich (Steinheim, Germany). Pancreatin (activity equal to 8 X USP specification) and bile extract [used as a heterogeneous source of bile salts (BS)] were obtained from Sigma (Steinheim, Germany). Tempolbenzoate (4-Benzoyloxy-2,2,6,6-tetramethylpiperidine-1-oxyl, TB) was purchased from Sigma-Aldrich (Steinheim, Germany). Acetonitrile and Methanol were of HPLC gradient grade and were purchased from Mallinckrodt Baker (Deventer, Netherlands). All other materials were of analytical grade and were used as received.

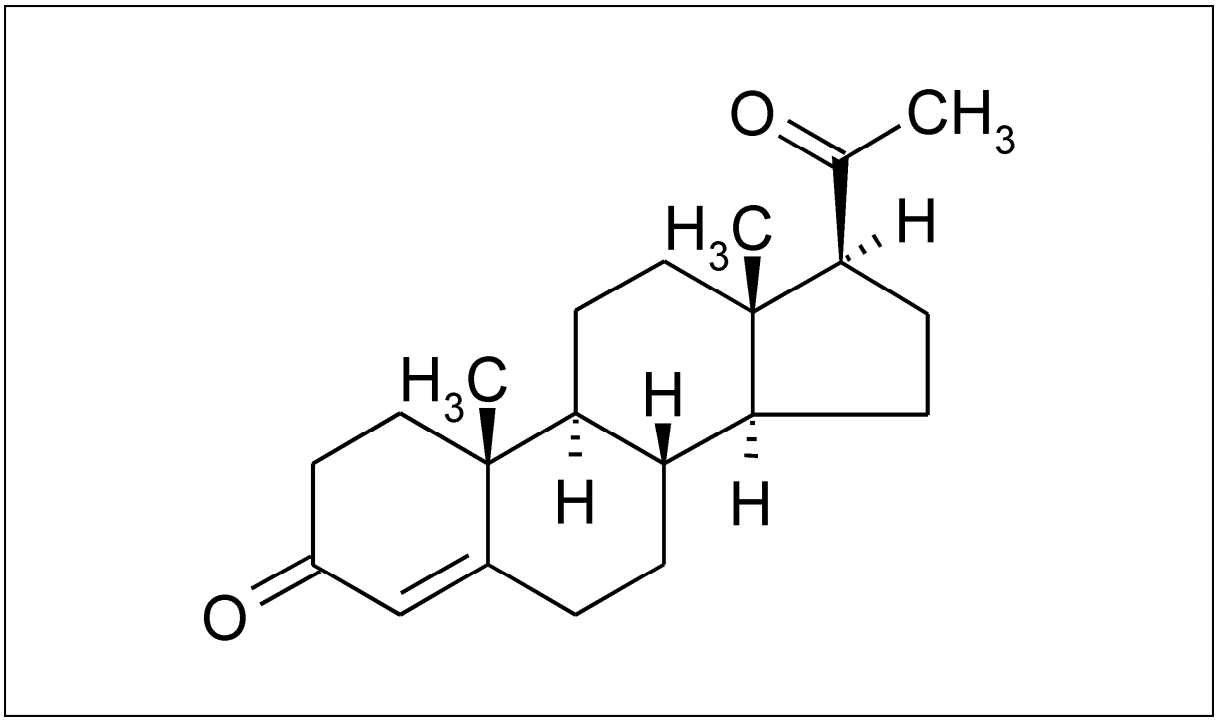

Figure 1.3 Chemical structure of progesterone.

3.3 Methods

3.3.1 Preparation and assessment of self-emulsifying mixtures

3.3.1.1 Preparation of Self-emulsifying mixtures

The compositions of the formulations prepared are listed in table 1.3. The preparation of the self-emulsifying (SE) mixtures involved the following steps:

- Mixing of Solutol® HS 15, Captex® and Capmul® at 50°C.
- Dissolving Progesterone in the lipid mixture.
- Cooling to room temperature.
- Equilibrating the mixtures for 24 h, to examine for any signs of phase separation.

Table 1.3 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

3.3.1.2 Assessment of self-emulsification

The USP 24 rotating paddle apparatus (Pharma Test PTW II, Hainburg, Germany), was used to evaluate the efficiency of self-emulsification of different mixtures. One gram of each mixture was added to 200 ml of distilled water with gentle agitation condition provided by a rotating paddle at 70 rpm and at a temperature of 37°C. The process of self-emulsification was visually monitored for the rate of emulsification and for the appearance of the produced emulsions.

3.3.1.3 Droplet size determination

Photon correlation spectroscopy (PCS) was used for determination of droplet diameter of the formed emulsions. The measurements were performed by means of a Malvern HPPS system (Malvern Instruments Ltd, United Kingdom) utilizing a backscatter angle of 173°. Samples were taken from the previous experiment (3.3.1.2) and were measured without dilution at 25C°. Mean hydrodynamic droplet diameter, expressed as z-average, was recorded using the Malvern HPPS software.

3.3.1.4 Equilibrium solubility measurements

Progesterone equilibrium solubility measurements were carried out in the following media: phosphate buffer pH 6.8, FaSSIF and FeSSIF (see section 3.3.1.6 for detailed composition). Furthermore, blank SE mixtures were mixed with different dissolution media (1% w/v) in order to evaluate the influence of the formulation on drug solubilization. For the assessment of the Progesterone equilibrium solubility in digested self-emulsifying systems under physiological conditions, blank aqueous digestion phases were obtained by carrying out the *in vitro* digestion experiment (see section 3.3.1.6) with drug free SE mixtures for 1 hour, 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.

Progesterone was added in excess to 5 ml of the dissolution medium in sealed tubes. Samples were incubated in an end-over-end mixer at 37°C for 24 hours and then centrifuged for 10 minutes in an Eppendorf centrifuge (MiniSpin, Eppendorf AG, Hamburg, Germany) at 13,000 rpm. The supernatant was filtered through a 0.2 µm Millipore filter, diluted to a suitable concentration range and analyzed by HPLC for Progesterone concentration.

3.3.1.5 ESR spectroscopy

Nitroxide loaded SE mixtures were produced and the microenvironment within the oil droplets after emulsification was evaluated using electron spin resonance (ESR) spectroscopy. The nitroxide spin probe tempol-benzoate (TB) was used as a model for poorly water-soluble drugs. One gram of each TB loaded self-emulsifying mixture (2 mmol/kg) was added to 200 ml of distilled water in dissolution apparatus rotating at a rate of 70 rpm and a temperature of 37°C. Samples were taken after 30 minutes 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).

3.3.1.6 In Vitro Digestion

Digestion experiments were carried out by the dispersion of 1% (w/v) of the SE mixtures, containing the drug dissolved at around 70% of saturation solubility in the corresponding mixture, in 7.5 ml digestion buffer (53.4% KH_2PO_4 1/15 M and 46.6% $Na_2HPO_4 \cdot 2H_2O$ 1/15 M, 150 mM NaCl, 5 mM $CaCl_2 \cdot 2H_2O$, pH 6.8) containing either a low (5/1.25 mM BS/PL) or a high (20/5 mM BS/PL) concentration of BS and PL to simulate the intestinal fluids in the fasting (FaSSIF) and the fed state (FeSSIF) respectively [210-213]. Figure 2.3 is illustrating the setup of the in vitro digestion experiment. Experiments were carried out in an end-over-end apparatus rotating at a rate of 15 rpm and a temperature of 37°C, and were started by the addition of 65.77 mg Pancreatin enzyme containing 450 U/ml of pancreatic lipase activity. 200 µl samples were taken at regular time intervals and centrifuged at 13,400 rpm for 4 minutes in an Eppendorf centrifuge (MiniSpin, Eppendorf AG, Hamburg, Germany) in order to separate the precipitate from the micellar phase. The supernatant was diluted with the mobile phase and analyzed for progesterone by HPLC using Merck Hitachi HPLC system consisting of a model AS 4000A auto-sampler, L 6200A programmable pump and a L 4250 UV-Vis detector (Merck, Darmstadt, Germany)

using a Hibar® RT 125-4 LiChrospher® 100 RP-18 (5 µm) column (Merck, Darmstadt, Germany). The mobile phase, composed of acetonitrile-water (70:30, v/v) adjusted to pH 3.5 with orthophosphoric acid, was pumped at a flow rate of 1 ml/min. 20 µl was injected and the column effluent was monitored at a wavelength of 240 nm. The retention time for progesterone was found to be 2.9 minutes.

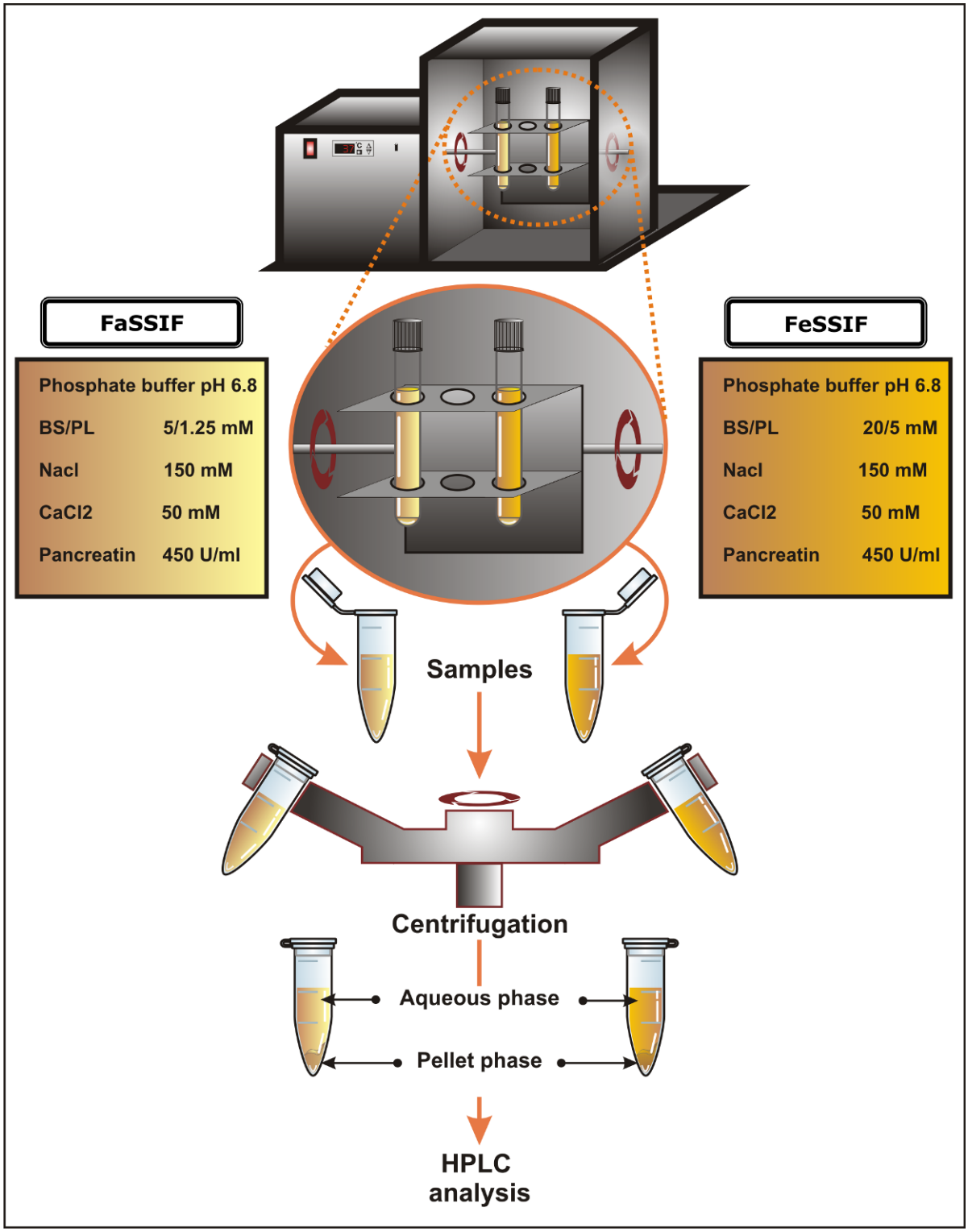

Figure 2.3 schematic presentation of the in vitro digestion experiment

3.3.1.7 Monitoring of In Vitro lipid digestion by ESR

ESR spectroscopy experiments were carried out to monitor the *in vitro* lipid digestion of a selected SE mixture. The lipophilic spin probe TB was mixed first with the SE mixture to obtain a final concentration of 1mM. 1 % of the nitroxide loaded SE mixture B5 was mixed with 7.5 ml of the previously described digestion buffer (see section 3.3.1.6 for details). Experiment was initiated by the addition of 65.77 mg Pancreatin (450 U/ml of pancreatic lipase activity) to the mixture. The mixture was then incubated in an end over end apparatus at 37 °C and pH was adjusted to 6.8 every 10 minutes. 100 µl samples were taken at fixed time intervals, filled into a capillary tube and measured by X-band ESR spectrometer. Control experiments of TB in different media were carried out to help understanding and interpreting the resulting spectra. The obtained spectra were simulated by means of simulation software (EPR center, Josef Stefan Institute, Ljubljana, Slovenia). For the control experiments blank aqueous digestion phases were obtained by carrying out the experiment with drug free self-emulsifying mixtures for 1 hour, 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.

3.3.2 Formulation and characterization of pellets

3.3.2.1 Formulation of pellets

Self-emulsifying mixture B5 was selected for incorporation into pellets, in three different levels (10, 20, and 40 %), by the mean of extrusion/ spheronization.

The self-emulsifying mixture was mixed with MCC in a kneader for 15 minutes. This was followed by addition of water until a mass suitable for extrusion is obtained. The wet mass produced was then extruded and then spheronized using the same method described in section 2.3.1.2. The produced pellets were then dried in a desiccator over silica gel at room temperature.

3.3.2.2 Electron Microscopy

Surface characteristics of pellets with 10, 20 and 40% content of SE mixture B5 were investigated by means of environmental scanning electron microscopy (ESEM). SEM micrographs were obtained using the same method described in section 2.3.7.

3.3.2.3 T_2 NMR distribution measurement

A benchtop NMR instrument (20 MHz, Oxford Instruments, Abingdon, Oxfordshire, UK) was used to measure T_2 distribution of pellets with 10, 20 and 40% SE mixture B5 incorporated into MCC. Moreover, T_2 distribution measurements were performed for the liquid SE mixture. The measurements were carried out utilizing a CPMG sequence using the following parameters: P90 3.65 µs, *tau* 135 µs, dwell time 10 µs.

3.3.2.4 In Vitro release testing

In vitro release experiments were performed in an end over end apparatus rotating at a rate of 15 rpm and a temperature of 37 °C. Therefore samples of pellets corresponding to 1% (w/v) final SE mixture concentration in the release media were assayed. The composition of the investigated pellets is shown in table 2.3.

Three different release media were chosen: phosphate buffer (pH 6.8) and *in vitro* digestion buffer with Pancreatin under either FaSSIF or FeSSIF conditions. Pancreatin powder containing 450 U/ml of pancreatic lipase activity was added at the beginning of the digestion experiment. 200 µl samples were taken at regular time intervals and centrifuged at 13,400 rpm for 4 minutes in order to separate a dispersed phase and a pellet phase. The supernatant was diluted with the mobile phase and analyzed for Progesterone by the previously described HPLC method. All experiments were carried out in triplicate.

Table 2.3 Composition of the investigated pellets

Ingredients	Pellets composition (% w/w)	
	Progesterone pellets	Pellets for ESR
MCC PH 101	58.7	60
Solutol HS15	16	16
Captex 355	16	16
Capmul MCM	8	8
Progesterone	1.3	–
Tempol benzoate	–	2 mmol/Kg

3.4 Results

3.4.1 Preparation and in vitro assessment of the SE mixtures

Most of the SE mixtures produced belong to type IIIA in the lipid formulation classification system (LFCS) proposed by C. W. Pouton with an oil content of 40-80% (w/w), a water soluble surfactant (HLB >12) content of 20-40 % and hydrophilic cosolvent content of 0-40% [6, 43]. In this study, Captex® 355 EP/NF (triglycerides of caprylic/capric (C_8/C_{10}) acid) was used as the oil, and the corresponding mono- and di-glycerides mixture (Capmul® MCM) was used to increase the solubilization and self-emulsification capacity. Solutol® HS 15 (main component: polyoxyethylene-660-12-hydroxy stearate) was used as the water soluble surfactant (HLB= 14-16).

All the formulated mixtures were efficiently emulsified within the first minute of contact with the dispersion medium and there was no evidence of phase separation or any instability problem for at least 24 hours. The optical clarity of the produced emulsions was strongly dependent on the composition. It changed largely, from a bright white at low Solutol® HS15 concentration toward a clear or slightly bluish emulsion, with increasing Solutol® HS15 concentration in the formulation, indicating that the surfactant plays a crucial role in determining the properties and functionality of the resulting dispersion.

Droplet diameter, expressed as Z-average (the intensity weighted mean hydrodynamic size of the ensemble collection of particles), of the emulsion resulted from the dispersion of different SE mixtures was determined by means of PCS. The results of droplet size determination experiment of the different formulation studied are shown in figure 3.3. The concentration of Solutol® HS in the formulation noticeably affects the nature of the emulsion formed. Increasing Solutol® HS concentration leads to an obvious improvement in the optical clarity, which correlates well with the determined particle size of the corresponding dispersion. It was also noticeable that increasing the amount of Captex®, the medium chain triglyceride oil, and decreasing the amount of Capmul®, in the formulation with a 2:1 w/w Captex® to Capmul® ratio, decreased the emulsification efficiency and caused higher particle sizes and lower optical clarity. This finding corresponds to the decreased polarity of the oily phase. At higher Solutol® HS 15 concentrations the decrease in the droplet diameter in relation to the Solutol® HS 15 concentration was very small for both

Captex®/Capmul® ratios. This can be attributed to the fact that Solutol® HS 15 is a micelle forming agent.

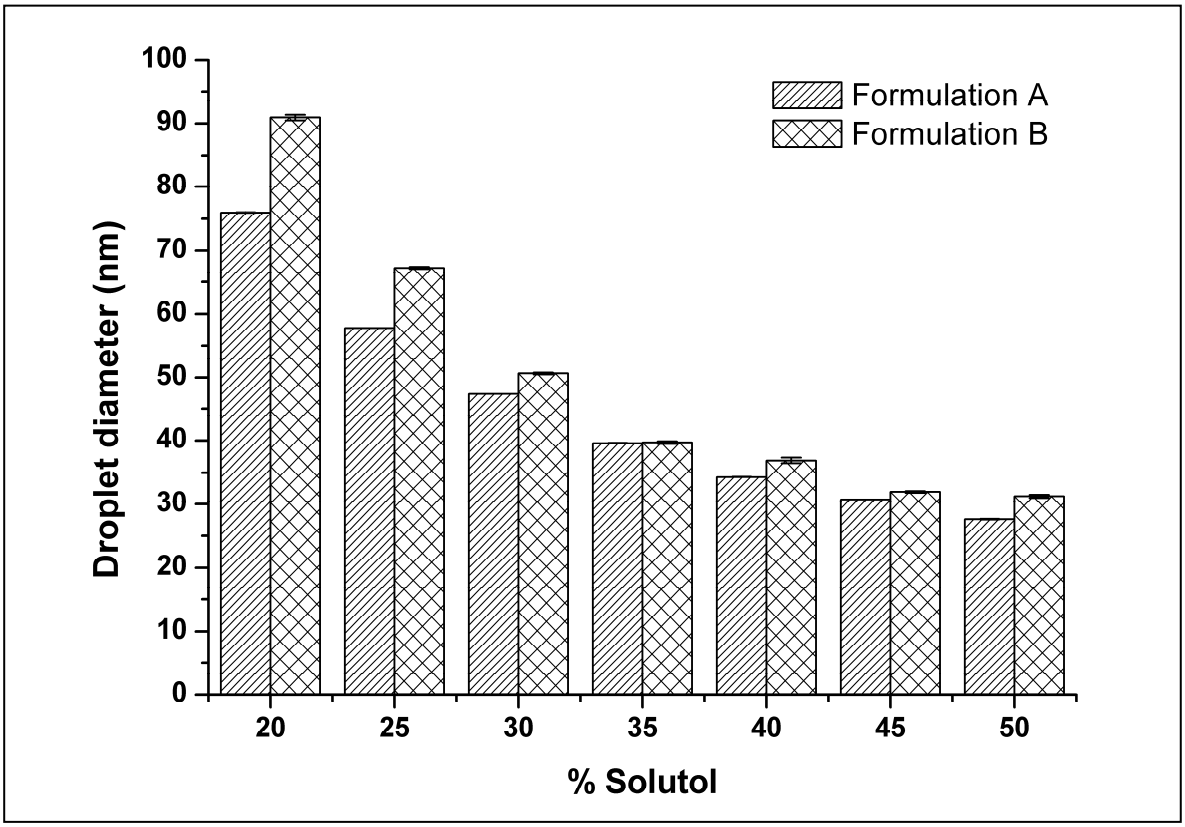

Figure 3.3 Effect of Solutol content on the droplet diameter (mean ± SD, n=3) of the emulsions produced by dispersing SE mixtures in water.

The preference between different Captex® to Capmul® ratios will depend on the solubilization characteristics of the drug. For instance, a higher proportion of triglyceride oil will be required for highly hydrophobic drugs but for less hydrophobic drugs an improvement of solvent capacity could be achieved through increasing the proportion of mixed mono- and di-glycerides. In general, a compromise between drug loading capacity and efficient emulsification has to be achieved.

ESR experiments were carried out to assess the microenvironment and to monitor partitioning and localization of the spin probe in different environments within the produced emulsions. In addition, ESR spectra of TB in different formulation components were recorded and simulated to to serve as references (Figure 4.3). Figure 4.3 (right diagram) shows the sensitivity of the line shape to the viscosity of the different media.

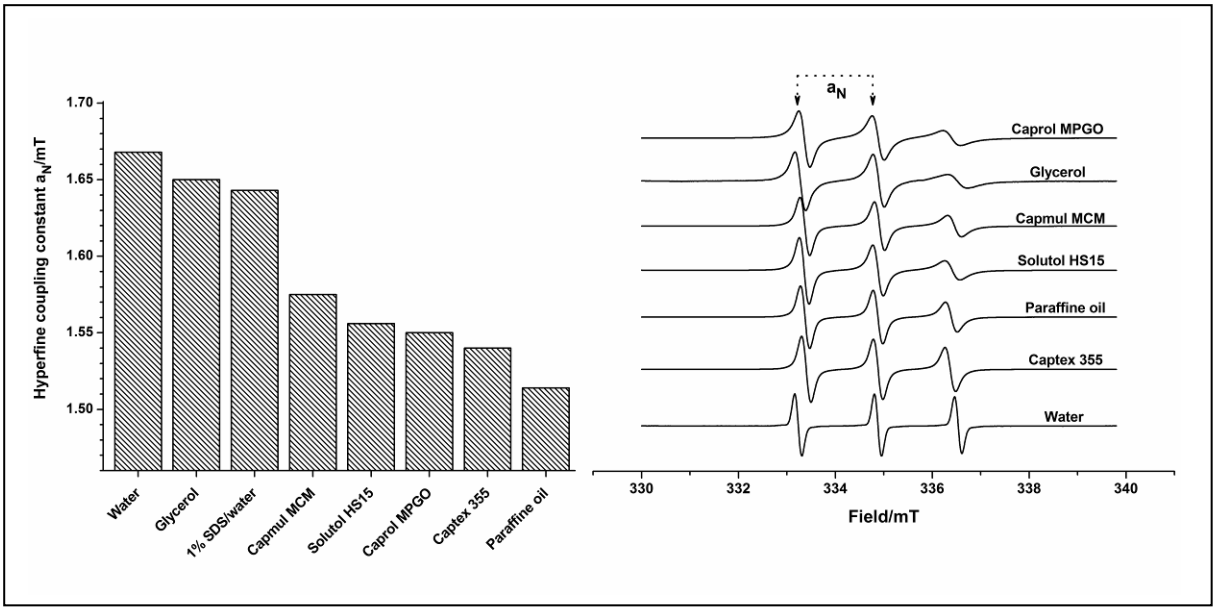

Figure 4.3 ESR spectra of TB in environments with different polarity and viscosity. The hyperfine coupling constant a_N is sensitive to the polarity of the environment (left diagram) which is used to monitor the partitioning of spin probes between compartments with different polarity. Please note the effect of increasing the viscosity on the broadening of the line width (right diagram).

In a low viscosity medium like water, the rotation (tumbling) of the spin probe is very fast and the rotations around the x, y and z axes cannot be distinguished resulting in an almost complete averaging of their individual contributions seen as 3 sharp lines. As the viscosity increases (from Captex ® 355 to Caprol ® MPGO), the rotation around the three axes is no longer the same and its slowed down around one or more axes leading to distortion of the line shape manifested as broadening of the lines with a special effect on the third line. In Captex® 355, the mobility of the spin probe is still 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 spectrum is still showing relatively high mobility of the spin probe. From the line width of the spectra, the rotational correlation time (τ_R) can be determined and it can be related to the viscosity of the media. The left diagram in figure 4.3 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. 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) which lies in between the polarity of water and paraffin oil. This

confirms with the function of Capmul ® MCM in the formulation which is increasing the solubilization and self-emulsification capacity. Figure 5.3 shows an illustration of the results of the ESR experiments.

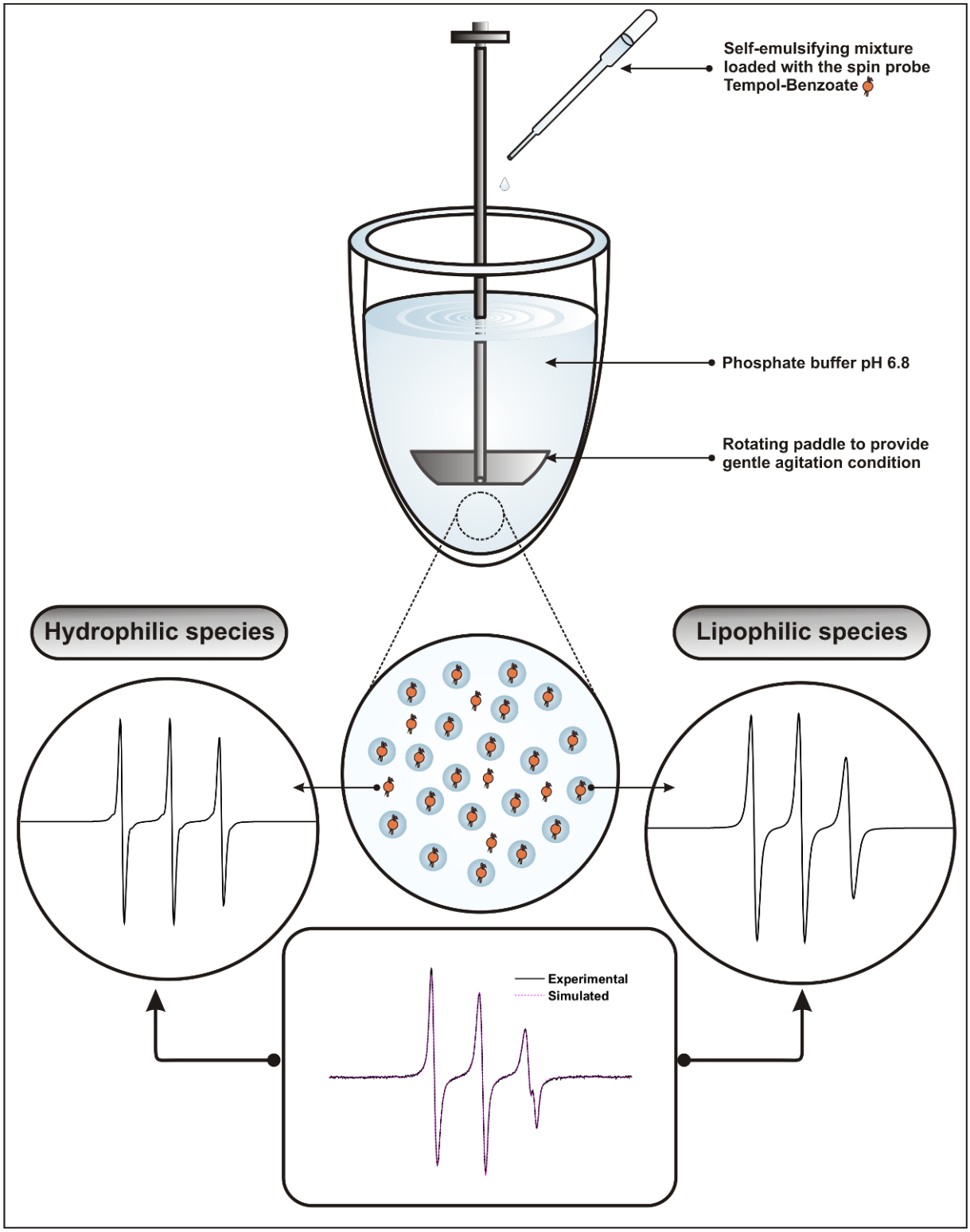

Figure 5.3 Schematic presentation of the in vitro ESR experiment.

TB was used as a model for moderately lipophilic drugs with a logP of 2.46 [176]. The general pattern of the ESR spectra of TB in diluted SEDDS was very similar with splitting of the third line caused by the distribution of the spin probe in two different polarity environments (Figure 6.3).

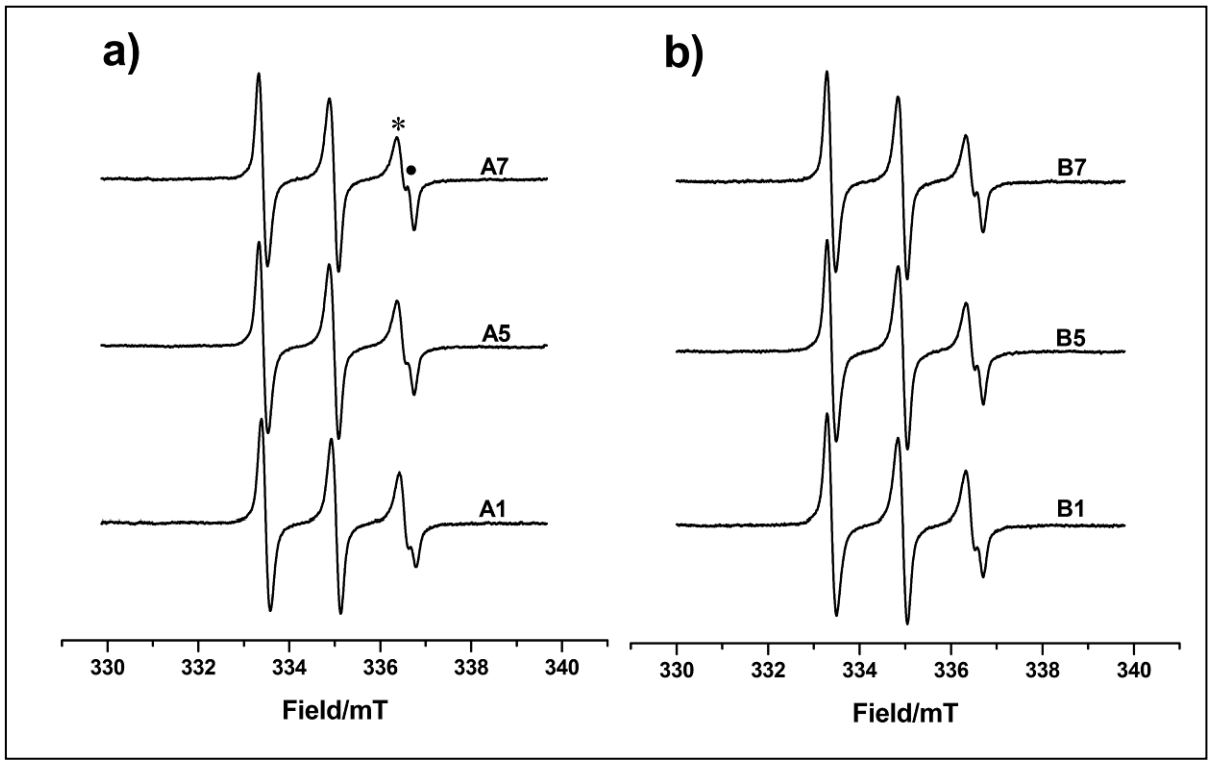

Figure 6.3 ESR spectra of TB in the emulsion resulted from the dispersion of SE mixtures a) from formulation A b) from formulation B in phosphate buffer pH 6.8. (* refers to the lipophilic while • refers to the hydrophilic species).

Spectra of the diluted SEDDS were successfully simulated with good fit into 2 spectra (Figure 7.3 a), a lipophilic spectrum [with less splitting (a_N=1.53 mT for formulation A and 1.52 mT for formulation B), lower polarity and higher viscosity, (Figure 7.3 b)], and a hydrophilic spectrum [with higher splitting (a_N=1.67 mT), higher polarity and lower viscosity, (Figure 7.3 c)]. The polarity of the lipophilic compartment was slightly higher in system A, with 1:1 Captex® 355 to Capmul® MCM ratio, compared to system B, 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.

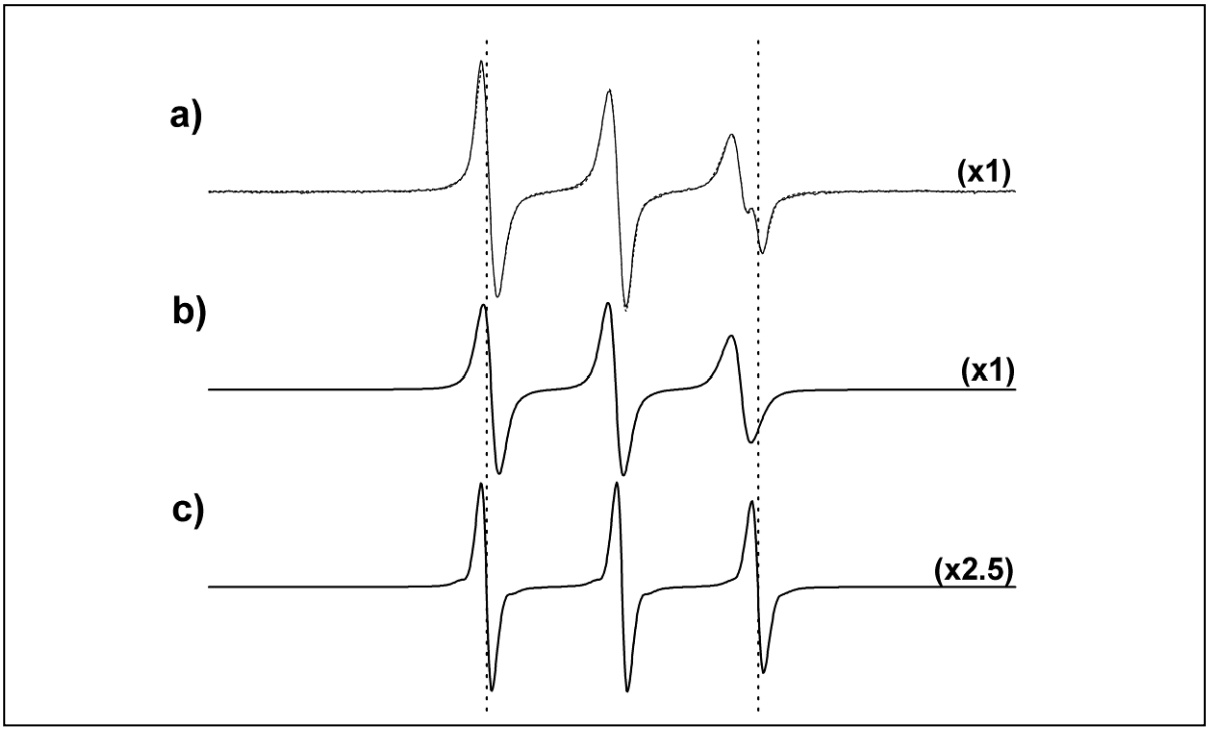

Figure 7.3 ESR simulation spectrum of TB in the emulsion resulted from the dispersion of SE mixture B5 in phosphate buffer. a) Experimental spectrum (bold line) and simulated spectrum (dotted line), b) spectrum of the lipophilic species c) hydrophilic species.

The percentage of TB localized in the lipophilic compartment is decreasing with increasing the surfactant fraction in the self-emulsifying mixture (Figure 8.3). As the oil to Solutol® HS15 ratio decreases the percentage of the spin probe localized in the lipophilic environment decreases which was caused by the increased hydrophilicity. Moreover, it was found that formulation A provides more attraction to TB in all oil to Solutol® HS15 ratios (Figure 8.3). In addition, it has been found that the micropolarity inside the emulsion droplet of both formulations was constant regardless of the varying oil and surfactant ratios.

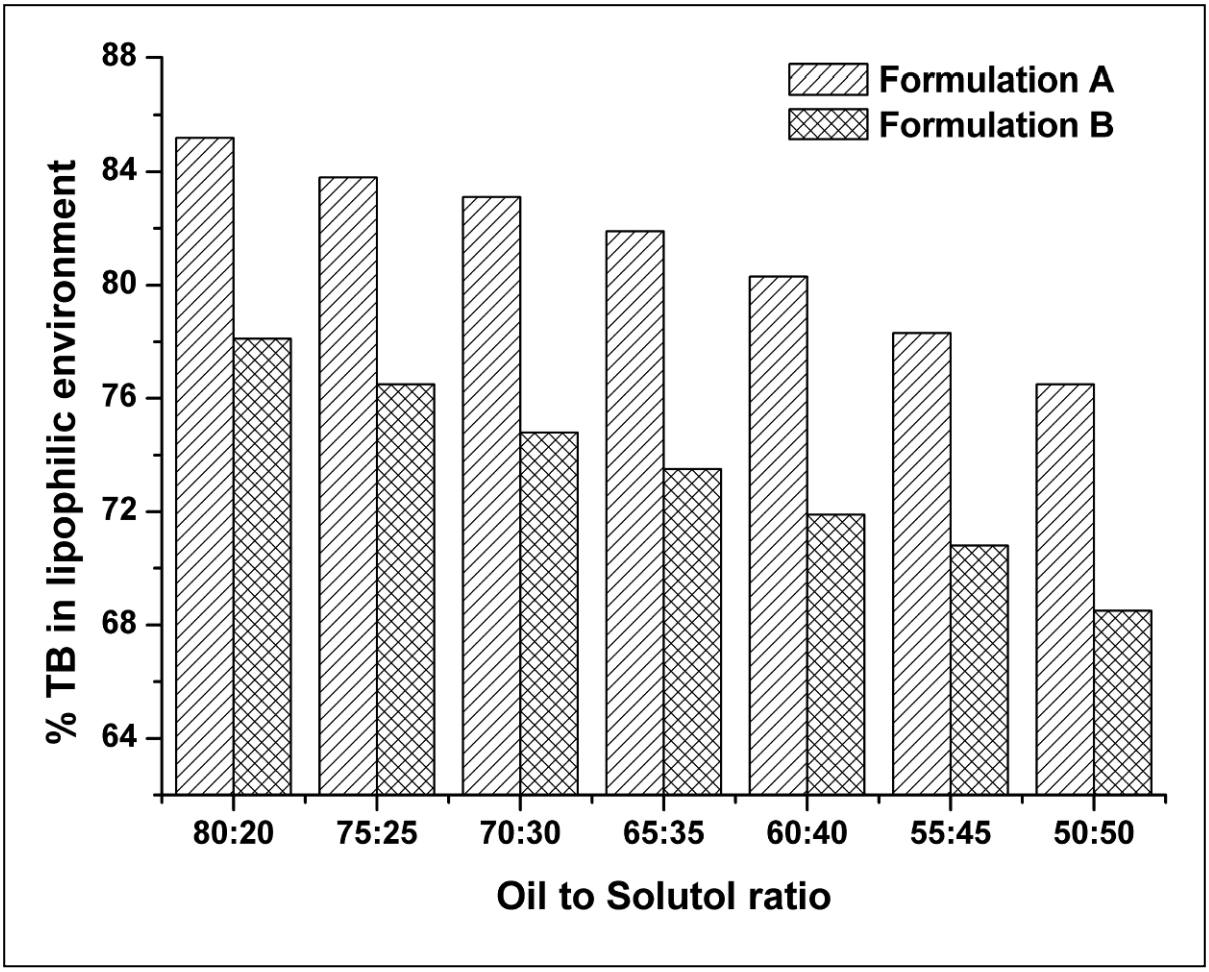

Figure 8.3 Effect of changing the oil to Solutol ratio on the distribution of TB in the lipophilic phase of the dispersed SE mixtures.

3.4.2 Equilibrium solubility

The equilibrium solubility of Progesterone in different vehicles is given in figure 9.3. The solubility increases from plain buffer solution to FaSSIF and FeSSIF media which corresponds to the higher concentration of lecithin and bile acids. A high solubility was achieved with 1% w/v of the self-emulsifying mixtures B1 and B5 in buffer.

Interestingly, the solubilization capacity was different when dispersions of SE mixtures in FaSSIF and FeSSIF were investigated. Depending on the formulation, the presence of mixed micelles and lecithin had either a positive or negative effect on the solubilization capacity. This finding could be attributed to interactions of formulation components with micelles or vesicular structures which lead to a change in the polarity of the system.

The aqueous phases which were obtained after digestion of the diluted SE mixtures showed a significant lower solubilization capacity for progesterone. Again, the FaSSIF system solubilized less drug molecules compared to the FeSSIF system. The digestion of the lipid phase in the SE mixtures B1 and B5 loaded FeSSIF and FaSSIF systems leads to the formation of digestion products of the SE mixture (e.g. hydroxystearate, free PEG, middle chain fatty acids, monoglycerides), and digestion products of the mixed micellar phase (e.g. lysolecithin and long chain free fatty acids). As a result, the polarity of the lipids increases and the composition and structures of the lipid phase will change, which will affect the drug solubility.

Overall, the results of the solubility study show that (i) mixed micelles increase the solubility, (ii) diluted SE mixtures B1 and B5 (1% in phosphate buffer) have a high solubilization capacity, (iii) the presence of bile salts and phospholipids affects the solubilization capacity of diluted SE mixtures B1 and B5 and (iv) the solubilization capacity of enzymatic digests is lower compared to undigested SE mixtures.

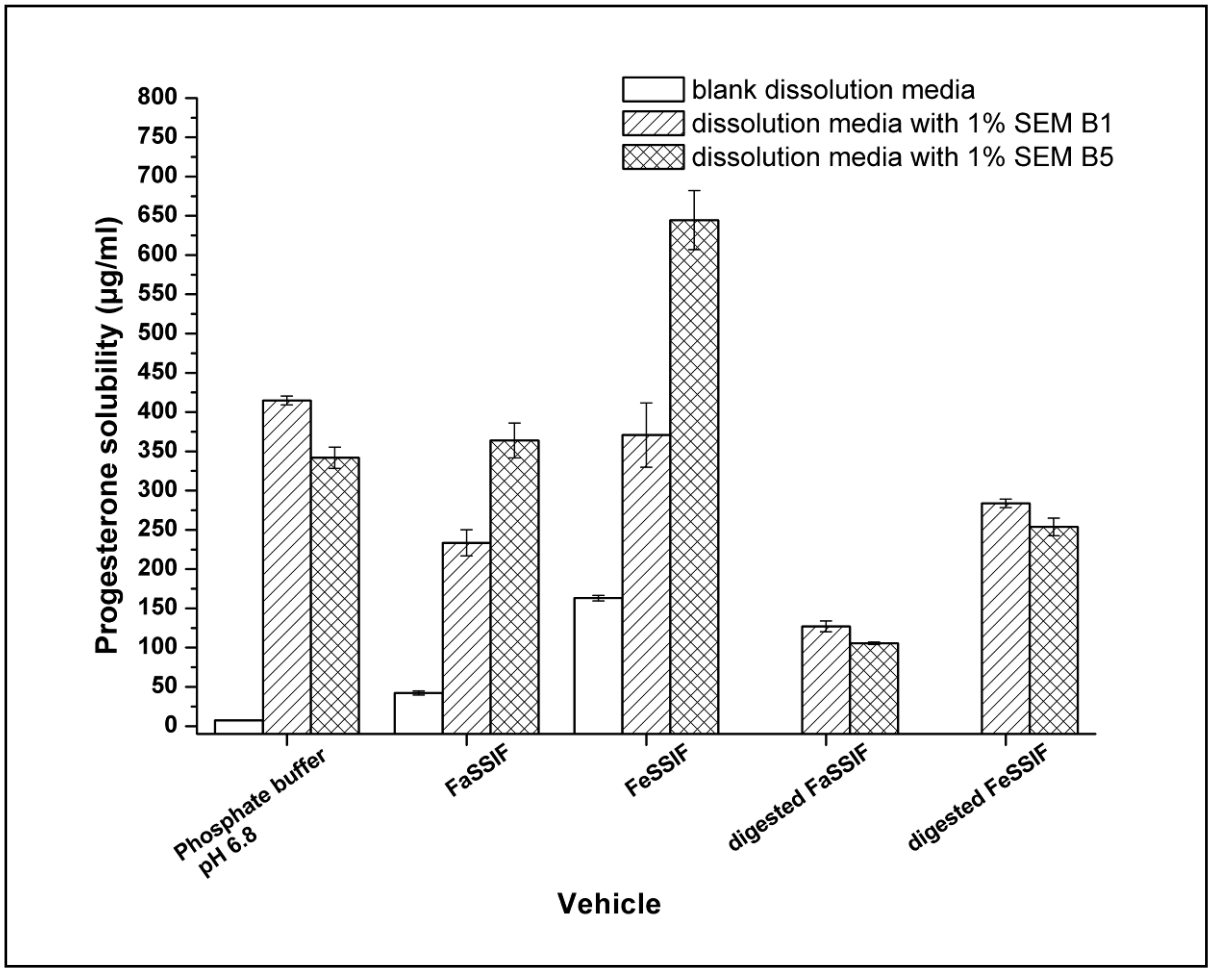

Figure 9.3 Equilibrium solubility (mean ± SD, n=3) of Progesterone in different media.

3.4.2 In Vitro digestion

The solubilization of progesterone during in vitro digestion of SE mixture B1 and B5 was monitored in both FaSSIF and FeSSIF conditions. Digests were centrifuged and were consequently separated into a dispersed phase and a pellet phase with no evident oil phase which complies with the data presented in different studies [142, 210]. Results of the digestion experiment are shown in figure 10.3. For formulation B1 in FeSSIF condition, progesterone concentration was reaching and maintaining the saturated solubility for at least one hour after which dropping of the solubilization capacity was noticed. Precipitation of progesterone in FeSSIF occurs more rapidly for formulation B5, which could be attributed to the lower oil content in the formulation leading to the liberation of a lower amount of monoglycerides and fatty acids as digestion proceeds [210, 213]. In FaSSIF digestion experiment, precipitation starts at earlier stage for both formulations which is attributable to the lower concentration of BS and PL in the digestion media.

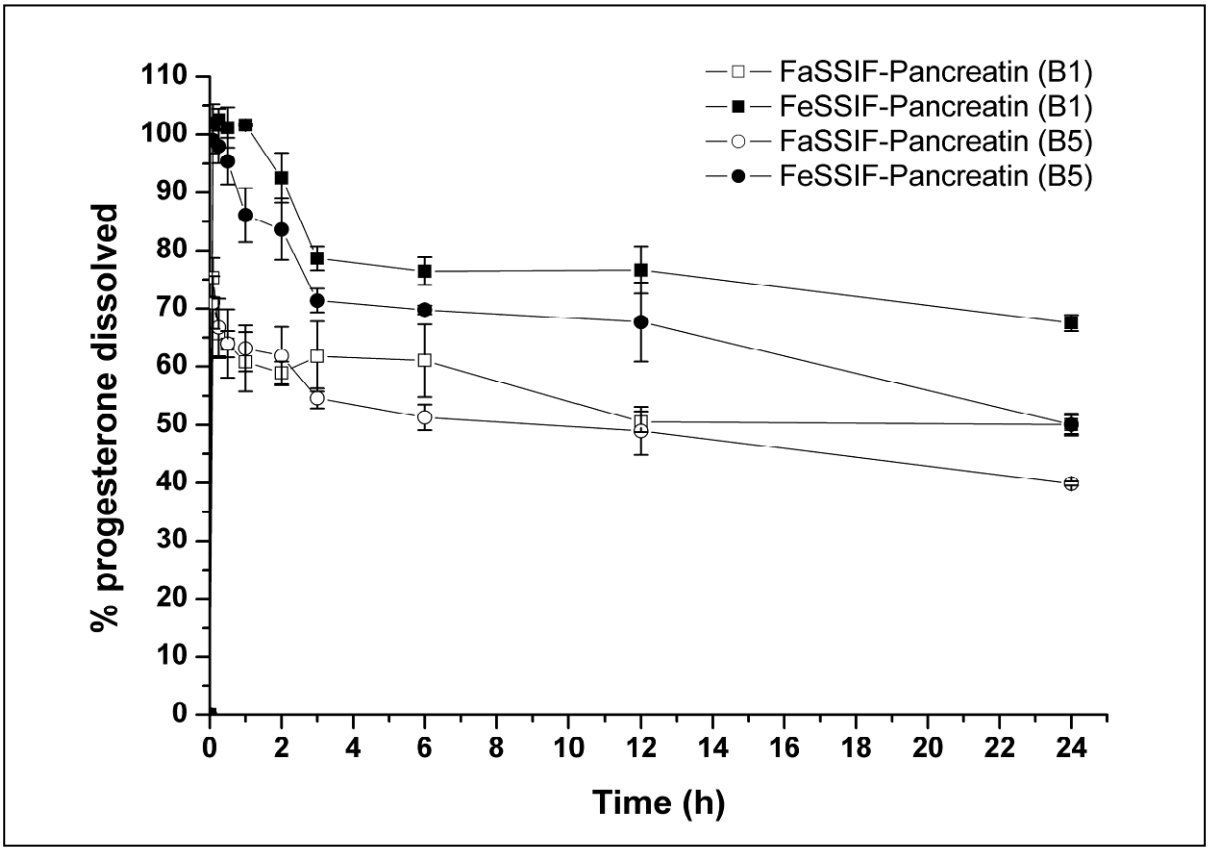

Figure 10.3 Mean (±SD, n=3) percentage of progesterone dissolved as a function of time (h), after the digestion of 1% (w/v) of the SE mixtures B1 in the digestion buffer in FaSSIF (□) and in FeSSIF (■), and SE mixture B5 in FaSSIF (○) and FeSSIF (●). Experiments were initiated by the addition of Pancreatin enzyme (450 U/ml of pancreatic lipase activity).

Furthermore, these data are in agreement with the results obtained from the equilibrium solubility study of progesterone. The results clearly indicate that (i) enzymatic digestion decreases the solubilization capacity for progesterone, (ii) the solubility is enhanced under FeSSIF conditions, (iii) the digested formulation B1 solubilize slightly more drug compared to the digested B5 SE mixture.

3.4.3 Monitoring of In Vitro lipid digestion by ESR

To have an insight into different phases formed during in vitro digestion of the self-emulsifying mixture B5, and to investigate the distribution and localization of TB, as a model for poorly soluble drugs, in such phases, an ESR in vitro digestion experiment was performed. There were no need for centrifugation and separation of different phases formed, and direct measurement of the digestion mixture was carried out at different time intervals after starting the experiment.

Values of rotational correlation time, related to mobility of the spin probe and microviscosity of the media, and hyperfine splitting constant (hfs), related to the micropolarity were obtained from simulating TB spectra by means of ESR spectra simulation software. The results obtained from the control experiment of TB in different media are shown in figure 11.3. There were no difference between water and phosphate buffer with both having small rotational correlation time, reflecting low viscosity, and high hyperfine coupling constant, reflecting high polarity (Figure 11.3 a and b). In mixed micelles and aqueous digestion media, the rotational correlation time increases reflecting a large increase in viscosity compared to water and phosphate buffer. Moreover, a large difference was noticed between the hydrophilic and the lipophilic species in mixed micelles and aqueous digestion media in both FaSSIF and FeSSIF conditions (Figure 11.3 a). The difference in viscosity was more noticeable in the case of mixed micelles in FaSSIF condition compared to other media. A smaller difference in hyperfine coupling constant, and hence in polarity, was noticed between the FeSSIF and the FaSSIF conditions in both mixed micelles and aqueous digestion media (Fig.11.3 b).

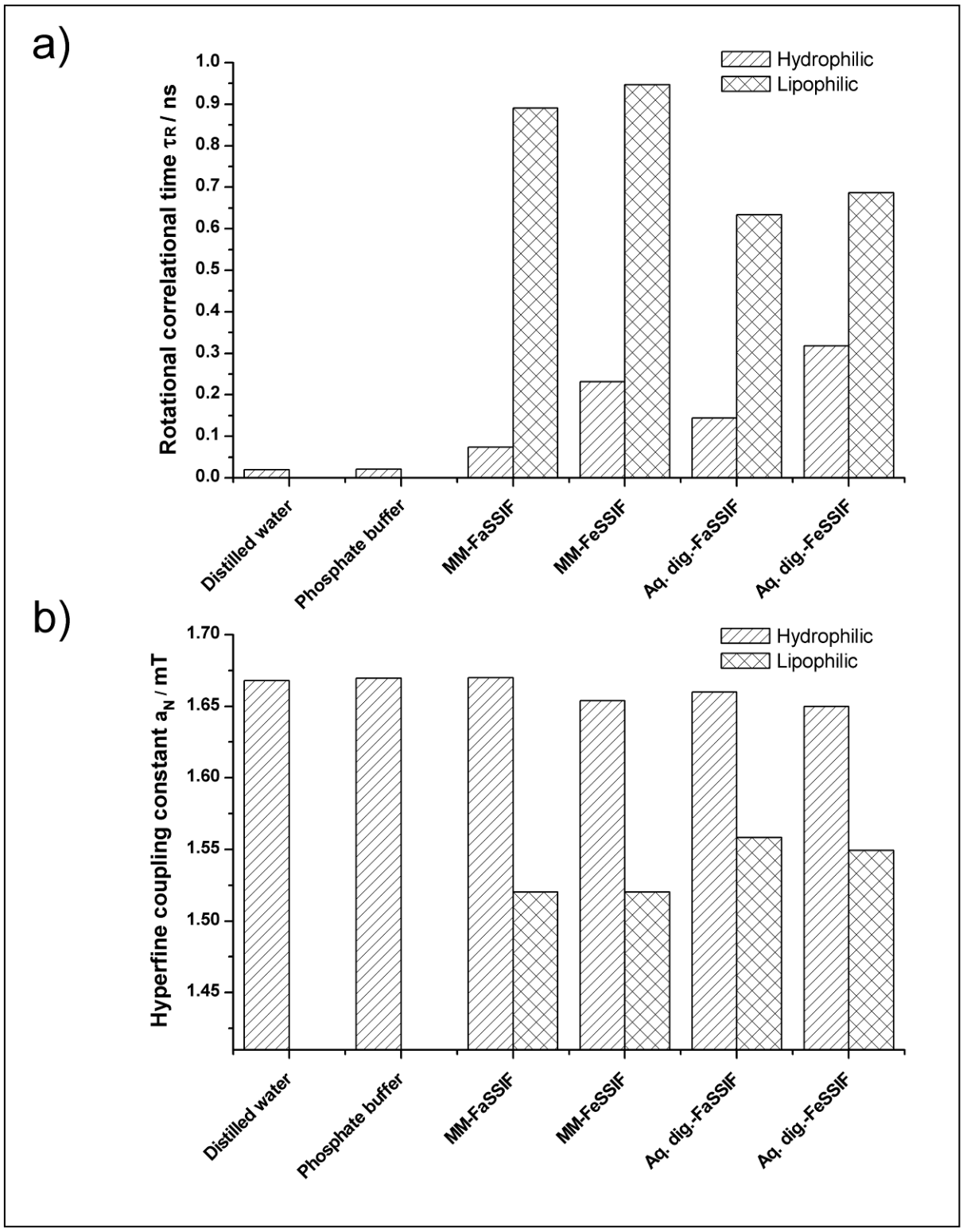

Figure 11.3 Values of a) Rotational correlation time and, b) Hyperfine coupling constant, for TB in different control media.

The 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 (Figure 12.3). In the spectra of the hydrophilic 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.

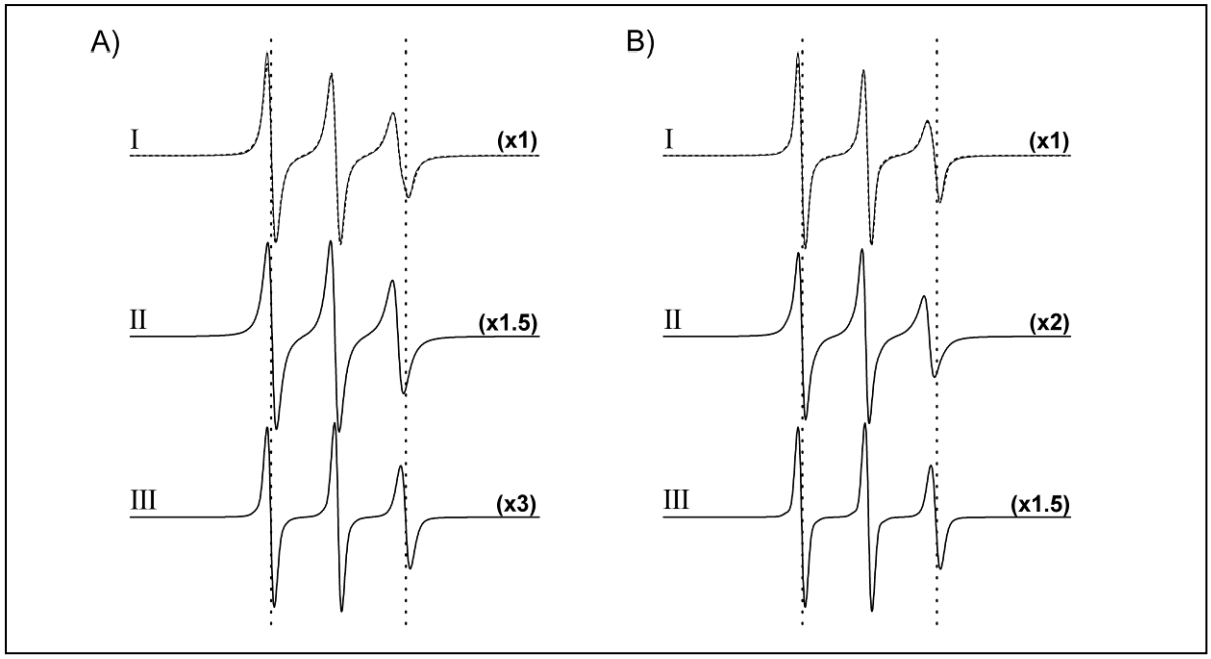

Figure 12.3 ESR simulation spectra of TB after A) 0 min, and B) 30 min of the digestion of 1% (w/v) of The SE mixture B5 in the digestion buffer under FaSSIF conditions. I) Experimental spectrum (bold line) and simulated spectrum (dotted line),II) spectrum of the lipophilic species, III) spectrum of the hydrophilic species.

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 minutes and to 1.58 mT after 10 minutes, which indicates an increase in polarity. The polarity of the lipid phase remains constant after 10 minutes 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 indicates 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 less polar.

In a further step, the quantitative distribution of the spin probe in different environments was calculated. As shown in figure 13.3, the percentage of TB localized in the hydrophilic environment increases significantly within the first 5 minutes under both FaSSIF and FeSSIF digestion conditions and it remains constant for the remaining time. This change in the distribution and localization of the spin probe is caused by enzymatic induced hydrolysis of the apolar triglycerides and the formation of the more polar fatty acids and monoglycerides and the localization of the spin probe into the core of either polar colloidal structures, like mixed micelles, or into the interior of less polar structures, like vesicles or liquid crystalline phases.

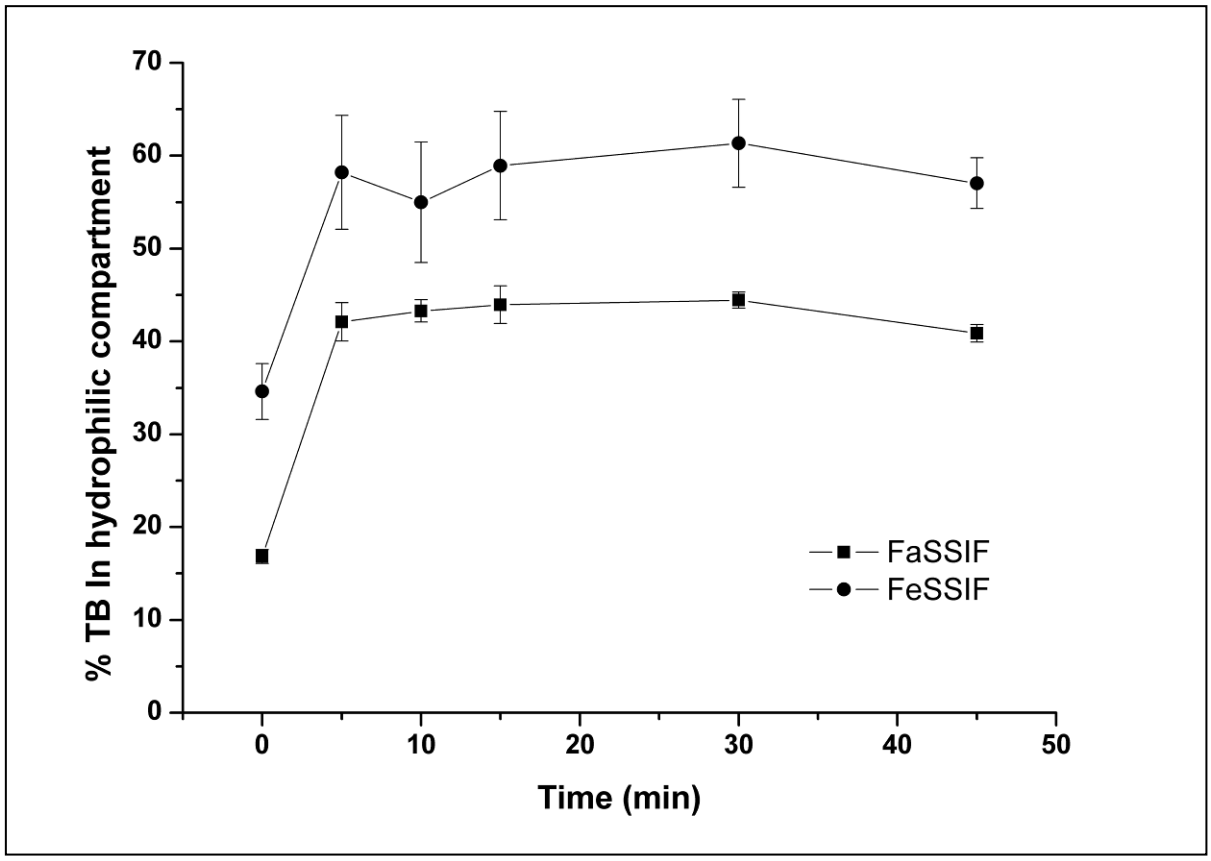

Figure 13.3 Distribution of TB in the hydrophilic compartment as a function of time, after the digestion of 1% (w/v) of the SE mixture B5 in the digestion buffer under FaSSIF (■), and FeSSIF (●) conditions.

The rotational correlation time, related to the mobility of the spin probe and the viscosity of the media, was monitored throughout the digestion of the formulation. For the lipophilic species in both conditions, the microviscosity increases significantly

within the first 5 minutes of the digestion process whereas the viscosity of the hydrophilic species remain almost unchanged for the entire experiment time (Figure 14.3 a and b).

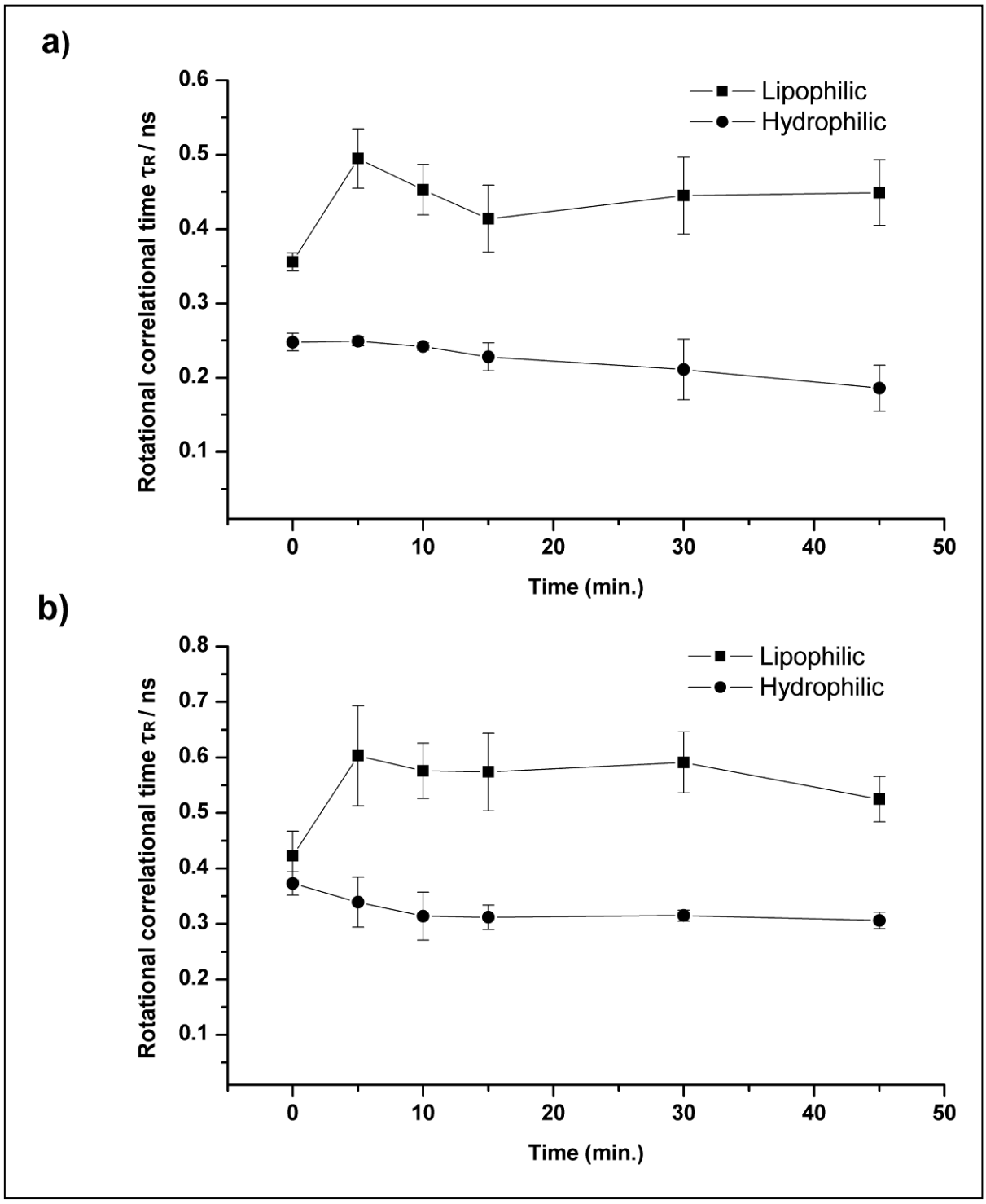

Figure 14.3 Change of rotational correlation time, for lipophilic and hydrophilic species, as a function of time after the digestion of 1% (w/v) of the SE mixture B5 in the digestion buffer under a) FaSSIF and b) FeSSIF conditions.

3.4.4 Formulation and assessments of SE pellets

3.4.4.1 Pellets size and shape analysis

Because of its superior emulsification properties, formulation B5 was selected for incorporation into MCC in different ratios to assess the possibility of pellets production. Pellets were produced using extrusion/spheronization technique. Assessment of the quality of the produced pellets was made by evaluating there size and shape utilizing the same technique described in sections 2.3.2 and 2.3.3 . Details of the results of size and shape analysis are shown in table 3.3 which includes the values of the geometrical mean diameter (D_g) and the geometrical standard deviation, the interquartile range (IQR, expected to include about 50% of the data), the mean Feret diameter ($D_{feret.mean}$) and the aspect ratio. The results show that it was possible to produce pellets with all MCC-lipid ratios used with the exception of the formulation containing equal parts of the components where the extrudate stuck together to form a big mass rather than pellets.

Table 3.3 Results of the size and shape analysis of the pellets

Pellets composition (%w/w)		Sieve analysis (100 g)			Image analysis	
SE mixture (B5)	MCC	Dg (µm)	σ_g	IQR (µm)	$D_{feret.mean.}$(µm)	Aspect ratio
10	90	1103.3	1.16	243	1100.45 (145.7)	1.054 (0.051)
20	80	1274.6	1.38	267	1240.32 (143.2)	1.065 (0.045)
40	60	1353.2	1.59	320	1412.68 (194.6)	1.087 (0.092)

3.4.4.2 ESEM investigations

ESEM investigations show that pellets with different incorporation levels of SE mixture B5 exhibit different surface structures. Micrographs with the lowest magnification show that all produced pellets were spherical in shape, which confirms with the aspect ratio measurements (Figure15.3). More details about the surface characteristics of pellets were obtained with increasing the magnification. Figure 15.3a shows typical features of the surface of MCC pellets with a highly rough and porous surface with apparent cellulose fibers network. For the pellets with 10% SE mixture, the surface become smoother, less porous but the cellulose fibers network

were still observed on the surface (Figure 15.3b). As the SE mixture load increases to 20 and 40 %, the pellets surface becomes smoother. There is obviously an incorporation of the SE mixture within the cellulose fibers network which causes a decrease of the pellet porosity (Fig. 15.3 c and d).

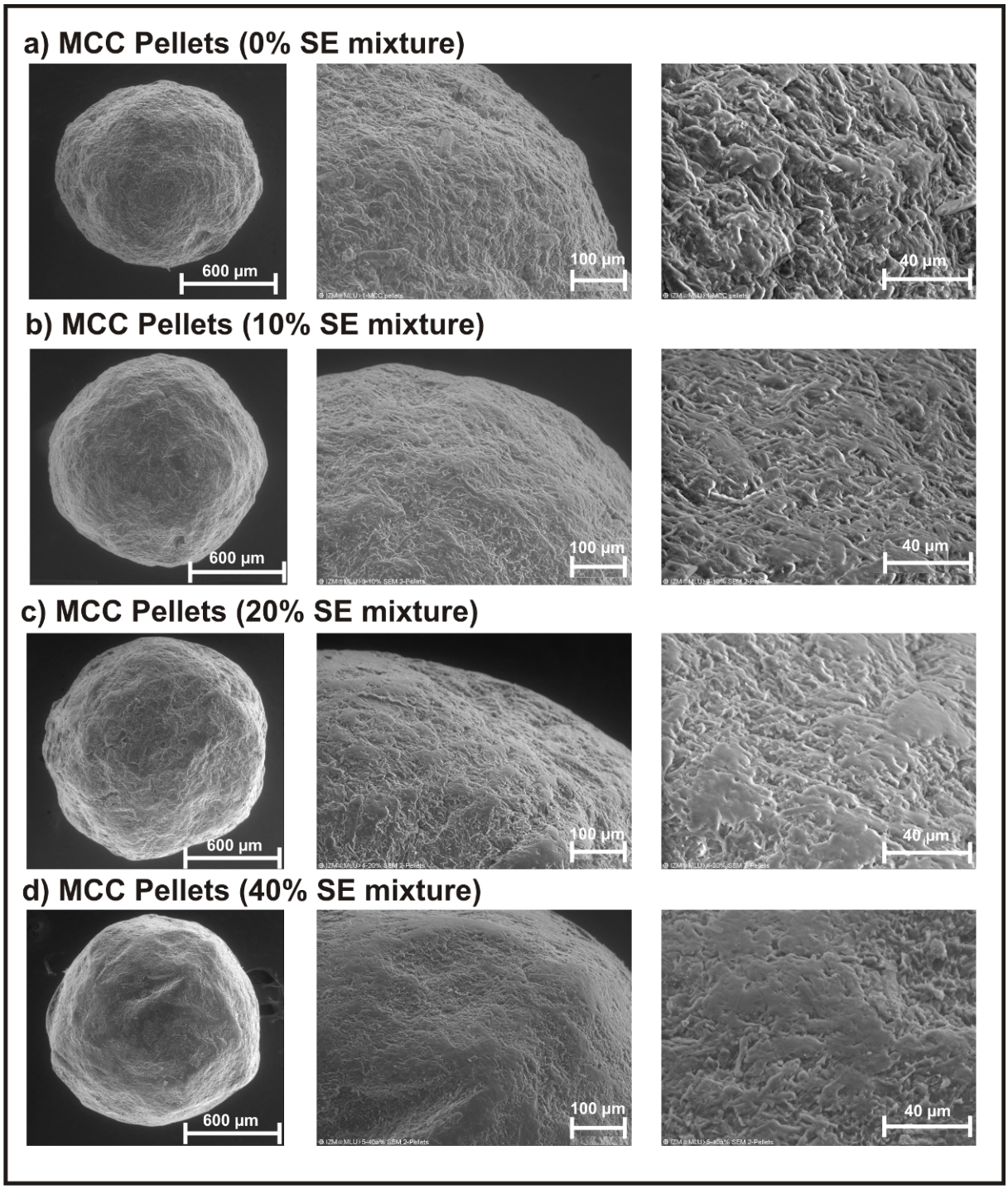

Figure 15.3 ESEM Pictures of self-emulsifying pellets with different load of the liquid self-emulsifying lipid mixture.

3.4.4.3 Assessment of friability

Friability testing of the produced pellets was conducted using the same method described in section 2.3.5. The results show that the friability was less than 1 % for lipid loads below 20 % and less than 1.5 % for the high lipid load of 40 % (Figure 16.3). Interestingly, the small lipid load of 10 % seems to cause even a slight decrease of the friability compared to the MCC control pellets. Generally, friability is increasing with increasing lipid load. The liquid lipid, which is adsorbed between the solid MCC weakens the interactions between the MCC fibers. Nevertheless, the low friability values are very encouraging.

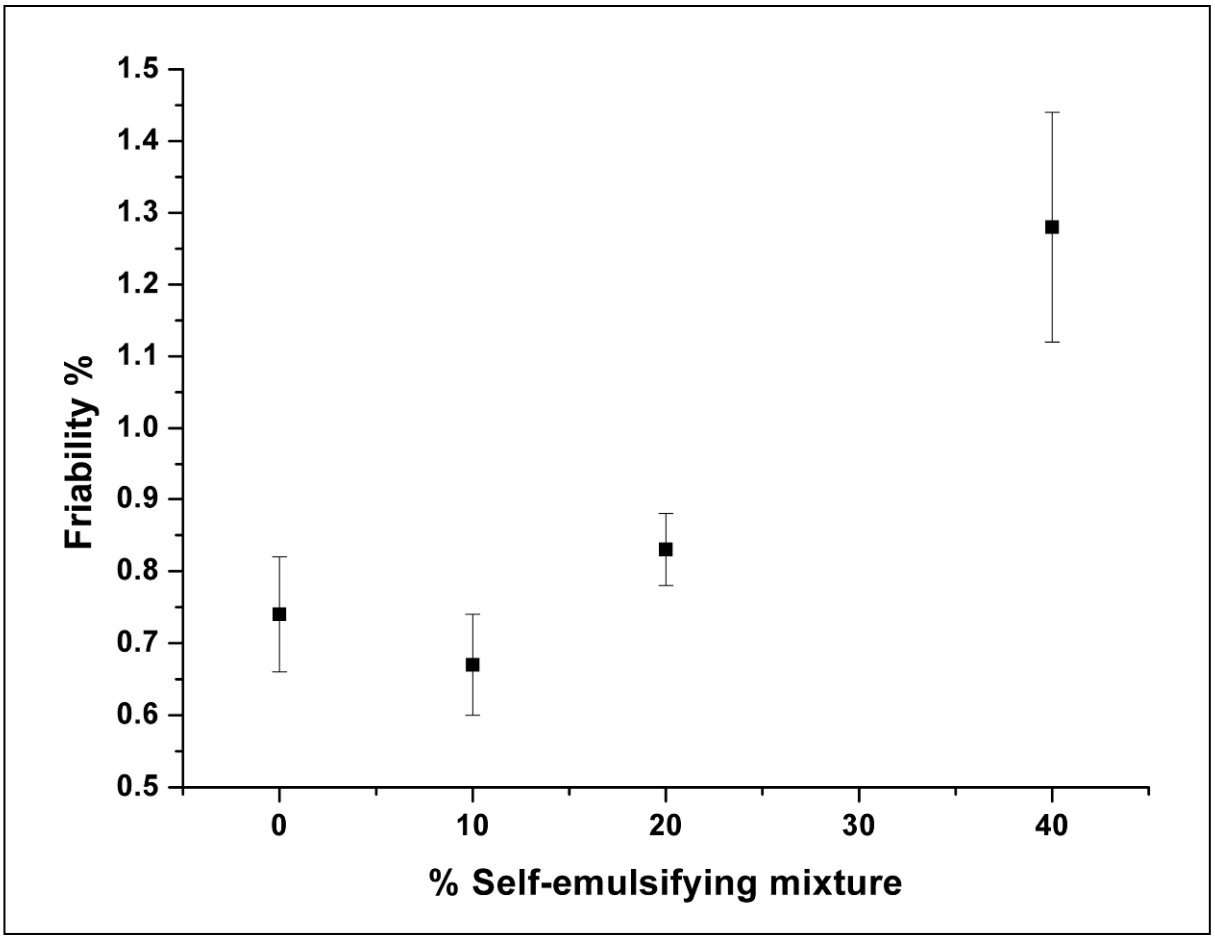

Figure 16.3 Friability % (mean ± SD, n=3) of MCC pellets prepared with different % of self-emulsifying mixture incorporation.

3.4.4.4 NMR T_2 Distribution measurement

^{1}H-NMR was used extensively for the determination of the distribution and the dynamic state of water in different pharmaceutical excipients and food components [214-216]. However, there were no studies reporting the use of this method for the characterization of the physical state and the distribution of lipids in the field of drug delivery. In this study, a new method was introduced for the evaluation of the physical state and the incorporation of the liquid SE mixture B5 inside solid MCC pellets. T_2 distribution measurements were carried out before and after incorporation of the mixture into MCC pellets in three different levels (10, 20, and 40%). Additionally, physical mixtures of the SE mixture with MCC in the same levels were evaluated to serve as references. Figure 17.3 shows the results of T_2 distribution measurements for the physical mixture of the SE mixture before and after physical mixing with MCC in three different levels. For the SE mixture alone the distribution obtained was typical to liquid lipid mixtures (figure 17.3).

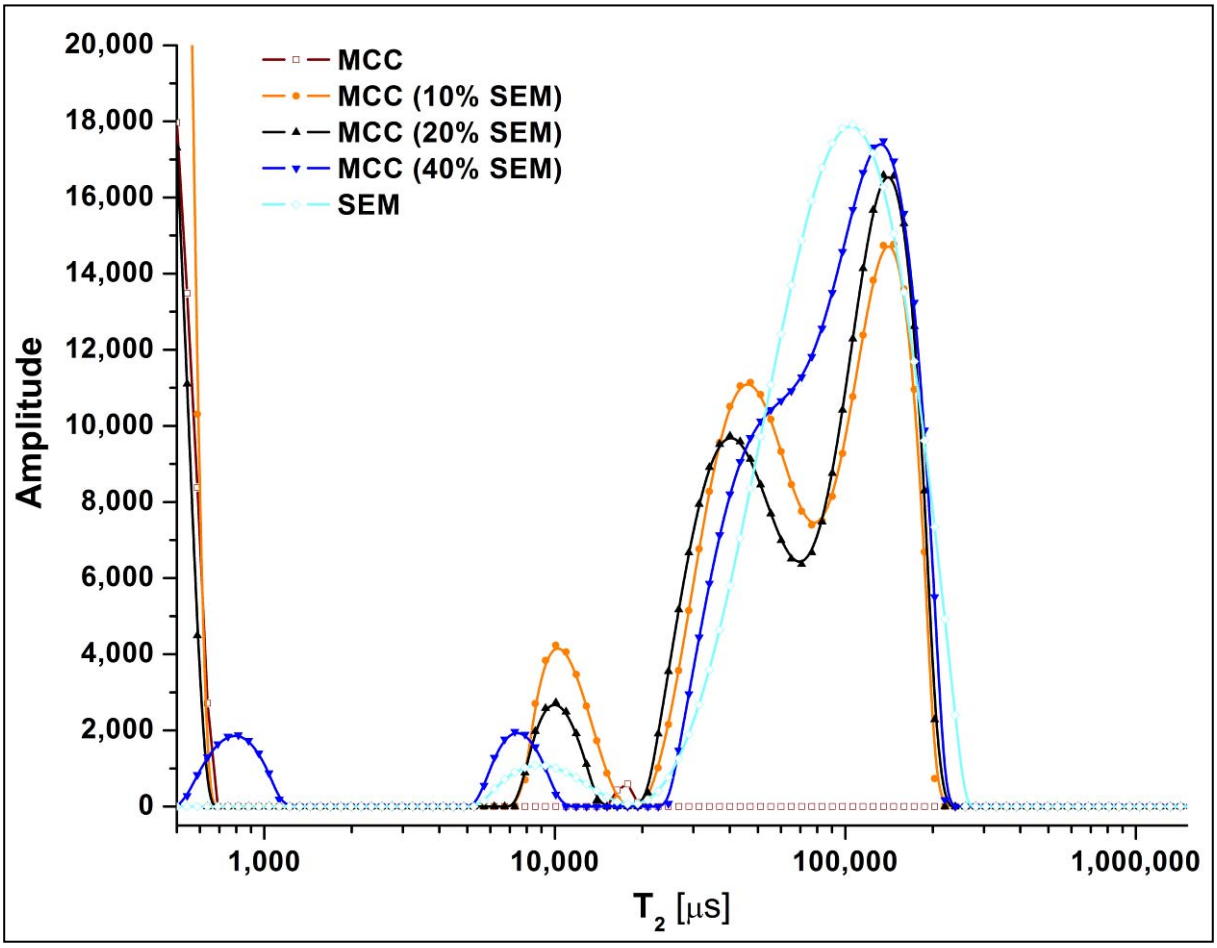

Figure 17.3 NMR T2 distribution of MCC (□), SE mixture B5 (◊), and physical mixtures of MCC with 10 (●), 20 (▲) and 40 % (▼) SE mixture B5.

For the physical mixtures, T_2 distribution were close to that obtained for the SE mixture with the existence of a moderately immobilized species indicating a little incorporation of the SE mixture into the MCC in all levels. For the pellets, dropping of the relaxation time was observed for the 10% SE mixture loaded MCC, indicating that the lipid mixture was strongly immobilized within the MCC matrix. As the SE mixture load increases to 20%, the existence of more mobile species was observed. In the pellets with 40% SEDDS, the freely moving liquid mixture was observed again and the existence of highly (I), medium (II) and low (III) bound species was noticed (figure 18.3). Additionally, in all the produced levels the immobilization was much higher in pellets than that observed in the physical mixture indicating the big effect of the extrusion/spheronization process in incorporating the SE mixture into the MCC.

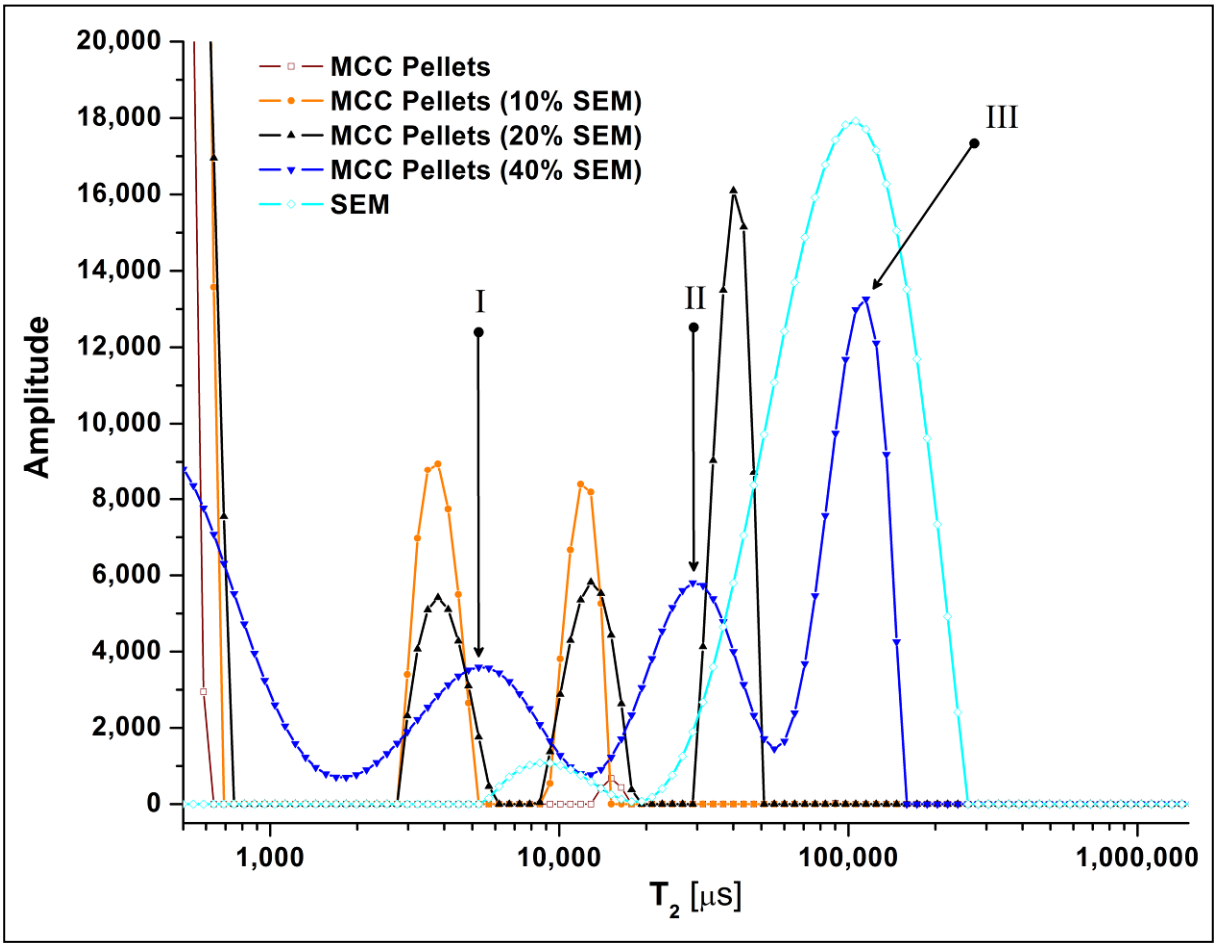

Figure 18.3 NMR T2 distribution of MCC (■), SE mixture B5 (♦), and pellets with 10 (●),20 (▲)and 40 % (▼) of incorporated SE mixture B5.

3.4.4.5 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 (see section 2.3.8 for details). Additionally, the ESR spectra of TB in different formulation components were recorded as references (Figure 19.3). 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 higher than that in self-emulsifying pellets due to the partial immobilization of the self-emulsifying mixture within MCC.

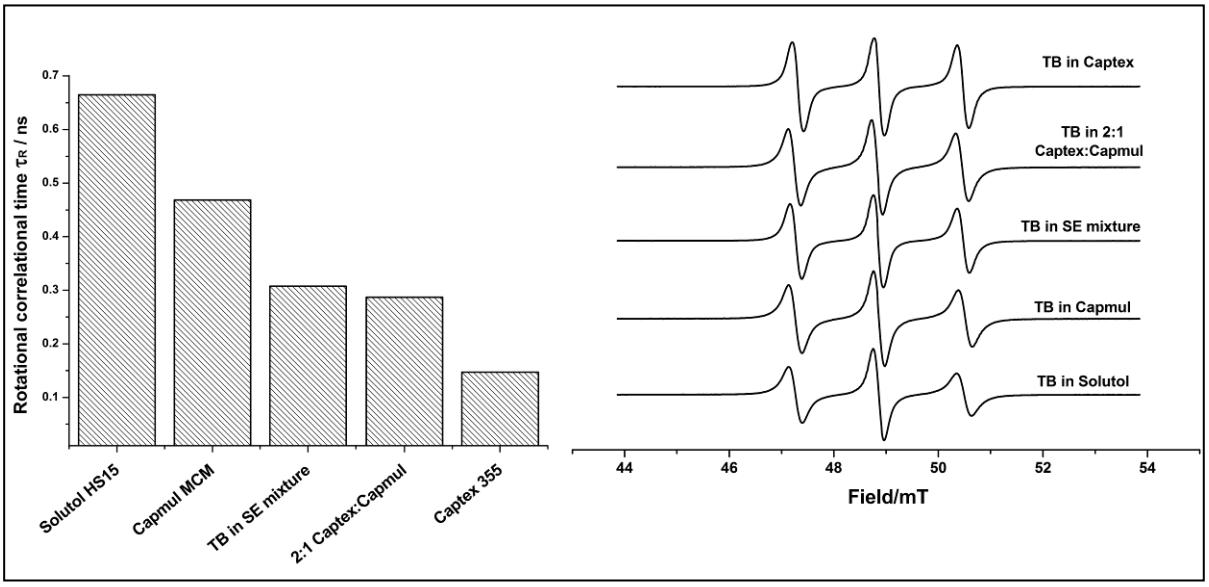

Figure 19.3 ESR spectra of TB in different formulation components and mixtures, reflecting the sensitivity of the line shape (right) and rotational correlation time (left) to the viscosity of different media.

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. Immediately after pellets exposure to the dissolution media inside the flow cell, the mobility of the spin probe changed rapidly within the first 5 minutes and was comparable to that in the free self-

emulsifying mixture (Figure 20.3). 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 minutes.

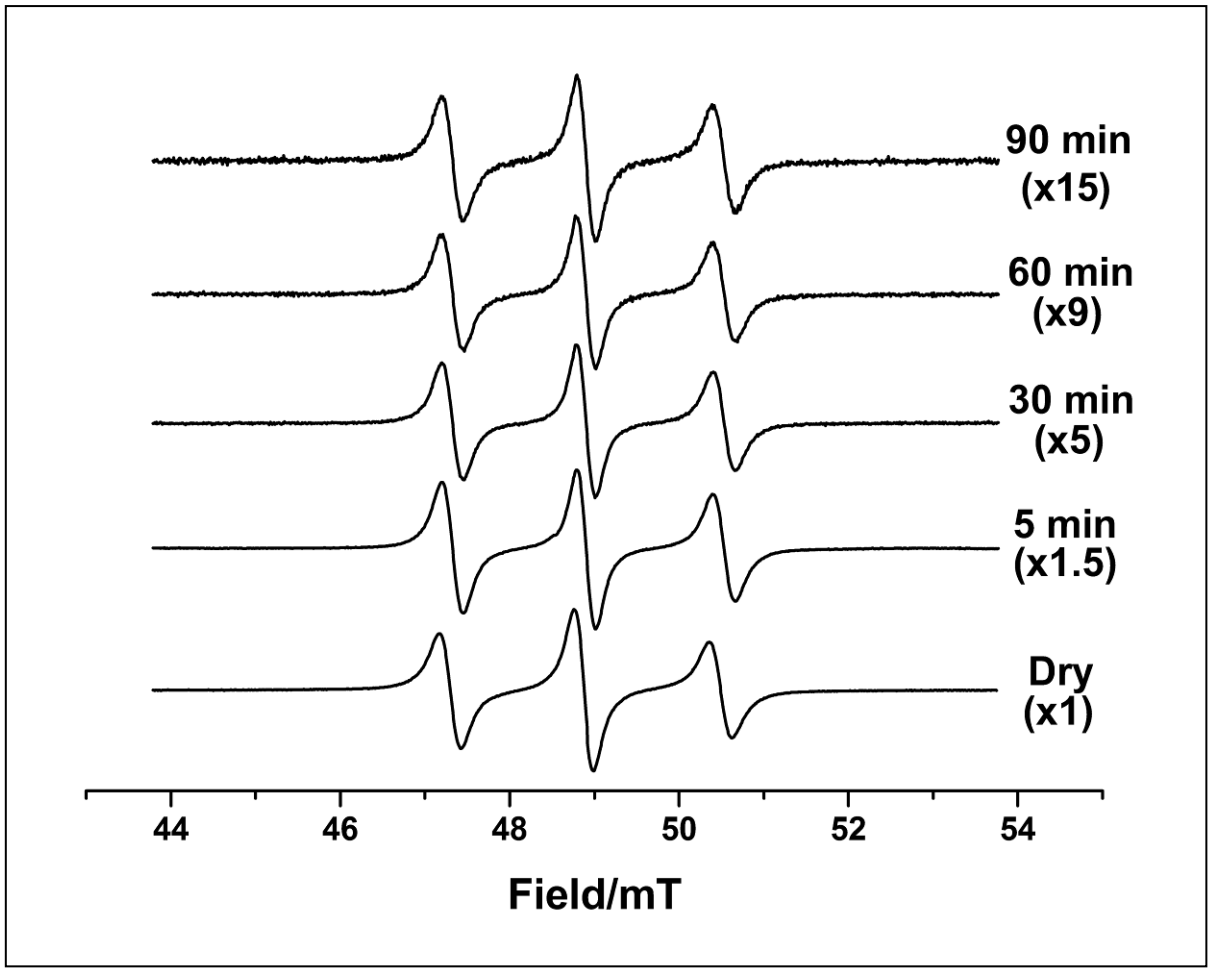

Figure 20.3 ESR spectra of TB loaded pellets before, 5, 30, 60 and 90 min after buffer exposure in the flow cell system.

3.4.4.6 In Vitro release testing

In vitro release experiments were conducted to evaluate the effect of different media on the release of Progesterone from the pellets. The results are shown in figure 21.3. In phosphate buffer, Progesterone was completely released within the first 2 hours of the study and no precipitation was noticed until the end of the experiment. On the other hand, release of Progesterone in the digestion media under

both FaSSIF and FeSSIF conditions was incomplete and precipitation of Progesterone was observed in the first few minutes of the experiment. Therefore, the presence of mixed micelles interferes with the performance of the SE lipid system under digestion conditions and leads to a decreased solubilization of the drug. Enhanced Progesterone precipitation in the FeSSIF and FaSSIF digestion media compared to the mixed micelle free medium could be attributable to several reasons including:

- Decreased digestion rate in the buffer medium due to insufficient solubilization of the digestion products.
- The presence of mixed micelles changes the composition of the lipid phase in the SE mixtures (e.g. translocation of SE components in the mixed micelles and of Bile acids/lecithin into the SE droplets) which results finally in a decreased solubilization capacity compared to the parent SE system.

It is also likely that both reasons contribute to the Progesterone precipitation in the FeSSIF and FaSSIF media.

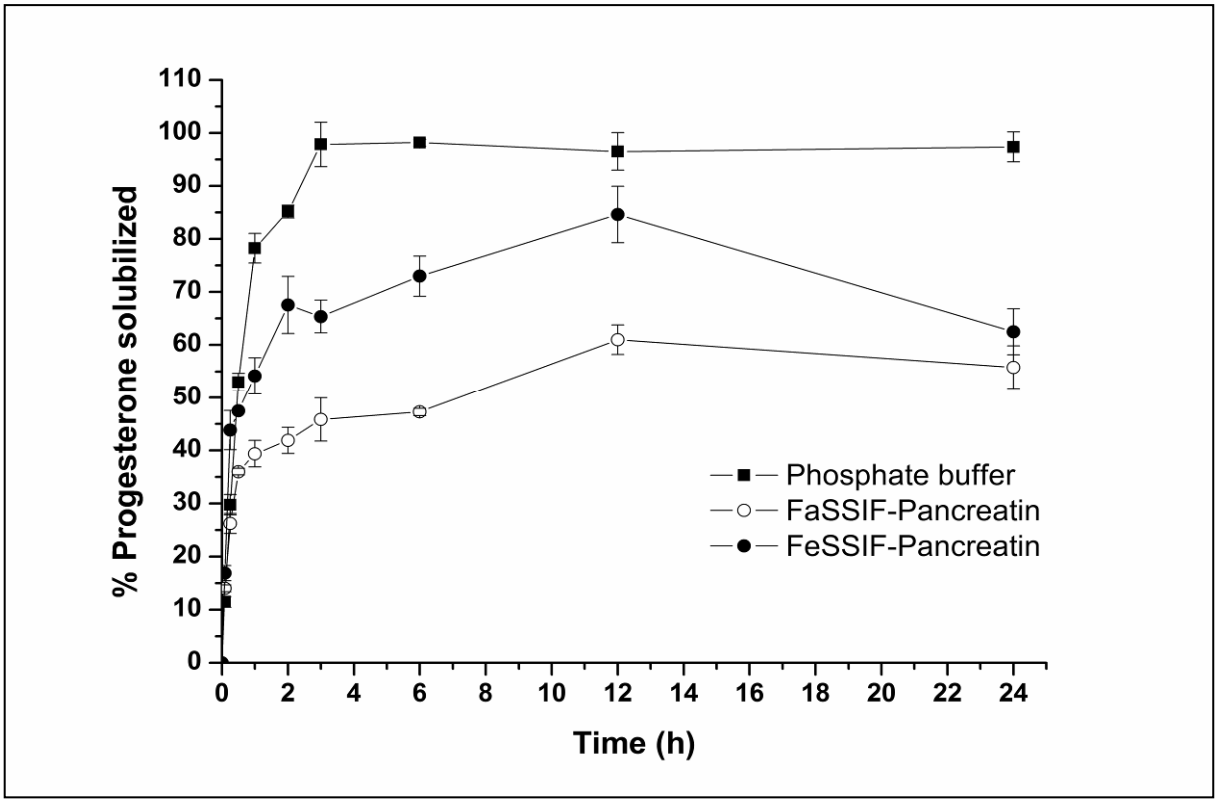

Figure 21.3 Mean (±SD, n=3) percentage of progesterone released as a function of time (h) from progesterone loaded SE pellets in 7.5 ml phosphate buffer, pH 6.8,(■), and in digestion buffer in both FaSSIF (○) and FeSSIF (●) conditions. Sample of pellets corresponding to 1% (w/v) final SE mixture concentration in the release media was assayed.

3.5 Conclusion

Stable isotropic SE mixtures have been formulated using a mixture of medium chain mono- and di-glycerides, medium chain triglycerides, and Solutol® HS 15 as a surfactant. The droplet sizes after dilution decreased with increasing Solutol® HS 15 content and were lower for more polar lipid mixtures (e.g. increased ratio of partial glycerides compared to triglycerides). SE mixtures are able to solubilize progesterone in buffer. The results have shown the potential advantages of using ESR spectroscopy to asses the micropolarity within the emulsion droplet of diluted SE mixtures. This can help in developing an optimum mixture for a certain drug in order to achieve a compromise between solubilization of the drug and its distribution into the aqueous media of the GIT after dispersion. After dilution of different spin probe loaded self-emulsifying mixtures, ESR spectra with a splitting of third line were obtained. This indicates the availability of two environments, a lipophilic highly viscous environment and a very hydrophilic environment with low viscosity.

Digestion of the lipid phase decreases the solubilization capacity for progesterone. It was also shown that the solubilization capacity strongly depends on the concentration of endogenously secreted materials such as BS and PL. 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 formulation triglycerides resulting in the formation of fatty acids and monoglycerides. In the presence of endogenous materials like bile salts, phospholipids and cholesterol, fatty acids and monoglycerides are then incorporated into different phases with different polarity and viscosity. The spin probe 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.

The liquid lipid SE mixture was successfully transformed into solid pellets by means of extrusion/spheronization with a maximum load of 40%. The pellets had a uniform size, a spherical shape and low friability. ESEM pictures show the adsorption of the liquid oil between the MCC fibers. Moreover, the self-emulsifying properties are

still preserved in the pellets. Therefore, extrusion/spheronization of SEDDS systems is an alternative to encapsulation in gelatine capsules.

The NMR results have shown the potential advantages of using NMR T_2 distribution measurements for the characterization of lipids. A higher degree of immobilization was achieved with the pellets formulated using extrusion /spheronization compared to the physical mixtures of the SE mixture and MCC.

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.

4 Summary

4.1 English version

The idea of combining advantages of pellets, as a solid multiparticulate drug delivery system, and SEDDS, which was successfully used to improve bioavailability of lipophilic drugs, was the main motive behind the current research work. Two different self-emulsifying systems have been developed for testing the feasibility of incorporating them into MCC pellets by means of extrusion/spheronization.

The first self-emulsifying mixture was composed of a mixture of Cithrol GMS (C18 mono- and di-glycerides) and Solutol HS15. This mixture was incorporated into MCC and pellets were produced using Extrusion/spheronization technique. Furthermore, reference pellets containing GMS and MCC were formulated. Pellets were characterized for size, shape, friability and dissolution for diazepam loaded pellets. In addition, nitroxide loaded pellets were produced and the microenvironment inside the pellets during the release process was monitored in an online process using ESR spectroscopy. Results demonstrated the possibility of producing self-emulsifying pellets containing 50% of the Self-emulsifying mixture. The produced pellets were of good physical properties concerning shape, size distribution and friability. In contrast to GMS-MCC pellets, they were able to introduce the lipophilic dyes or drugs into the aqueous phase of the dissolution media. This shows that GMS alone is not self-emulsifying and solutol addition was necessary to achieve self-emulsification properties. The ESR results show that the lipophilic spin probe was mainly localized in the lipid environment inside the pellets for the whole release period. The release data showed a noticeable improvement in the in vitro dissolution of diazepam in comparison with the non-emulsifying formulation. Additionally, a supersaturated state for the water soluble diazepam was achieved and maintained in a dissolution study with a higher diazepam load.

The second self-emulsifying mixture was composed of a mixture of medium chain mono- and di-glycerides (Capmul®), medium chain tri-glycerides mixture (Captex®) and Solutol® HS15. Self-emulsifying mixtures with different composition were formulated and droplet diameter of the emulsion resulted from the dilution of SE mixtures were measured using PCS. ESR was used to monitor the microenvironment of the diluted mixtures and to evaluate the polarity of emulsion droplets. Additionally,

in vitro digestion experiments were carried out to asses the change in the solubilization capacity of progesterone after dilution and enzymatic digestion of self-emulsifying mixtures in simulated intestinal fluids in both the fasted and the fed state. In vitro digestion was also monitored by ESR spectroscopy to have an insight on the characteristics of the different phases formed during digestion process and to evaluate the distribution and localization of the nitroxide spin probe TB in these phases. Self-emulsifying mixture that combines good emulsification properties, acceptable solubilization of the model drug and optimum surfactant concentration was then incorporated into MCC and self-emulsifying pellets were produced by means of extrusion/spheronization. The formulated pellets were then characterized for size and shape. Furthermore, NMR T2 distribution measurements were carried out to evaluate the incorporation of different levels of self-emulsifying mixture into MCC pellets. TB loaded self-emulsifying pellets were produced and an ESR flow through experiment was carried out to monitor the microenvironment within pellets during the release process. Dissolution experiment was designed to evaluate the release of progesterone from pellets in different release media. The results show that the optical clarity and droplet diameter of the dispersed SE mixtures were largely affected by changing oil to Solutol ratio. The percent of TB localized in the lipophilic environment of the diluted self-emulsifying mixture was decreasing with increasing the surfactant fraction. Different phases with variable viscosity and polarity have been resulted from the digestion of SE mixtures. This leads to a change in the solubilization capacity of progesterone. Pellets with good properties, concerning size and shape, have been produced through the incorporation of a selected SE mixture into MCC. NMR T_2 distribution measurements showed that as the lipid load increases the existence of highly, medium and low bound species was noticed within pellets.

4.1 German version

Die Motivation für diese Arbeit war es, die Vorteile von selbstemulgierenden Drug-Delivery-Systemen (SEDDS), die zur Verbesserung der Bioverfügbarkeit schwerlöslicher Arzneistoffe verwendet werden, mit den Vorteilen von Pellets als feste multipartikuläre Drug-Delivery-Systeme zu kombinieren,. Zwei verschiedene selbstemulgierende Systeme wurden entwickelt, um zu untersuchen, ob sie durch Extrusion/ Spheronisation in MCC-Pellets eingebaut werden können.

Das erste selbstemulgierende System bestand aus Cithrol GMS (C18-Mono- und Diglyceride) und Solutol HS15. Diese Mischung wurde in MCC eingearbeitet und dann zur Herstellung von Pellets nach der Extrusions-/ Spheronisationsmethode verwendet. Weiterhin wurden Referenz-Pellets hergestellt, welche nur Cithrol GMS und MCC enthielten. Die Pellets wurden hinsichtlch ihrer Größe, Gestalt und ihres Abriebs untersucht. Für die mit Diazepam beladenen Pellets wurden Freisetzungs-Tests durchgeführt. Zusätzlich wurden auch Pellets hergestellt, die eine Nitroxid-Spinsonde als Modellarzneistoff enthielten. Mit Hilfe der ESR Spektroskopie war es so möglich, das Mikromilieu innerhalb der Pellets während des gesamten Freisetzungsprozesses zu untersuchen.

Die Ergebnisse dieser Untersuchungen zeigten, dass es möglich ist selbstemulgierende Pellets mit einem Anteil von 50 % des selbstemulgierenden Systems herzustellen. Die hergestellten Pellets hatten eine gute Qualität zeichneten sich durch eine homogene Größe, sphärische Gestalt und geringen Abrieb aus. Im Gegensatz zu den GMS-MCC-Pellets waren sie in der Lage, die lipophile Spinsonde und Diazepam in das wässrige Freisetzungsmedium freizusetzen. Daraus folgt, dass die Zugabe von Solutol HS15 notwendig ist, um ein selbstemulgierendes System zu erhalten.

Die ESR-Daten ergaben, dass die lipophile Spinsonde während der gesamten Freisetzungsperiode hauptsächlich in den hydrophoben Bereichen innerhalb der Pellets lokalisiert ist. Durch die Freisetzungsversuche mit den Diazepam beladenen Pellets konnte gezeigt werden, dass die in-vitro Freisetzung von Diazepam deutlich? verbessert wurde im Vergleich zu nichtemulgierenden Systemen. Durch Verwendung höherer Diazepam-Konzentrationen war es sogar möglich, einen übersättigten Zustand für Diazepam in Wasser zu erreichen und diesen über den Freisetzungszeitraum zu erhalten.

Das zweite selbstemulgierende System enthielt ein Gemisch aus mittelkettigen Mono- und Diglyceriden (Capmul®), mittelkettigen Triglyceriden (Captex®) und Solutol HS15. Selbstemulgierende Systeme mit unterschiedlicher Zusammensetzung wurden hergestellt. Die jeweils nach Verdünnung resultierenden Tröpfchengrößen wurden mittels Photonenkorrelationsspektroskopie (PCS) bestimmt. Durch ESR Spektroskopie war es möglich die Polaritätsverhältnisse in diesen Emulsionssystemen zu bestimmen.

Außerdem wurden in-vitro Verdauungsexperimente durchgeführt, um zu untersuchen ob die Solubilisierung von Progesteron durch Verdünnung und enzymatische Verdauung der selbstemulgierenden Systeme in simulierten intestinalen Flüssigkeiten (im nüchternen und postprandialen Zustand) beeinflusst wird. Der Verdauungsprozess wurde auch durch ESR Spektroskopie beobachtet, um einen Einblick in die Eigenschaften der verschiedenen Phasen zu erhalten, die sich beim Verdauungsprozess bilden. Außerdem wurde das Verteilungsverhalten und die bevorzugte Lokalisation der Spinsonde Tempolbenzoat (TB) in diesen Phasen untersucht.

Die Lipidsysteme, die sowohl gute selbstemulgierende Eigenschaften, als auch ausreichende Arzneistoff-Solubilisation und optimale Tensid-Konzentration aufwiesen, wurden ausgewählt und zusammen mit MCC zu selbstemulgierenden Pellets verarbeitet. Diese Pellets wurden hinsichtlich Gestalt und Größe untersucht. Außerdem wurden mittels ^{1}H-NMR T2-Relaxationszeitverteilungen bestimmt, um die Inkorporation des selbstemulgierenden Systems in die MCC-Pellets zu untersuchen.

Selbstemulgierende Pellets, die TB als Modellarzneistoff enthielten, wurden hergestellt und für Freisetzungsversuche mittles einer ESR-Durchflusszelle verwendet. Außerdem wurde auch die Freisetzung von Progesteron aus den Pellets in verschiedenen Freisetzungsmedien untersucht. Die Ergebnisse zeigen, dass die optische Transparenz und die Tröpfchengröße der dispergierten SE-Systeme stark vom Verhältnis zwischen Öl und Solutol abhängen. Ein steigender Anteil an Solutol führte zu einem höheren Prozentsatz an TB in der hydrophilen Phase innerhalb des dispergierten selbstemulgierenden Systems. Verdauungsprozesse führen zu einer Bildung verschiedener Phasen, die sich hinsichtlich ihrer Viskosität und Polarität unterscheiden lassen. Dadurch kommt es zu einer Veränderung in der Solubilisierungskapazität für Progesteron.

Einige selbstemulgierende Systeme wurde ausgewählt und nach Mischen mit MCC dazu verwendet, Pellets mit guten Eigenschaften hinsichtlich Größe und Gestalt herzustellen. NMR Relaxationszeitmessungen zur Bestimmung von T2 Verteilungen zeigten, dass mit steigender Lipidkonzentration stark, mittel und schwach gebundene Lipidanteile nachweisbar sind.

5. References

[1] C.A. Lipinski. Drug-like properties and the causes of poor solubility and poor permeability. *J. Pharmacol. Toxicol. Meth.* 44:235-249 (2000).

[2] D. Horter and J.B. Dressman. Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract. *Adv. Drug Deliv. Rev.* 25:3-14 (1997).

[3] C.A. Lipinski, F. Lombardo, B.W. Dominy, and P.J. Feeney. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* 23:3-25 (1997).

[4] C.A. Lipinski, F. Lombardo, B.W. Dominy, and P.J. Feeney. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* 46:3-26 (2001).

[5] M. Gibaldi, S. Feldman, and N.D. Weiner. Hydrodynamic and diffusional considerations in assessing effects of surface active agents on dissolution rate of drugs. *Chem. Pharmaceut. Bull.* 18:715-723 (1970).

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

[7] M. Gibaldi and S. Feldman. Mechanisms of surfactant effects on drug absorption. *J. Pharm. Sci.* 59:579-589 (1970).

[8] M. Gibaldi. Introduction to biopharmaceutics, Lea & Febiger 1971.

[9] L.X. Yu, G.L. Amidon, J.E. Polli, H. Zhao, M.U. Mehta, D.P. Conner, V.P. Shah, L.J. Lesko, M.L. Chen, V.H.L. Lee, and A.S. Hussain. Biopharmaceutics classification system: The scientific basis for biowaiver extensions. *Pharm. Res.* 19:921-925 (2002).

[10] S. Pinnamaneni, N.G. Das, and S.K. Das. Formulation approaches for orally administered poorly soluble drugs. *Pharmazie.* 57:291-300 (2002).

[11] J.C. Chaumeil. Micronization: A method of improving the bioavailability of poorly soluble drugs. *Meth. Find. Exp. Clin. Pharmacol.* 20:211-215 (1998).

[12] G.T. Mcinnes, M.J. Asbury, L.E. Ramsay, J.R. Shelton, and I.R. Harrison. Effect of micronization on the bioavailability and pharmacologic activity of spironolactone. *J. Clin. Pharmacol.* 22:410-417 (1982).

[13] R.H. Muller, C. Jacobs, and O. Kayser. Nanosuspensions as particulate drug formulations in therapy rationale for development and what we can expect for the future. *Adv. Drug Deliv. Rev.* 47:3-19 (2001).

[14] V.B. Patravale, A.A. Date, and R.M. Kulkarni. Nanosuspensions: A promising drug delivery strategy. *J. Pharm. Pharmacol.* 56:827-840 (2004).

[15] D. Duchene and G. Ponchel. Bioadhesion of solid oral dosage forms, why and how? *Eur. J. Pharm. Biopharm.* 44:15-23 (1997).

[16] C. Nyström. Dissolution properties of soluble drugs: Theoretical background and possibilities to improve the dissolution behaviour. In R.H. Müller, S. Benita, and B. Böhm (eds.), *Emulsions and nanosuspensions for the formulation of poorly soluble drugs*, Medpharm Scientific Publishers, Stuttgart, 1998, p. 143.

[17] E. MeriskoLiversidge, P. Sarpotdar, J. Bruno, S. Hajj, L. Wei, N. Peltier, J. Rake, J.M. Shaw, S. Pugh, L. Polin, J. Jones, T. Corbett, E. Cooper, and G.G. Liversidge. Formulation and antitumor activity evaluation of nanocrystalline suspensions of poorly soluble anticancer drugs. *Pharm. Res.* 13:272-278 (1996).

[18] E. Merisko-Liversidge, G.G. Liversidge, and E.R. Cooper. Nanosizing: A formulation approach for poorly-water-soluble compounds. *Eur. J. Pharm. Sci.*18:113-120 (2003).

[19] P. Gassmann, M. List, A. Schweitzer, and H. Sucker. Hydrosols - alternatives for the parenteral application of poorly water-soluble drugs. *Eur. J. Pharm. Biopharm.* 40:64-72 (1994).

[20] C. Jacobs, O. Kayser, and R.H. Muller. Nanosuspensions as a new approach for the formulation for the poorly soluble drug tarazepide. *Int. J. Pharm.* 196:161-164 (2000).

[21] C. Jacobs, O. Kayser, and R.H. Muller. Production and characterisation of mucoadhesive nanosuspensions for the formulation of bupravaquone. *Int. J. Pharm.* 214:3-7 (2001).

[22] O. Kayser. A new approach for targeting to cryptosporidium parvum using mucoadhesive nanosuspensions: Research and applications. *Int. J. Pharm.* 214:83-85 (2001).

[23] S.R. Vippagunta, H.G. Brittain, and D.J.W. Grant. Crystalline solids. *Adv. Drug Deliv. Rev.* 48:3-26 (2001).

[24] J. Haleblia and W. McCrone. Pharmaceutical applications of polymorphism. *J. Pharm. Sci.* 58:911-929 (1969).

[25] S. Agatonovic-Kustrin, V. Wu, T. Rades, D. Saville, and I.G. Tucker. Powder diffractometric assay of two polymorphic forms of ranitidine hydrochloride. *Int. J. Pharm.* 184:107-114 (1999).

[26] A.M. Kaushal, P. Gupta, and A.K. Bansal. Amrphous drug delivery systems: Molecular aspects, design, and performance. *Crit. Rev. Ther. Drug Carrier Syst.* 21:133-193 (2004).

[27] A.H. Goldberg, M. Gibaldi, J.L. Kanig, and Mayersoh.M. Increasing dissolution rates and gastrointestinal absorption of drugs via solid solutions and eutectic mixtures .4. Chloramphenicol-urea system. *J. Pharm. Sci.* 55:581-583 (1966).

[28] V. Andronis and G. Zografi. Molecular mobility of supercooled amorphous indomethacin, determined by dynamic mechanical analysis. *Pharm. Res.* 14:410-414 (1997).

[29] C. Leuner and J. Dressman. Improving drug solubility for oral delivery using solid dispersions. *Eur. J. Pharm. Biopharm.* 50:47-60 (2000).

[30] J. Breitenbach. Melt extrusion: From process to drug delivery technology. *Eur. J. Pharm. Biopharm.* 54:107-117 (2002).

[31] M. Mayersoh and M. Gibaldi. New method of solid-state dispersion for increasing dissolution rates. *J. Pharm. Sci.* 55:1323-1324 (1966).

[32] A.H. Goldberg, M. Gibaldi, and J.L. Kanig. Increasing dissolution rates and gastrointestinal absorption of drugs via solid solutions and eutectic mixtures .3. Experimental evaluation of griseofulvin-succinic acid solid solution. *J. Pharm. Sci.* 55:487-492 (1966).

[33] A.H. Goldberg, M. Gibaldi, and J.L. Kanig. Increasing dissolution rates and gastrointestinal absorption of drugs via solid solutions and eutectic mixtures .2. Experimental evaluation of a eutectic mixture - urea-acetaminophen system. *J. Pharm. Sci.* 55:482-487 (1966).

[34] G.V. Betageri and S.R. Dipali. Preparation and in-vitro dissolution profiles of tolazamide-polyethylene glycol solid dispersions. *Drug Dev. Ind. Pharm.* 21:1347-1352 (1995).

[35] V.L. Bassani, D. Krieger, D. Duchene, and D. Wouessidjewe. Enhanced water-solubility of albendazole by hydroxypropyl-beta-cyclodextrin complexation. *Journal of Inclusion Phenomena and Molecular Recognition in Chemistry.* 25:149-152 (1996).

[36] A.A. Rasool, A.A. Hussain, and L.W. Ditter. Solubility enhancement of some water-insoluble drugs in the presence of nicotinamide and related-compounds. *J. Pharm. Sci.* 80:387-393 (1991).

[37] L. Szente and J. Szejtli. Highly soluble cyclodextrin derivatives: Chemistry, properties, and trends in development. *Adv. Drug Deliv. Rev.* 36:17-28 (1999).

[38] P. Montassier, D. Duchene, and M.C. Poelman. Inclusion complexes of tretinoin with cyclodextrins. *Int. J. Pharm.* 153:199-209 (1997).

[39] J.W. Wong and K.H. Yuen. Improved oral bioavailability of artemisinin through inclusion complexation with beta- and gamma-cyclodextrins. *Int. J. Pharm.* 227:177-185 (2001).

[40] S. Chakrabarti and F.M. Belpaire. Bioavailability of phenytoin in lipid containing dosage forms in rats. *J. Pharm. Pharmacol.* 30:330-331 (1978).

[41] T. Tokumura, Y. Tsushima, K. Tatsuishi, M. Kayano, Y. Machida, and T. Nagai. Enhancement of the oral bioavailability of cinnarizine in oleic-acid in beagle dogs. *J. Pharm. Sci.* 76:286-288 (1987).

[42] A.K. Trull, K.K.C. Tan, L. Tan, G.J.M. Alexander, and N.V. Jamieson. Absorption of cyclosporine from conventional and new microemulsion oral formulations in liver-transplant recipients with external biliary diversion. *Br. J. Clin. Pharmacol.* 39:627-631 (1995).

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

[44] A.J. Humberstone and W.N. Charman. Lipid-based vehicles for the oral delivery of poorly water soluble drugs. *Adv. Drug Deliv. Rev.* 25:103-128 (1997).

[45] C.J.H. Porter, N.L. Trevaskis, and W.N. Charman. Lipids and lipid-based formulations: Optimizing the oral delivery of lipophilic drugs. *Nature Reviews Drug Discovery.* 6:231-248 (2007).

[46] R.G. Strickley. Solubilizing excipients in oral and injectable formulations. *Pharm. Res.* 21:201-230 (2004).

[47] L.J. Naylor, V. Bakatselou, and J.B. Dressman. Comparison of the mechanism of dissolution of hydrocortisone in simple and mixed micelle systems. *Pharm. Res.* 10:865-870 (1993).

[48] L.J. Naylor, V. Bakatselou, N. RodriguezHornedo, N.D. Weiner, and J.B. Dressman. Dissolution of steroids in bile salt solutions is modified by the presence of lecithin. *Eur. J. Pharm. Biopharm.* 41:346-353 (1995).

[49] J.B. Dressman, M. Vertzoni, K. Goumas, and C. Reppas. Estimating drug solubility in the gastrointestinal tract. *Adv. Drug Deliv. Rev.* 59:591-602 (2007).

[50] T.S. Wiedmann, W. Liang, and L. Kamel. Solubilization of drugs by physiological mixtures of bile salts. *Pharm. Res.* 19:1203-1208 (2002).

[51] T.S. Wiedmann and L. Kamel. Examination of the solubilization of drugs by bile salt micelles. *J. Pharm. Sci.* 91:1743-1764 (2002).

[52] M.G. Wakerly, C.W. Pouton, B.J. Meakin, and F.S. Morton. Self-emulsification of vegetable oil-nonionic surfactant mixtures - a proposed mechanism of action. *Acs Symposium Series.* 311:242-255 (1986).

[53] S.A. Charman, W.N. Charman, M.C. Rogge, T.D. Wilson, F.J. Dutko, and C.W. Pouton. Self-emulsifying drug delivery systems - formulation and biopharmaceutic evaluation of an investigational lipophilic compound. *Pharm. Res.* 9:87-93 (1992).

[54] N.H. Shah, M.T. Carvajal, C.I. Patel, M.H. Infeld, and A.W. Malick. Self-emulsifying drug-delivery systems (sedds) with polyglycolyzed glycerides for improving in-vitro dissolution and oral absorption of lipophilic drugs. *Int. J. Pharm.* 106:15-23 (1994).

[55] D.Q.M. Craig, H.S.R. Lievens, K.G. Pitt, and D.E. Storey. An investigation into the physicochemical properties of self-emulsifying systems using low-frequency dielectric-spectroscopy, surface-tension measurements and particle-size analysis. *Int. J. Pharm.* 96:147-155 (1993).

[56] R.N. Gursoy and S. Benita. Self-emulsifying drug delivery systems (sedds) for improved oral delivery of lipophilic drugs. *Biomed. Pharmacother.* 58:173-182 (2004).

[57] C.W. Pouton. Self-emulsifying drug delivery systems - assessment of the efficiency of emulsification. *Int. J. Pharm.* 27:335-348 (1985).

[58] C.W. Pouton. Effects of the inclusion of a model drug on the performance of self-emulsifying formulations *J. Pharm. Pharmacol.* 37:1P (1985).

[59] M. Kimura, M. Shizuki, K. Miyoshi, T. Sakai, H. Hidaka, H. Takamura, and T. Matoba. Relationship between the molecular-structures and emulsification properties of edible oils. *Biosci. Biotechnol. Biochem.* 58:1258-1261 (1994).

[60] D.J. Hauss, S.E. Fogal, J.V. Ficorilli, C.A. Price, T. Roy, A.A. Jayara, and J.J. Keirns. Lipid-based delivery systems for improving the bioavailability and lymphatic transport of a poorly water-soluble ltb4 inhibitor. *J. Pharm. Sci.* 87:164-169 (1998).

[61] M.J. Groves and D.A. Degalindez. Self-emulsifying action of mixed surfactants in oil. *Acta Pharm. Suecica.* 13:361-372 (1976).

[62] M. Sekine, E. Maeda, K. Sasahara, R. Okada, K. Kimura, M. Fukami, and S. Awazu. Improvement of bioavailability of poorly absorbed drugs .3. Oral acute toxicity and local irritation of medium chain glyceride. *J. Pharmacobio-Dynam.* 8:633-644 (1985).

[63] E.S. Swenson and W.J. Curatolo. Intestinal permeability enhancement for proteins, peptides and other polar drugs - mechanisms and potential toxicity .2. *Adv. Drug Deliv. Rev.* 8:39-92 (1992).

[64] C.W. Pouton. Formulation of self-emulsifying drug delivery systems. *Adv. Drug Deliv. Rev.* 25:47-58 (1997).

[65] T. Lindmark, T. Nikkila, and P. Artursson. Mechanisms of absorption enhancement by medium-chain fatty-acids in intestinal epithelial caco-2 cell monolayers. *J. Pharmacol. Exp. Therapeut.* 275:958-964 (1995).

[66] T. Gershanik and S. Benita. Self-dispersing lipid formulations for improving oral absorption of lipophilic drugs. *Eur. J. Pharm. Biopharm.* 50:179-188 (2000).

[67] W.N. Charman and V.J. Stella. Transport of lipophilic molecules by the intestinal lymphatic-system. *Adv. Drug Deliv. Rev.* 7:1-14 (1991).

[68] R. Holm, C.J.H. Porter, A. Mullertz, H.G. Kristensen, and W.N. Charman. Structured triglyceride vehicles for oral delivery of halofantrine: Examination of intestinal lymphatic transport and bioavailability in conscious rats. *Pharm. Res.* 19:1354-1361 (2002).

[69] R. Holm, C.J.H. Porter, G.A. Edwards, A. Mullertz, H.G. Kristensen, and W.N. Charman. Examination of oral absorption and lymphatic transport of halofantrine in a triple-cannulated canine model after administration in self-microemulsifying drug delivery systems (smedds) containing structured triglycerides. *Eur. J. Pharm. Sci.* 20:91-97 (2003).

[70] J.M. Kovarik, E.A. Mueller, J.B. Vanbree, W. Tetzloff, and K. Kutz. Reduced interindividual and intraindividual variability in cyclosporine pharmacokinetics from a microemulsion formulation. *J. Pharm. Sci.* 83:444-446 (1994).

[71] P.P. Constantinides, J.P. Scalart, C. Lancaster, J. Marcello, G. Marks, H. Ellens, and P.L. Smith. Formulation and intestinal-absorption enhancement evaluation of water-in-oil microemulsions incorporating medium-chain glycerides. *Pharm. Res.* 11:1385-1390 (1994).

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

[73] M. Sekine, H. Terashima, K. Sasahara, K. Nishimura, R. Okada, and S. Awazu. Improvement of bioavailability of poorly absorbed drugs .2. Effect of medium chain glyceride base on the intestinal-absorption of cefmetazole sodium in rats and dogs. *J. Pharmacobio-Dynam.* 8:286-295 (1985).

[74] M. Sekine, K. Sasahara, R. Okada, and S. Awazu. Improvement of bioavailability of poorly absorbed drugs .4. Mechanism of the promoting effect of medium chain glyceride on the rectal absorption of water-soluble drugs. *J. Pharmacobio-Dynam.* 8:645-652 (1985).

[75] J. Unowsky, C.R. Behl, G. Beskid, J. Sattler, J. Halpern, and R. Cleeland. Effect of medium chain glycerides on enteral and rectal absorption of beta-lactam and aminoglycoside antibiotics. *Chemotherapy.* 34:272-276 (1988).

[76] G. Beskid, J. Unowsky, C.R. Behl, J. Siebelist, J.L. Tossounian, C.M. Mcgarry, N.H. Shah, and R. Cleeland. Enteral, oral, and rectal absorption of ceftriaxone using glyceride enhancers. *Chemotherapy.* 34:77-84 (1988).

[77] P.P. Constantinides. Lipid microemulsions for improving drug dissolution and oral absorption - physical and biopharmaceutical aspects. *Pharm. Res.* 12:1561-1572 (1995).

[78] T.R. Kommuru, B. Gurley, M.A. Khan, and I.K. Reddy. Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q(10): Formulation development and bioavailability assessment. *Int. J. Pharm.* 212:233-246 (2001).

[79] G. Buckton. Surfactants. In G. Buckton (ed.), *Interfacial phenomena in drug delivery and targeting*, Harwood academic publishers, Chur, 1995, pp. 135-161.

[80] D.W. Osborne, C.A. Middleton, and R.L. Rogers. Alcohol-free microemulsions. *Journal of Dispersion Science and Technology.* 9:415-423 (1988).

[81] G.M. Eccleston. Microemulsions. In J. Swarbrickand J.C. Boylan (eds.), *Encyclopedia of pharmaceutical technology*, Vol. 9, Marcel Dekker, New York, 1992, pp. 375-421.

[82] D. Attwood. Microemulsions. In J. Kreuter (ed.), *Colloidal drug delivery systems*, Marcel Dekker, New York, 1994, pp. 31-71.

[83] M.G. Wakerly, C.W. Pouton, B.J. Meakin, and F.S. Morton. The effect of surfactant hlb on the self-emulsifying efficiency of nonionic surfactant-vegetable oil mixture. *J. Pharm. Pharmacol.* 38:2P (1986).

[84] A.T.M. Serajuddin, P.C. Sheen, D. Mufson, D.F. Bernstein, and M.A. Augustine. Effect of vehicle amphiphilicity on the dissolution and bioavailability of a poorly water-soluble drug from solid dispersions. *J. Pharm. Sci.* 77:414-417 (1988).

[85] D.W. Holt, E.A. Mueller, J.M. Kovarik, J.B. Vanbree, and K. Kutz. The pharmacokinetics of sandimmun-neoral - a new oral formulation of cyclosporine. *Transplant. Proc.* 26:2935-2939 (1994).

[86] E.A. Mueller, J.M. Kovarik, J.B. Vanbree, W. Tetzloff, J. Grevel, and K. Kutz. Improved dose linearity of cyclosporine pharmacokinetics from a microemulsion formulation. *Pharm. Res.* 11:301-304 (1994).

[87] L. Vernillet, J.M. Kovarik, R. Freiburghaus, P. Schaub, E.A. Mueller, W. Niederberger, and H. Zehender. Blood cyclosporine-A and metabolite kinetic profiles

after administration of sandimmune soft gelatin capsules and neoral in transplant recipients. *Transplant. Proc.* 26:2964-2968 (1994).

[88] J. Vonderscher and A. Meinzer. Rationale for the development of sandimmune-neoral. *Transplant. Proc.* 26:2925-2927 (1994).

[89] M.Y. Levy and S. Benita. Drug release from submicronized o/w emulsion - a new invitro kinetic evaluation model. *Int. J. Pharm.* 66:29-37 (1990).

[90] M.G. Wakerly, C.W. Pouton, and B.J. Meakin. Evaluation of the self-emulsifying performance of a non-ionic surfactant-vegetable oil mixture. *J. Pharm. Pharmacol.* 39:6P (1987).

[91] P. Gao, M.E. Guyton, T.H. Huang, J.M. Bauer, K.J. Stefanski, and Q. Lu. Enhanced oral bioavailability of a poorly water soluble drug pnu-91325 by supersaturatable formulations. *Drug Dev. Ind. Pharm.* 30:221-229 (2004).

[92] P. Gao, B.D. Rush, W.P. Pfund, T.H. Huang, J.M. Bauer, W. Morozowich, M.S. Kuo, and M.J. Hageman. Development of a supersaturable sedds (s-sedds) formulation of paclitaxel with improved oral bioavailability. *J. Pharm. Sci.* 92:2386-2398 (2003).

[93] T. Gershanik and S. Benita. Positively charged self-emulsifying oil formulation for improving oral bioavailability of progesterone, Vol. 1, Informa Healthcare, 1996, pp. 147 - 157.

[94] T. Gershanik, S. Benzeno, and S. Benita. Interaction of a self-emulsifying lipid drug delivery system with the everted rat intestinal mucosa as a function of droplet size and surface charge. *Pharm. Res.* 15:863-869 (1998).

[95] T. Gershanik, E. Haltner, C.M. Lehr, and S. Benita. Charge-dependent interaction of self-emulsifying oil formulations with caco-2 cells monolayers: Binding, effects on barrier function and cytotoxicity. *Int. J. Pharm.* 211:29-36 (2000).

[96] E. Atef. Formulation and in-vitro and in-vivo testing of phenytoin self-emulsifying drug delivery system (SEDDS). Ph.D. Thesis, Massachusetts college of pharmacy and health sciences, Boston, 2004.

[97] H. Reiss. Entropy-induced dispersion of bulk liquids. *J. Colloid. Interface. Sci.* 53:61-70 (1975).

[98] G. Buckton. Interfacial phenomena, surface tension and liquid/liquid interface. In G. Buckton (ed.), *Interfacial phenomena in drug delivery and targeting*, Harwood academic publishers, Chur, 1995, pp. 1-25.

[99] T. Dabros, A. Yeung, J. Masliyah, and J. Czarnecki. Emulsification through area contraction. *J. Colloid. Interface. Sci.* 210:222-224 (1999).

[100] M.J. Groves and R.M.A. Mustafa. Measurement of spontaneity of self-emulsifiable oils. *J. Pharm. Pharmacol.* 26:671-681 (1974).

[101] M.J. Groves, R.M.A. Mustafa, and J.E. Carless. Phase studies of mixed phosphated surfactants, normal-hexane and water. *J. Pharm. Pharmacol.* 26:616-623 (1974).

[102] M.J. Rang and C.A. Miller. Spontaneous emulsification of oils containing hydrocarbon, nonionic surfactant, and oleyl alcohol. *J. Colloid. Interface. Sci.* 209:179-192 (1999).

[103] D.Q.M. Craig, S.A. Barker, D. Banning, and S.W. Booth. An investigation into the mechanisms of self-emulsification using particle-size analysis and low-frequency dielectric-spectroscopy. *Int. J. Pharm.* 114:103-110 (1995).

[104] H. Toguchi, Y. Ogawa, K. Iga, T. Yashiki, and T. Shimamoto. Gastrointestinal absorption of ethyl 2-chloro-3-[4-(2-methyl-2-phenylpropyloxy)phenyl]propionate from different dosage forms in rats and dogs. *Chem. Pharmaceut. Bull.* 38:2792-2796 (1990).

[105] H. Toguchi, Y. Ogawa, and T. Shimamoto. Effects of the physicochemical properties of the emulsion formulation on the bioavailability of ethyl 2-chloro-3-[4-(2-methyl-2-phenylpropyloxy)phenyl]propionate in rats. *Chem. Pharmaceut. Bull.* 38:2797-2800 (1990).

[106] K.J. Palin, A.J. Phillips, and A. Ning. The oral absorption of cefoxitin from oil and emulsion vehicles in rats. *Int. J. Pharm.* 33:99-104 (1986).

[107] R.A. Myers and V.J. Stella. Systemic bioavailability of penclomedine (nsc-338720) from oil-in-water emulsions administered intraduodenally to rats. *Int. J. Pharm.* 78:217-226 (1992).

[108] V. Stella, J. Haslam, N. Yata, H. Okada, S. Lindenbaum, and T. Higuchi. Enhancement of bioavailability of a hydrophobic amine anti-malarial by formulation with oleic-acid in a soft gelatin capsule. *J. Pharm. Sci.* 67:1375-1377 (1978).

[109] T.T. Kararli, T.E. Needham, M. Griffin, G. Schoenhard, L.J. Ferro, and L. Alcorn. Oral delivery of a renin inhibitor compound using emulsion formulations. *Pharm. Res.* 9:888-893 (1992).

[110] D.J. Hauss, S.C. Mehta, and G.W. Radebaugh. Targeted lymphatic transport and modified systemic distribution of ci-976, a lipophilic lipid-regulator drug, via a formulation approach. *Int. J. Pharm.* 108:85-93 (1994).

[111] C. Malcolmson and M.J. Lawrence. A comparison of the incorporation of model steroids into nonionic micellar and microemulsion systems. *J. Pharm. Pharmacol.* 45:141-143 (1993).

[112] R.J. Ptachcinski, R. Venkataramanan, and G.J. Burckart. Clinical pharmacokinetics of cyclosporine. *Clin. Pharmacokinet.* 11:107-132 (1986).

[113] A. Meinzer, E.A. Mueller, and J. Vonderscher. Microemulsion - a suitable galenical approach for the absorption enhancement of low soluble compounds. *B T Gattefosse* 21-26 (1995).

[114] F.G.J. Poelma, R. Breas, and J.J. Tukker. Intestinal-absorption of drugs .3. The influence of taurocholate on the disappearance kinetics of hydrophilic and lipophilic drugs from the small-intestine of the rat. *Pharm. Res.* 7:392-397 (1990).

[115] Y.Y. Chiu, K. Higaki, B.L. Neudeck, J.L. Barnett, L.S. Welage, and G.L. Amidon. Human jejunal permeability of cyclosporin a: Influence of surfactants on p-glycoprotein efflux in caco-2 cells. *Pharm. Res.* 20:749-756 (2003).

[116] C.J.H. Porter. Lipid-based delivery systems for poorly water soluble drugs: Biopharmaceutical implications. *Eur. J. Pharm. Sci.* 28:S8-S8 (2006).

[117] K. Kawakami, T. Yoshikawa, Y. Moroto, E. Kanaoka, K. Takahashi, Y. Nishihara, and K. Masuda. Microemulsion formulation for enhanced absorption of poorly soluble drugs - i. Prescription design. *J. Control. Release.* 81:65-74 (2002).

[118] J.M. Dintaman and J.A. Silverman. Inhibition of p-glycoprotein by d-alpha-tocopheryl polyethylene glycol 1000 succinate (tpgs). *Pharm. Res.* 16:1550-1556 (1999).

[119] B.K. Nordskog, C.T. Phan, D.F. Nutting, and P. Tso. An examination of the factors affecting intestinal lymphatic transport of dietary lipids. *Adv. Drug Deliv. Rev.* 50:21-44 (2001).

[120] J.Y. Kiyasu, B. Bloom, and I.L. Chaikoff. The portal transport of absorbed fatty acids. *J. Biol. Chem.* 199:415-419 (1952).

[121] S.M. Caliph, W.N. Charman, and C.J.H. Porter. Effect of short-, medium-, and long-chain fatty acid-based vehicles on the absolute oral bioavailability and intestinal lymphatic transport of halofantrine and assessment of mass balance in lymph-cannulated and non-cannulated rats. *J. Pharm. Sci.* 89:1073-1084 (2000).

[122] R.L. Juliano and V. Ling. Surface glycoprotein modulating drug permeability in chinese-hamster ovary cell mutants. *Biochimica Et Biophysica Acta.* 455:152-162 (1976).

[123] Y.L. Lo, C.Y. Hsu, and J.D. Huang. Comparison of effects of surfactants with other mdr reversing agents on intracellular uptake of epirubicin in caco-2 cell line. *Anticancer Res.* 18:3005-3009 (1998).

[124] L. Yu, A. Bridgers, J. Polli, A. Vickers, S. Long, A. Roy, R. Winnike, and M. Coffin. Vitamin e-tpgs increases absorption flux of an hiv protease inhibitor by enhancing its solubility and permeability1. *Pharm. Res.* 16:1812-1817 (1999).

[125] W. Schwizer, K. Asal, C. Kreiss, C. Mettraux, J. Borovicka, B. Remy, C. Guzelhan, D. Hartmann, and M. Fried. Role of lipase in the regulation of upper gastrointestinal function in humans. *Am. J. Physiol. Gastrointest. Liver Physiol.* 273:G612-G620 (1997).

[126] J. Borovicka, W. Schwizer, C. Mettraux, C. Kreiss, B. Remy, K. Asal, J.B.M.J. Jansen, I. Douchet, R. Verger, and M. Fried. Regulation of gastric and pancreatic lipase secretion by cck and cholinergic mechanisms in humans. *Am. J. Physiol. Gastrointest. Liver Physiol.* 273:G374-G380 (1997).

[127] H. Moreau, A. Bernadac, Y. Gargouri, F. Benkouka, R. Laugier, and R. Verger. Immunocytolocalization of human gastric lipase in chief cells of the fundic mucosa. *Histochem.* 91:419-423 (1989).

[128] A.B.R. Thomson, M. Keelan, M.L. Garg, and M.T. Clandinin. Intestinal aspects of lipid absorption - in review. *Can. J. Physiol. Pharmacol.* 67:179-191 (1989).

[129] T.H. Liao, M. Hamosh, and P. Hamosh. Fat digestion in the stomach - mechanism of action and substrate-specificity of lingual lipase. *Federation Proceedings.* 40:941-941 (1981).

[130] M. Hamosh, J.W. Scanlon, D. Ganot, M. Likel, K.B. Scanlon, and P. Hamosh. Fat digestion in the newborn - characterization of lipase in gastric aspirates of premature and term infants. *J. Clin. Investig.* 67:838-846 (1981).

[131] C.K. Abrams, M. Hamosh, T.C. Lee, A.F. Ansher, M.J. Collen, J.H. Lewis, S.B. Benjamin, and P. Hamosh. Gastric lipase - localization in the human stomach. *Gastroenterology.* 95:1460-1464 (1988).

[132] F. Carriere, J.A. Barrowman, R. Verger, and R. Laugier. Secretion and contribution to lipolysis of gastric and pancreatic lipases during a test meal in humans. *Gastroenterology.* 105:876-888 (1993).

[133] C. Renou, F. Carriere, E. Ville, P. Grandval, M. Joubert-Collin, and R. Laugier. Effects of lansoprazole on human gastric lipase secretion and intragastric lipolysis in healthy human volunteers. *Digestion.* 63:207-213 (2001).

[134] G.C. Ferreira and J.S. Patton. Inhibition of lipolysis by hydrocarbons and fatty alcohols. *J. Lipid Res.* 31:889-897 (1990).

[135] J.K. Embleton and C.W. Pouton. Structure and function of gastro-intestinal lipases. *Adv. Drug Deliv. Rev.* 25:15-32 (1997).

[136] C. Chapus, M. Semeriva, C. Bovierlapierre, and P. Desnuelle. Mechanism of pancreatic lipase action .1. Interfacial activation of pancreatic lipase. *Biochemistry.* 15:4980-4987 (1976).

[137] F.H. Mattson and R.A. Volpenhein. The digestion and absorption of triglycerides. *J. Biol. Chem.* 239:2772-2777 (1964).

[138] W.C. Duane, R.L. Ginsberg, and L.J. Bennion. Effects of fasting on bile-acid metabolism and biliary lipid-composition in man. *J. Lipid Res.* 17:211-219 (1976).

[139] J.M. Laher and J.A. Barrowman. Polycyclic-hydrocarbon and polychlorinated biphenyl solubilization in aqueous-solutions of mixed micelles. *Lipids.* 18:216-222 (1983).

[140] J.O. Christensen, K. Schultz, B. Mollgaard, H.G. Kristensen, and A. Mullertz. Solubilisation of poorly water-soluble drugs during in vitro lipolysis of medium- and long-chain triacylglycerols. *Eur. J. Pharm. Sci.* 23:287-296 (2004).

[141] K.J. MacGregor, J.K. Embleton, J.E. Lacy, E.A. Perry, L.J. Solomon, H. Seager, and C.W. Pouton. Influence of lipolysis on drug absorption from the gastro-intestinal tract. *Adv. Drug Deliv. Rev.* 25:33-46 (1997).

[142] L. Sek, C.J.H. Porter, A.M. Kaukonen, and W.N. Charman. Evaluation of the in-vitro digestion profiles of long and medium chain glycerides and the phase behaviour of their lipolytic products. *J. Pharm. Pharmacol.* 54:29-41 (2002).

[143] I. Ghebre-Sellassie. Pellets: A general overview. In I. Ghebre-Sellassie (ed.), *Pharmaceutical pelletization technology*, Marcel Dekker, New York, 1989, pp. 1-13.

[144] S.R. Bechard and J.C. Leroux. Coated pelletized dosage form - effect of compaction on drug release. *Drug Dev. Ind. Pharm.* 18:1927-1944 (1992).

[145] C. Eskilson. Controlled release by microencapsulation .1. *Manufacturing Chemist.* 56:33-39 (1985).

[146] H. Bechgaard and K. Ladefoged. Distribution of pellets in gastrointestinal-tract - influence on transit-time exerted by density or diameter of pellets. *J. Pharm. Pharmacol.* 30:690-692 (1978).

[147] H. Bechgaard and G.H. Nielsen. Controlled-release multiple-units and single-unit doses - literature-review. *Drug Dev. Ind. Pharm.* 4:53-67 (1978).

[148] T. Govender and C.M. Dangor. Formulation and preparation of controlled release pellets of salbutamol by the air suspension technique. *J. Microencapsul.* 14:445-455 (1997).

[149] A.D. Reynolds. A new technique for production of spherical particles. *Manufacturing Chemist.* 41:40-43 (1970).

[150] J.M. Newton. Extrusion and extruders. In J. Swarbrickand J.C. Boylan (eds.), *Encyclopedia of pharmaceutical technology*, Marcel Dekker, New York, 2002, pp. 1220-1236.

[151] D.C. Hicks and H.L. Freese. Extrusion and spheronizing equipment. In I. Ghebre-Sellassie (ed.), *Pharmaceutical pelletization technology*, Marcel Dekker, New York, 1989, pp. 71-100.

[152] R.C. Rowe. Spheronization - a novel pill-making process. *Pharmacy International.* 6:119-123 (1985).

[153] N. Nakahara. US patent, 1966.

[154] I.M. Jalal, W.E. Smith, and Malinows.Hj. Tablet granulations composed of spherical-shaped particles. *J. Pharm. Sci.* 61:1466-1468 (1972).

[155] Malinows.Hj and W.E. Smith. Effects of spheronization process variables on selected tablet properties. *J. Pharm. Sci.* 63:285-288 (1974).

[156] C.W. Woodruff and N.O. Nuessle. Effect of processing variables on particles obtained by extrusion-spheronization processing. *J. Pharm. Sci.* 61:787-790 (1972).

[157] J.W. Conine and H.R. Hadley. Preparation of small solid pharmaceutical spheres. *Drug & Cosmetic Industry.* 106:38-41 (1970).

[158] L. Baert and J.P. Remon. Influence of amount of granulation liquid on the drug-release rate from pellets made by extrusion spheronization. *Int. J. Pharm.* 95:135-141 (1993).

[159] P. Kleinebudde and H. Lindner. Experiments with an instrumented twin-screw extruder using a single-step granulation extrusion process. *Int. J. Pharm.* 94:49-58 (1993).

[160] G.P. Millili and J.B. Schwartz. The strength of microcrystalline cellulose pellets - the effect of granulating with water ethanol mixtures. *Drug Dev. Ind. Pharm.* 16:1411-1426 (1990).

[161] R.E. Oconnor and J.B. Schwartz. Spheronization .2. Drug release from drug-diluent mixtures. *Drug Dev. Ind. Pharm.* 11:1837-1857 (1985).

[162] A.B.B. Bashaiwoldu, F. Podczeck, and M. Newton. A study on the effect of drying techniques on the mechanical properties of pellets and compacted pellets. *Eur. J. Pharm. Sci.* 21:119-129 (2004).

[163] P.J. Harrison, J.M. Newton, and R.C. Rowe. The characterization of wet powder masses suitable for extrusion spheronization. *J. Pharm. Pharmacol.* 37:686-691 (1985).

[164] M.R. Harris and I. Ghebre-Sellassie. Formulation variables. In I. Ghebre-Sellassie (ed.), *Pharmaceutical pellitization technology*, Vol. 37, Marcel Dekker, New York, 1989, pp. 217-239.

[165] C. Vervaet, L. Baert, and J.P. Remon. Extrusion-spheronisation - a literature-review. *Int. J. Pharm.* 116:131-146 (1995).

[166] J.W. Wallace. Cellulose derivatives and natural products utilized in pharmaceutics. In J. Swarbrickand J.C. Boylan (eds.), *Encyclopedia of pharmaceutical technology*, Vol. 2, Marcel Dekker, New York, 1991, pp. 319-337.

[167] K.E. Fielden, J.M. Newton, P. Obrien, and R.C. Rowe. Thermal studies on the interaction of water and microcrystalline cellulose. *J. Pharm. Pharmacol.* 40:674-678 (1988).

[168] P. Kleinebudde. The crystallite-gel-model for microcrystalline cellulose in wet-granulation, extrusion, and spheronization. *Pharm. Res.* 14:804-809 (1997).

[169] P. Fajer. Electron spin resonance spectroscopy labeling in peptide and protein analysis. In R. Meyers (ed.), *Encyclopedia of analytical chemistry*, John Wiley & Sons, New York, 2000.

[170] K. Mäder, A. Domb, and H.M. Swartz. Gamma-sterilization-induced radicals in biodegradable drug delivery systems. *Appl. Radiat. Isot.* 47:1669-1674 (1996).

[171] J. Raffi, S. Gelly, L. Barral, F. Burger, P. Piccerelle, P. Prinderre, M. Baron, and A. Chamayou. Electron paramagnetic resonance of radicals induced in drugs and excipients by radiation or mechanical treatments. *Spectrochim. Acta Mol. Biomol. Spectros.* 58:1313-1320 (2002).

[172] K. Mäder, H.M. Swartz, R. Stosser, and H.H. Borchert. The application of epr spectroscopy in the field of pharmacy. *Pharmazie.* 49:97-101 (1994).

[173] T. Yamaguchi, S. Itai, H. Hayashi, S. Soda, A. Hamada, and H. Utsumi. In vivo esr studies on pharmacokinetics and metabolism of parenteral lipid emulsion in living mice. *Pharm. Res.* 13:729-733 (1996).

[174] M. Petelin, Z. Pavlica, S. Bizimoska, and M. Sentjurc. In vivo study of different ointments for drug delivery into oral mucosa by epr oximetry. *Int. J. Pharm.* 270:83-91 (2004).

[175] R.S. Macomber. Magnetic properties of nuclei. *A complete introduction to modern nmr spectroscopy*, John Wiley & Sons, New York, 1998, pp. 6-21.

[176] A. Rübe. Development and physico-chemical characterization of nanocapsules. Ph.D. thesis, Martin-Luther University, 2006.

[177] D.J. Lurie and K. Mäder. Monitoring drug delivery processes by epr and related techniques - principles and applications. *Adv. Drug Deliv. Rev.* 57:1171-1190 (2005).

[178] M. Hemminga and I. Van den Dries. Spin label applications to food science. In L.J. Berliner (ed.), *Spin labeling, the next millennium*, Plenum publishing corporation, New York 1998.

[179] W.K. Subczynski, J. Wojas, V. Pezeshk, and A. Pezeshk. Partitioning and localization of spin-labeled amantadine in lipid bilayers: An EPR study. *J. Pharm. Sci.* 87:1249-1254 (1998).

[180] K. Jores, W. Mehnert, and K. Mäder. Physicochemical investigations on solid lipid nanoparticles and on oil-loaded solid lipid nanoparticles: A nuclear magnetic resonance and electron spin resonance study. *Pharm. Res.* 20:1274-1283 (2003).

[181] I. Katzhendler, K. Mäder, and M. Friedman. Correlation between drug release kinetics from proteineous matrix and matrix structure: EPR and NMR study. *J. Pharm. Sci.* 89:365-381 (2000).

[182] I. Katzhendler, K. Mäder, R. Azoury, and M. Friedman. Investigating the structure and properties of hydrated hydroxypropyl methylcellulose and egg albumin matrices containing carbamazepine: EPR and NMR study. *Pharm. Res.* 17:1299-1308 (2000).

[183] A. Brunner, K. Mäder, and A. Gopferich. pH and osmotic pressure inside biodegradable microspheres during erosion. *Pharm. Res.* 16:847-853 (1999).

[184] K. Mäder, B. Bittner, Y. Li, W. Wohlauf, and T. Kissel. Monitoring microviscosity and microacidity of the albumin microenvironment inside degrading microparticles from poly(lactide-co-glycolide) (PLG) or ABA-triblock polymers containing hydrophobic poly(lactide-co-glycolide) A blocks and hydrophilic poly(ethyleneoxide) B blocks. *Pharm. Res.* 15:787-793 (1998).

[185] C. Kroll, W. Herrmann, R. Stosser, H.H. Borchert, and K. Mäder. Influence of drug treatment on the microacidity in rat and human skin - an in vitro electron spin resonance imaging study. *Pharm. Res.* 18:525-530 (2001).

[186] C. Tuleu, M. Newton, J. Rose, D. Euler, R. Saklatvala, A. Clarke, and S. Booth. Comparative bioavailability study in dogs of a self-emulsifying formulation of progesterone presented in a pellet and liquid form compared with an aqueous suspension of progesterone. *J. Pharm. Sci.* 93:1495-1502 (2004).

[187] S.M. Khoo, A.J. Humberstone, C.J.H. Porter, G.A. Edwards, and W.N. Charman. Formulation design and bioavailability assessment of lipidic self-emulsifying formulations of halofantrine. *Int. J. Pharm.* 167:155-164 (1998).

[188] J. Chatchawalsaisin, F. Podczeck, and J.M. Newton. The preparation by extrusion/spheronization and the properties of pellets containing drugs, microcrystalline cellulose and glyceryl monostearate. *Eur. J. Pharm. Sci.* 24:35-48 (2005).

[189] E. Franceschinis, D. Voinovich, M. Grassi, B. Perissutti, J. Filipovic-Grcic, A. Martinac, and F. Meriani-Merlo. Self-emulsifying pellets prepared by wet granulation in high-shear mixer: Influence of formulation variables and preliminary study on the in vitro absorption. *Int. J. Pharm.* 291:87-97 (2005).

[190] M. Newton, J. Petersson, F. Podczeck, A. Clarke, and S. Booth. The influence of formulation variables on the properties of pellets containing a self-emulsifying mixture. *J. Pharm. Sci.* 90:987-995 (2001).

[191] A. Besheer, K.M. Wood, N.A. Peppas, and K. Mäder. Loading and mobility of spin-labeled insulin in physiologically responsive complexation hydrogels intended for oral administration. *J. Control. Release.* 111:73-80 (2006).

[192] A. Martin. Physical pharmacy, Lippincott Williams &Wilkins, Maryland, 1993.

[193] A.M. Bouwman, J.C. Bosma, P. Vonk, J.H.A. Wesselingh, and H.W. Frijlink. Which shape factor(s) best describe granules? *Powder Technology.* 146:66-72 (2004).

[194] R. Dreu, J. Sirca, K. Pintye-Hodi, T. Burjan, O. Planinsek, and S. Srcic. Physicochemical properties of granulating liquids and their influence on microcrystalline cellulose pellets obtained by extrusion-spheronisation technology. *Int. J. Pharm.* 291:99-111 (2005).

[195] R. Chopra, F. Podczeck, J.M. Newton, and G. Alderborn. The influence of pellet shape and film coating on the filling of pellets into hard shell capsules. *Eur. J. Pharm. Biopharm.* 53:327-333 (2002).

[196] B.D. Tarr and S.H. Yalkowsky. Enhanced intestinal absorption of cyclosporine in rats through the reduction of emulsion droplet size. *Pharm. Res.* 6:40-43 (1989).

[197] J.S. Patton and M.C. Carey. Watching fat digestion. *Science.* 204:145-148 (1979).

[198] M.C. Carey, D.M. Small, and C.M. Bliss. Lipid digestion and absorption. *Annu. Rev. Physiol.* 45:651-677 (1983).

[199] D.G. Fatouros, G.R. Deen, L. Arleth, B. Bergenstahl, F.S. Nielsen, J.S. Pedersen, and A. Mullertz. Structural development of self nano emulsifying drug delivery systems

(SNEDDS) during in vitro lipid digestion monitored by small-angle x-ray scattering. *Pharm. Res.* 24:1844-1853 (2007).

[200] J.E. Staggers, O. Hernell, R.J. Stafford, and M.C. Carey. Physical-chemical behavior of dietary and biliary lipids during intestinal digestion and absorption .1. Phase-behavior and aggregation states of model lipid systems patterned after aqueous duodenal contents of healthy adult human-beings. *Biochemistry.* 29:2028-2040 (1990).

[201] M.W. Rigler, R.E. Honkanen, and J.S. Patton. Visualization by freeze-fracture, invitro and invivo, of the products of fat digestion. *J. Lipid Res.* 27:836-857 (1986).

[202] G.A. Kossena, W.N. Charman, B.J. Boyd, and C.I.H. Porter. Influence of the intermediate digestion phases of common formulation lipids on the absorption of a poorly water-soluble drug. *J. Pharm. Sci.* 94:481-492 (2005).

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

[204] F.J. Alvarez and V.J. Stella. The role of calcium-ions and bile-salts on the pancreatic lipase-catalyzed hydrolysis of triglyceride emulsions stabilized with lecithin. *Pharm. Res.* 6:449-457 (1989).

[205] N.H. Zangenberg, A. Mullertz, H.G. Kristensen, and L. Hovgaard. A dynamic in vitro lipolysis model II: Evaluation of the model. *Eur. J. Pharm. Sci.* 14:237-244 (2001).

[206] N.H. Zangenberg, A. Mullertz, H.G. Kristensen, and L. Hovgaard. A dynamic in vitro lipolysis model I. Controlling the rate of lipolysis by continuous addition of calcium. *Eur. J. Pharm. Sci.* 14:115-122 (2001).

[207] S. Klein. Optimierung eines pankreatin-assays als prädiktives in vitro modell zur bioverfügbarkeit von wirkstoffformulierungen Vol. Diploma Thesis, Martin Luther University, 2006.

[208] L. Sek, C.J.H. Porter, and W.N. Charman. Characterisation and quantification of medium chain and long chain triglycerides and their in vitro digestion products, by hptlc coupled with in situ densitometric analysis. *J. Pharmaceut. Biomed. Anal.* 25:651-661 (2001).

[209] A. Rube, S. Klein, and K. Mäder. Monitoring of in vitro fat digestion by electron paramagnetic resonance spectroscopy. *Pharm. Res.* 23:2024-2029 (2006).

[210] A.M. Kaukonen, B.J. Boyd, C.J.H. Porter, and W.N. Charman. Drug solubilization behavior during in vitro digestion of simple triglyceride lipid solution formulations. *Pharm. Res.* 21:245-253 (2004).

[211] O. Hernell, J.E. Staggers, and M.C. Carey. Physical-chemical behavior of dietary and biliary lipids during intestinal digestion and absorption .2. Phase-analysis and aggregation states of luminal lipids during duodenal fat digestion in healthy adult human-beings. *Biochemistry.* 29:2041-2056 (1990).

[212] S.D. Ladas, P.E.T. Isaacs, G.M. Murphy, and G.E. Sladen. Comparison of the effects of medium and long-chain triglyceride containing liquid meals on gallbladder and small intestinal function in normal man. *Gut.* 25:405-411 (1984).

[213] A.M. Kaukonen, B.J. Boyd, W.N. Charman, and C.J.H. Porter. Drug solubilization behavior during in vitro digestion of suspension formulations of poorly water-soluble drugs in triglyceride lipids. *Pharm. Res.* 21:254-260 (2004).

[214] H.R. Tang, J. Godward, and B. Hills. The distribution of water in native starch granules - a multinuclear NMR study. *Carbohydr. Polymer.* 43:375-387 (2000).

[215] E. Chiotelli, G. Pilosio, and M. Le Meste. Effect of sodium chloride on the gelatinization of starch: A multimeasurement study. *Biopolymers.* 63:41-58 (2002).

[216] R.R. Li, W.L. Kerr, R.T. Toledo, and J.A. Carpenter. ^{1}H NMR studies of water in chicken breast marinated with different phosphates. *J. Food Sci.* 65:575-580 (2000).

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Publications

Research articles

Ahmed Abdalla, Karsten Mäder, **Preparation and characterization of a self-emulsifying pellet formulation,** Eur. J. Pharm. Biopharm., 66:220-226, 2007.

Ahmed Abdalla, Sandra Klein and Karsten Mäder, **A new self-emulsifying drug delivery system (SEDDS) for poorly soluble drugs: Characterization, dissolution, in vitro digestion and incorporation into solid pellets,** *Eur. J. Pharm. Sci.,* DOI: 10.1016/j.ejps.2008.09.006, 2008.

Ahmed Abdalla and Karsten Mäder, **ESR studies on the influence of physiological dissolution and digestion media on the lipid phase characteristics of SEDDS and SEDDS pellets,** *Int. J. Pharm.,* DOI: 10.1016/j.ijpharm.2008.09.014, 2008.

Ahmed Abdalla, Karsten Mäder, and Hendrik Metz, **Characterization of lipid status in self-emulsifying pellets by bench-top NMR, in process.**

Poster and oral presentations

Ahmed Abdalla, Karsten Mäder, **Preparation and characterization of a self-emulsifying pellet formulation**, Poster, 33rd Annual Meeting of the controlled release society, Vienna, 2006.

Ahmed Abdalla, Karsten Mäder, **Development and characterization of self-emulsifying pellet formulations for poorly soluble drugs**, oral presentation, Bayer Bitterfield, 2008.

Ahmed Abdalla, Sandra Klein, Hendrik Metz and Karsten Mäder, **Formulation and characterization of self-emulsifying pellet formulations for poorly soluble drugs**, Poster, 6th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Barcelona, 2008.

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Erklärung

Hiermit erkläre ich, dass ich mich mit der vorliegenden Arbeit erstmals um die Erlangung eines Doktorgrades bewerbe.

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