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Research paper

Electrolyte- and pH-stabilities of aqueous solid lipid nanoparticle (SLN™) dispersions in artificial gastrointestinal media

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Abstract

The influence of artificial gastrointestinal (GI) media on the physical stability of solid lipid nanoparticle (SLN) formulations consisting of different lipids and various surfactants/stabilizers has been investigated in vitro, with respect to ionic strength and pH. Laser diffractometry and zeta potential measurements were the techniques applied. Some SLN formulations already showed aggregation/particle growth in the presence of electrolytes at neutral pH. Other lipid nanodispersions remained physically stable with respect to the influence of electrolytes, but were pH-sensitive. It was possible to produce SLN that were GIT (gastrointestinal tract) stable by an optimized stabilizer composition. There is no optimal surfactant mixture for stabilization of any lipid, e.g. SLN consisting of the lipid Cutina CP showed GIT stability in combination with the stabilizer sugar ester S1670, whereas the stabilization with the surfactant mixture Tween 80/Span 85 was not effective. Vice versa, the emulsifier Pluronic F68 stabilized the lipid Compritol ATO 888 but not the lipid Imwitor 900 sufficiently to avoid aggregation of the SLN dispersion in artificial GI media. The stabilizing properties depend obviously on the specific interactions of the lipid matrix with the emulsifier, e.g. anchoring of the stabilizer on the lipid surface and density on the surface.

Keywords: Solid lipid nanoparticles; SLN; Gastrointestinal; Stability; Electrolytes; pH

1. Introduction

Solid lipid nanoparticles (SLN™) [1] are an alternative delivery system for pharmaceutical and cosmetic ingredients to emulsions, liposomes and polymeric nanoparticles with increasing attention from different research groups [2–7]. The possible applications are through the parenteral, the peroral or the dermal route.

By oral delivery, these systems may provide sustained release inside the gastrointestinal tract [8] and drug protection against chemical degradation for labile drug molecules, e.g. peptide drugs. Incorporation of drugs into SLN opens the perspective of enhanced and/or less variable bioavailability and prolonged plasma levels [9–11], which is postulated due to a controlled, optimized release in combination with general adhesive properties of small particles [12]. Further advantages of SLN are the low toxicity of the physiological compounds (biological degradation of the lipid matrix) and the possibility of simple and cost-effective large-scale production by high-pressure homogenization.

The advantages of colloidal drug carriers (e.g. improved bioavailability [5,9]) described above, are in general linked to their size in the submicron range. The preservation of particle size of colloidal carrier systems after peroral administration is therefore a crucial point. Although studies of the physical stability of SLN formulations during storage [13,14] or formulation of pellets as carriers of SLN for oral administration [15] have been performed, no data has been published so far concerning physical stability in the gastrointestinal tract. The gastric environment (ionic strength, low pH) may destabilize the SLN and potentially lead to aggregation. However, regarding the complex in vivo situation in the gastrointestinal tract after peroral application, enzymatic degradation of the lipid matrix, which could lead to deaggregation, has to be taken into consideration. In a previous work, a special lipase degradation assay has been established by Müller et al. [16] to intensively investigate the degradation of SLN [17–20].

The present study was envisaged to show if the gastrointestinal medium has any influence on the physical stability of SLN formulations due to ionic strength and different pH values in vitro. Nevertheless, further studies have to be done to examine the in vitro/in vivo correlation of the influence of possible particle aggregation on bioavailability, e.g. the influence of enzymes.

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2. Materials and methods

2.1. Materials

Imwitor 900® (glycerol monostearate (40–50%)) and Dynasan 114® (glycerol trimyristate) were a gift of Condea (Witten, Germany), Compritol ATO 888® (glycerol tribehenate) and Geleol® (glycerol palmitostearate) of Gattfossé (Weil am Rhein, Germany). Cutina CP® (cetyl palmitate) was used from Henkel KG (Düsseldorf, Germany) and sugar ester S1670 (sucrose fatty acid ester) from Mitsubishi-Kagaku Foods (Tokyo, Japan). Pluronic F68® (Poloxamer 188, polyoxyethylene–polyoxyproylene copolymer) was provided by BASF AG (Ludwigshafen, Germany), Span 85 (sorbitan trioleate) by Uniqema (Everberg, Belgium) and Poloxamer 407 (polyoxyethylene–polyoxyproylene copolymer) by ICI (Essen, Germany). Tego Care 450® (polyglycerol methylglucodistearate) and Tagat S® (polyoxyethyleneglycerol monostearate) were kind gifts of Goldschmidt (Essen, Germany). Stearyl alcohol (octadecanol), sodium dodecyl sulfate (SDS), sodium cholate, Tween 80 (polyoxyethylenesorbitan monostearate) and all other chemicals were purchased from Sigma (Deisenhofen, Germany).

2.2. Preparation of the solid lipid nanoparticles

The lipids were heated to about 15–20°C above their melting point and added to the aqueous solution of the surfactants of the same temperature. The mixtures were stirred with a T 25 ultra-turrax (IKA, Staufen, Germany) for 1 min at 8000 rpm. The obtained pre-emulsions were homogenized at high pressure (500 bar, three cycles, 15–20°C above the melting point of the lipid) with an LAB 40 (APV Deutschland GmbH, Lübeck, Germany) and stored in the refrigerator at 4–6°C. Details of the production method are given elsewhere [1].

In the previous studies, a screening for each lipid was performed to determine the minimum surfactant concentration to yield physically stable SLN in a narrow size range (approximately 100–300 nm). These formulations were used in the present study. The concentration of surfactants needs to be minimized due to toxicological reasons.

2.3. Particle size analysis

Laser diffractometry was performed by a Coulter LS 230 (Coulter Electronics, Germany) yielding the volume distribution of the particles. The applied refractive index of the SLN dispersion medium is 1.33; of the lipid particles: real index 1.45635/imaginary index 0.01. Characterization parameters were the diameters LD50 and LD90, e.g. a diameter LD50 of 1 μm means that 50% of all particles possess a diameter of 1 μm or less.

2.4. Zeta potential

The particle charge was quantified as a zeta potential using a Zetasizer 4 (Malvern, UK). Measurements were performed in the original dispersion medium [21] in distilled water adjusted with sodium chloride to a conductivity of 50 μS [21] and in the artificial GI media (see below). The measured electrophoretic mobility was converted to the zeta potential using the Helmholtz–Smoluchowski equation.

2.5. Stability studies

Stability studies were carried out in simulated gastrointestinal (GI) fluids:

(a) pH 1.1: 12 ml HCl (32%) with 1188 ml H_2O; adjustment with NaCl to isoosmolarity;
(b) pH 3.5: 150 ml solution 1 (10.5 g citric acid + 100 ml NaOH (1 M) + 395.5 ml H_2O) with 100 ml HCl (0.2 M); adjustment with NaCl to isoosmolarity;
(c) pH 7.4: 68.0 g $NaH_2PO_4 \cdot H_2O$ with 15.6 g NaOH with 10 l H_2O; adjustment with NaCl to isoosmolarity.

Nine ml of the different simulated gastrointestinal media were added to 1 ml of the SLN dispersions containing 10% lipid, whereas 4 ml of the artificial GI media were added to 1 ml of the aqueous SLN formulations containing 5% lipid. The samples were investigated right after addition of the GI fluids to the SLN (t_0) and after 2-h incubation (t_2) for the determination of particle size distribution by laser diffractometry.

3. Results and discussion

The composition of the aqueous SLN dispersions with regard to the lipid matrix and the surfactant proved itself very important with respect to the influence on particle size from the production process [22], the physical long-term stability during storage [13,14], drug release profiles [23] or enzymatic degradation velocity [17–20]. Therefore, SLN formulations consisting of various lipids and different surfactants were produced (Table 1) to investigate the influence of artificial GI media on their physical stability. All the systems showed a particle size distribution in the nanoparticle range (LD90 151–494 nm).

After addition of these nanodispersions to the simulated gastrointestinal fluids, the GIT stability of the samples was investigated by observing potential changes of the particle size distribution. The SLN formulations were considered as physically stable, if no distinctive aggregation or particle growth in the microparticular size was detected by laser diffractometry. Unstable SLN dispersions showed pronounced aggregation directly after addition to the GI media (i.e. t_0) and a slight but steady increase in size at the end of the 2-h incubation period (i.e. t_2). Physically

Table 1
Composition of the different aqueous SLN formulations

SLN group	Lipid type	Concentration (%)	Code	Surfactants type	Concentration (%)
I	Cutina CP	10	Ia	Sugar ester S1670	3
			Ib	SDS	3
			Ic	Tego Care 450	1.2
			Id	Tween 80/Span 85 (7:3)	1
II	Compritol 888 ATO	5	IIa	Pluronic F68	1.2
			IIb	Sugar ester S1670	3
			IIc	SDS	3
III	Stearyl alcohol	10	IIIa	Tween 80	1.2
IV	Imwitor 900	5	IVa	Tween 80	1.2
			IVb	Sugar ester S1670	3
			IVc	Pluronic F68	1.2
		10	IVd	Tagat S/sodium cholate (5:1)	3
V	Geleol	5	Va	Pluronic F68	1.2
VI	Dynasan 114	5	VIa	Poloxamer 407	0.5
			VIb	Sodium cholate	0.5

stable formulations at t_0 showed no considerable changes in particle size distribution after 2 h (t_2). To analyze the extent of stability, we focus on the size data measured after 2-h incubation time (t_2).

Regarding the gastrointestinal environment, there are two main factors causing destabilization of aqueous nanodispersions: ionic strength and/or a low pH. Some SLN formulations showed distinctive particle growth or aggregation (detection of large particles/aggregates in the microparticular range (e.g. a LD90 up to 22 μm) in each simulated isotonic gastrointestinal medium, indicating the electrolyte concentration as the destabilizing factor (Table 2a and Fig. 1). At the low pH values, the formulations Ic and VIa showed heavy aggregation resulting in the formation of few but large particles that could no longer be subjected to particle size analysis by laser diffractometry since no sufficient concentration for correct measurements could be reached by adequate probe volumes. Besides being electro-

Table 2
(a)–(c) Particle sizes of the different aqueous SLN formulations before addition (= original SLN) and after addition to the isotonic artificial gastrointestinal media after 2-h incubation (t_2) by means of laser diffractometry

SLN code	Original SLN		pH 7.4		pH 3.5		pH 1.1	
	LD50 (μm)	LD90 (μm)	LD50 (μm)	LD90 (μm)	LD50 (μm)	LD90 (μm)	LD50 (μm)	LD90 (μm)
(a) *Electrolyte-sensitive SLN dispersions*								
Ic	0.282	0.494	0.172	11.203	–[a]	–[a]	–[a]	–[a]
Id	0.120	0.251	0.142	13.293	0.206	14.867	0.208	21.994
Va	0.091	0.159	0.151	6.569	0.158	6.387	0.262	10.874
VIa	0.249	0.403	0.438	1.360	–[a]	–[a]	–[a]	–[a]
(b) *pH-sensitive SLN dispersions*								
IIb	0.099	0.194	0.118	0.340	0.254	1.416	0.348	1.604
IVb	0.182	0.428	0.181	0.425	–[a]	–[a]	–[a]	–[a]
IVc	0.098	0.183	0.090	0.128	0.289	13.551	0.259	16.654
IVd	0.122	0.151	0.104	0.150	–[a]	–[a]	–[a]	–[a]
VIb	0.209	0.371	0.210	0.365	–[a]	–[a]	–[a]	–[a]
(c) *GIT-stable SLN dispersions*								
Ia	0.107	0.211	0.108	0.215	0.130	0.275	0.128	0.273
Ib	0.113	0.221	0.108	0.213	0.108	0.212	0.110	0.215
IIa	0.143	0.365	0.132	0.377	0.142	0.369	0.131	0.374
IIc	0.097	0.183	0.094	0.180	0.096	0.184	0.092	0.175
IIIa	0.182	0.393	0.164	0.376	0.209	0.403	0.174	0.375
IVa	0.097	0.179	0.092	0.139	0.092	0.188	0.093	0.175

[a] Heavy aggregation, particle size analysis not accessible anymore by laser diffractometry.

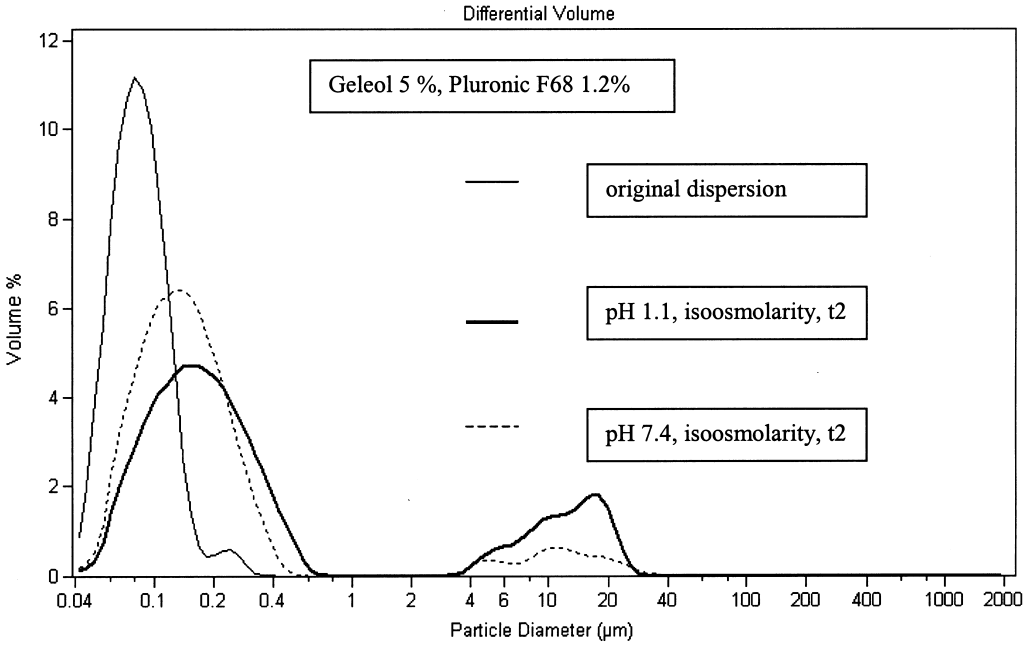

Fig. 1. Particle size distributions of the electrolyte-sensitive SLN formulation Va (Geleol 5%, Pluronic F68 1.2%) determined by laser diffractometry. Comparison of the particle size distribution of the original aqueous SLN formulation with the distributions after addition to isotonic artificial GI media (pH 1.1 and pH 7.4, 2-h incubation (t_2)).

lyte-sensitive, the pH value had an additionally destabilizing effect (see below).

Freitas and Müller [24] have already investigated the influence of different electrolytes on Compritol SLN. The destabilizing effect of electrolytes on emulsions has been reported before [25,26]. A diffuse layer of hydrated non-fixed ions has been assumed for particles in aqueous dispersions. A similar charge on the surface of particles leads to electrostatic repulsion. The zeta potential of the particles can be used as a measure of particle charge (and electrostatic repulsion) [21]. High electrolyte concentrations may cause a compression of the diffuse layer. Thus, the decrease of zeta potential and electrostatic repulsion results in destabilization.

This could be confirmed for the investigated electrolyte-sensitive SLN formulations, which showed a significantly decreased zeta potential in the isotonic artificial media compared to the measurements in the original SLN dispersion medium or in distilled water (50 μS) (Table 3a), going along with destabilization. Heavy aggregation at the lower pH values in some SLN dispersions prevented correct zeta potential measurements. Additional steric stabilization through non-ionic surfactants e.g. Pluronic F68, which can be used to avoid aggregation [27], was not sufficient to provide GIT stability in these SLN dispersions. A certain thickness of the polymer layer (>10 nm [21]) is necessary for efficient and complete steric stabilization. Depending on the particle size and hydrophobicity of the surface, the thickness of the coating layer of Pluronic F68 on polystyrene particles, for example, is only maximum about 7 nm [21]. High electrolyte concentrations may dehydrate the surfactant adsorption layer of Pluronic F68 and thereby further reduce its thickness and stabilizing effect [28]. Reich [29] described that, depending on the hydrophobicity of the surface of poly(D,L-lactide) (PLA) nanoparticles, Pluronic F68 shows a diverse adsorbing pattern, resulting in a different thickness of the adsorption layer and thus leading to physical stability differences in GI fluids. The surfactants in the electrolyte-sensitive SLN formulations showed insufficient steric stabilization to compensate for the decrease of the electrostatic repulsion due to a low layer thickness and density on the particle surface.

Other SLN dispersions were not influenced by the used electrolyte concentration, since they showed no distinctive changes of particle size distribution in the isotonic artificial GI fluid at neutral pH. However, these formulations were destabilized through a low pH (Table 2b). The pH-sensitive nanodispersions showed a similar behaviour at pH 3.5 and pH 1.1, i.e. a similar extended aggregation took place at the two pH values. The influence of a low pH was, in general, stronger than the influence of the electrolyte concentration, resulting in very large aggregates (IVb, IVd and VIb) that could not be analyzed by laser diffractometry (see above).

The dependence of the physical stability on the pH has already been reported for emulsions [21]. Schwarz and Mehnert [30] investigated the influence of the pH on drug-loaded SLN. A decreasing pH leads to an increasing protonation and decreasing deprotonation of the functional groups on the particle surface (e.g. NH_3/NH_4^+ and $COOH/COO^-$ in lecithin-stabilized emulsions). The zeta potential decreases and, after the 'point of zero', the zeta potential becomes positive, thus increasing again. Additionally, a decrease in pH results in an increased electrolyte concentra-

Table 3
(a)–(c) Zeta potential measurements (in mV) of the SLN formulations in distilled water (50 μS), in the original SLN dispersion medium and in the isotonic artificial GI media at pH values 7.4, 3.5 and 1.1; standard deviations were typically 0.2–1.5 mV

SLN	Distilled water (mV)	Original medium (mV)	pH 7.4 (mV)	pH 3.5 (mV)	pH 1.1 (mV)
(a) *Electrolyte-sensitive SLN dispersions*					
Ic	−43.6	–[a]	−28.7	–[b]	–[b]
Id	−33.6	−23.3	−11.0	−4.8	−2.6
Va	−10.5	−26.2	−6.4	−5.7	−2.5
VIa	−18.3	−9.3	–[b]	–[b]	–[b]
(b) *pH-sensitive SLN dispersions*					
IIb	−20.5	–[a]	−19.2	−6.4	−0.6
IVb	−35.8	–[a]	−20.3	–[b]	–[b]
IVc	−15.3	−24.6	−11.8	−4.1	−6.6
IVd	−21.9	−16.4	−7.3	–[b]	–[b]
VIb	−41.2	−39.0	−58.9	–[b]	–[b]
(c) *GIT-stable SLN dispersions*					
Ia	−31.7	–[a]	−22.8	−9.2	−7.9
Ib	−47.8	−53.6	−73.3	−61.0	−61.8
IIa	−15.8	−23.4	−10.7	−8.2	−11.2
IIc	−46.8	−44.2	−49.7	−48.8	−51.4
IIIa	−25.2	−24.1	−9.3	−7.9	−6.3
IVa	−10.6	−18.9	−8.9	−8.7	−9.2

[a] Measurements in original SLN dispersion medium not possible due to water-insoluble ingredients.
[b] Heavy aggregation.

tion, which can cause a compression of the diffuse layer. This leads to a decrease of the electrostatic repulsion resulting in destabilization of the nanodispersion.

The zeta potential measurements (Table 3b) of the pH-sensitive SLN formulations are in good agreement with the stability results (Table 2b). At neutral pH, a small decrease of the zeta potential compared to the zeta potential in distilled water (50 μS) or the original SLN medium occurred, whereas a greater decrease was observed at the lower pH values. Formulation VIb (Dynasan 114, sodium cholate) showed an even more increased zeta potential at pH 7.4 due to further adsorption of the electrolytes of the medium on the particle surface, thus increasing the electrostatic repulsion. At the lower pH values, the higher electrolyte concentration led to the compression of the diffuse layer resulting in aggregation of the particles (see above). The large decrease of the zeta potential of the formulations IVd and IVc at pH 7.4 did not lead to aggregation. In combination with the lipid Imwitor 900, the additional steric stabilization through the non-ionic surfactants Tagat S and Pluronic F68 seems to provide a sufficient thickness and density of the layer on the surface to stabilize the particles efficiently at neutral pH. At the lower pH values, this steric stabilization in the SLN formulations could not compensate the decrease of the electrostatic repulsion to prevent aggregation.

It was possible to produce aqueous SLN dispersions that were stable in every simulated gastrointestinal medium within the 2 h under investigation by an optimized combination and concentration of lipid and surfactant (Table 2c).

The ionic surfactant SDS proved itself to be very sufficient in stabilizing emulsions [21] and inorganic suspensions [31]. In general, with increasing salt concentration above 10^{-6} M, the particle charge will increase to a maximum and then diminish at higher ionic strengths (approximately 0.001–1 M) due to the compression of the diffuse layer. Ionic surfactants can be used to increase the particle charge even at high electrolyte concentrations. At this high ionic strength, the diffuse layer is covered by the adsorbed surfactant layer. SDS possesses charged groups, which are postulated to be located outside the diffuse layer thus preventing the decrease of the zeta potential [31]. This was confirmed by the high, even increased zeta potential values in the isotonic artificial media of the formulations Ib and IIc, stabilized with SDS (Table 3c).

The surfactant Tween 80 provided GIT stability in combination with the lipids Imwitor 900 and stearyl alcohol (IIIa, IVa). These formulations did not show high zeta potential values, neither in the original dispersion medium nor in the simulated GI fluids. The aggregation of the lipid particles is prevented in these formulations efficiently by steric stabilization. The low zeta potential is caused by the coverage of the diffuse layer by the uncharged polymer layer. This coating layer of Tween 80 had a sufficient thickness and density on the surface in combination with complete coverage of the particles surface to provide physical stability in the different media. This mechanism of steric stabilization can also be assumed for the GIT-stable formulations Ia and IIa, consisting of the emulsifiers, sugar ester S1670 and Pluronic F68.

The results so far have shown that SLN formulations consisting of different lipids could be stabilized by certain surfactants against the influence of electrolytes and/or a low pH.

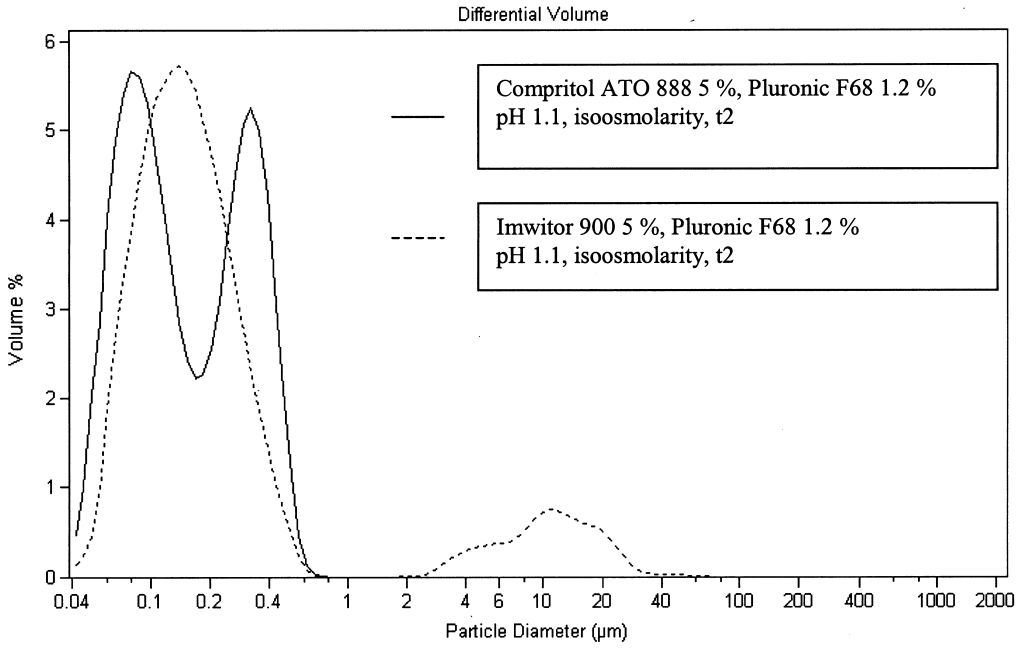

Fig. 2. Particle size distribution of SLN consisting of the same surfactant (Pluronic F68 1.2%) but different lipid matrix, determined by laser diffractometry. Comparison of the aqueous GIT-stable SLN formulation IIa (Compritol ATO 888 5%) with the aqueous pH-sensitive SLN formulation IVc (Imwitor 900 5%) after addition to the isotonic artificial GI media (pH 1.1, 2-h incubation (t_2)).

Comparing SLN consisting of the same lipid but stabilized by different surfactants and vice versa, we could conclude that neither certain lipids nor certain surfactants provide guaranteed stability in gastrointestinal media. Fig. 2 compares the physical stability of SLN formulations stabilized with the same surfactant, but consisting of different lipids. The non-ionic steric emulsifier Pluronic F68 was suitable for stabilizing the triglyceride Compritol ATO 888 in the artificial GI medium at pH 1.1. However, in combination with the lipid Imwitor 900, the resulting SLN dispersion proved to be pH-sensitive.

The data show that the GIT-stability is dependent on the optimized combination of the lipid with the surfactant. This is attributed to differences in the affinity of the emulsifier to the lipid–water interface during SLN production. The affinity of the surfactant to the particle surface is less entropy driven, but very much influenced by the hydrophobicity of the lipid surface. Due to these differences in hydrophobicities of different lipids, there are different affinities of the surfactants towards the surface as a result, thus leading to differences in surface coverage (no. of surfactant molecules per nm^2). Without complete coverage of the interface with emulsifier, particle aggregation is enhanced. The contact of uncovered lipid crystalline surfaces can lead to crystal growth between the particles. Jores et al. [32] have performed studies to determine an optimized surfactant concentration.

The affinity of the emulsifier for the lipid–water interface during SLN production also leads to differences in the anchoring of the lipophilic surfactant parts in the outer shell of the lipid particles, which are liquid during the hot homogenization procedure. These differences are a function of the compatibility and structural similarity of the lipid and the surfactant moiety. Both affinity of the surfactant to the lipid and anchoring of the surfactant in the particle surface result in a different thickness of the stabilizing layer and a different zeta potential. Thus, the same surfactant can lead to different stabilities in combination with different lipids and vice versa.

The analysis of the zeta potentials showed that the steric stabilization alone is not sufficient for GIT-stable SLN. It needs to be combined with a minimum of electrostatic stabilization (i.e. zeta potential approximately 8–9 mV). The results further showed that zeta potentials below approximately 6 mV lead to physically instable SLN in any case. For further investigations regarding GIT-stable SLN, these parameters can be used as selection criteria to sort out inefficient SLN.

According to steric stabilization theory [28], the thickness of the sterically stabilizing layer is of high importance. In case of polymeric particles, the layer thickness can be determined by a simple photon correlation spectroscopy (PCS) measurement (comparison of 'naked' particles vs. particles with adsorbed sterically stabilizing surfactant). This is not possible for SLN because the naked reference does not exist. At present, field-flow fractionation (FFF) measurements are being performed [33], which allow to determine the exact thickness of this stabilization layer. This will be the third criterion to select GIT-stable SLN.

Although particle growth and aggregation after peroral application is a crucial consideration, it has to be borne in mind that this in vitro study only observed the two factors, ionic strength and different pH values. The situation in vivo

is much more complex. Reich [29] found a complex influence – stabilizing and destabilizing – of different charged gastrointestinal proteins on the physical stability of PLA nanoparticles at pH 7.5. Of great importance is the enzymatic degradation of SLN in the gastrointestinal tract [17], which can lead to deaggregation of the lipid particles. Additionally, it has to be mentioned that this study was performed with drug-free SLN dispersions. Further influence on physical stability by incorporated drugs due to potential ionic properties and/or interaction with the particle interface is very likely and has to be considered.

4. Conclusion

From the results, it can be concluded that it is possible to produce GIT-stable SLN dispersions by optimizing the surfactant/mixture for each lipid in vitro. Pre-requisites for stability were identified, i.e. minimum of 8–9 mV zeta potential in combination with a steric stabilization. To complete this information, future FFF studies are necessary to measure the thickness of the sterically stabilizing surfactant layers, which is another appropriate criterion to select GIT-stable SLN.

In vivo, there are two cases possible concerning the influence of SLN aggregation on the bioavailability of orally administered incorporated drugs.

First, SLN need be to physically stable to achieve a high bioavailability. In this case, we need to apply the SLN, which were found to be stable in GI media in our in vitro test system.

Second, aggregation does not play a role. In spite of having a certain aggregation in the SLN, the bioavailability is also high.

To investigate this question of a possible influence of SLN aggregation on the bioavailability, drug-loaded non-aggregating (stable) and aggregating (GIT-unstable) SLN need to be administered in a comparative in vivo study. The presented in vitro study provides the basis for the development of these SLN for the in vivo experiments.

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