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Chemical stability of lipid excipients in SLN-production of test formulations, characterisation and short-term stability

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The study investigates the chemical stability of lipids used as excipients in the production of solid lipid nanoparticles (SLN). A total of 17 SLN formulations was produced using different lipids. Most of the formulations were produced using identical binary surfactant mixtures and concentrations to study the effect of the chemical nature of the lipid on its stability in SLN. In some formulations surfactants were exchanged to study the contribution of the surfactant. The particles were characterised by photon correlation spectroscopy, laser diffractometry, zeta potential determination and differential scanning calorimetry, the latter to assess potential effects of lipid crystallinity and modifications on lipid stability. Lipid analysis was performed by gas chromatography using a sampling preparation and analysis procedure especially developed for SLN. This short-term study provides primarily information about the stability of the lipid under production conditions, that means high pressure homogenisation (cavitation) at high temperature. No degradation products could be detected for all lipids, the production process itself did not impair excipient stability.

1. Introduction

About ten years ago solid lipid nanoparticles (SLN) have been invented as a novel, alternative drug delivery system (Müller and Lucks 1996). At the beginning of the nineties only three research groups were working with SLN producing them by high pressure homogenisation (Müller et al. 1995; Westesen et al. 1993) or alternatively by a microemulsion technique (Gasco 1993). The SLN delivery system attracted increasing interest and meanwhile research groups all over the world are working with this system (Müller et al. 1995, 2000).

However, it took until some years ago to have the first data published about the chemical stability of pharmaceutical drugs and cosmetic active ingredients incorporated into SLN (Jenning 1999). By now, intensive studies about lipid crystallisation including polymorphic transitions have been performed (Freitas and Müller 1999; Wissing and Müller 2002a, 2002b) however no attention was given to the chemical stability of the lipid matrix – an important prerequisite for a developed formulation to be introduced to the clinic and the pharmaceutical market.

In 1999 an analytical method was published for the determination of the chemical stability of lipids in the matrix of SLN (Radomska et al. 1999). It is based on a gas chromatography (GC) analysis in combination with a method to extract the lipid from aqueous SLN dispersions. Exemplarily three SLN formulations based on three different lipids were chosen to establish the method and to perform a first stability study, the dispersions were stored at room temperature. However, no systematic stability study was performed.

This paper describes the first part of a systematic stability study investigating the effect of the chemical nature of the lipid and concentration of surfactant but also the influence of storage conditions. It describes the development of the test formulations, their characterisation and short-term stability.

2. Investigations, results and discussion

To investigate the effect of the chemical nature of the lipid matrix, glycerides with increasing length of the chain of the fatty acid were used (Dynasan 112 to Dynasan 118). As lipids with C22, the fatty acids Compritol 888 and Precirol Ato 5 were included in the study. Primarily to study the effect of a different ratio of mono-, di- and triglycerides various Softisan lipids and Imwitor 900 were selected. The first SLN produced ever were made from lipids for suppositories, that means Witepsol (Müller and Lucks 1996). Two Witepsol lipids S51 and S55 – having a very different hydroxyl number – were also used as lipids with different emulsifying capacity for water. It was assumed that a different content of emulsifying mono- and diglycerides (e.g. quantified by hydroxyl number) might lead to a different content of water in the SLN lipid matrix thus potentially affecting lipid stability. The existence of water inside the particle matrix of SLN was previously shown by ESR measurements when lipids with highly emulsifying properties are used for particle production (Zimmerman 2001). An overview of the lipids used and their relevant properties gives Table 1.

Table 1: Chemical composition, melting point and hydroxyl value of lipids used as SLN matrix

Liped	Glycerides (%)			Melting point (°C)	Hydroxyl value (mg KOH/g)
	mono-	di-	tri-		
Dynasan 112	–	3	96	43–47	max. 10
Dynasan 114	–	4	95	55–58	max. 10
Dynasan 116	–	3	96	61–65	max. 10
Dynasan 118	–	2	97	70–76	max. 10
Softisan 138	–	11	88	37–40	max. 15
Softisan 142	–	10	89	42–44	max. 15
Softisan 154	–	3	96	53–58	max. 10
Compritol 888	12–18	52–54	28–32	~70	80–105
Imwitor 900	40–50	~40	~5	56–61	300–330
Precirol Ato 5	8–15	~54	~30	52–55	90–110
Witepsol S51	4	33	62	30–32	55–70
Witepsol S55	10	14	75	33.5–35.5	50–65

Surfactants will distribute between the water phase and melted lipid phase during the production of SLN. Surfactant contained in the lipid phase can lead to solubilisation of water, which consequently might affect lipid stability. To avoid potential differences in the uptake of water by the lipid phase due to the variation in solubilisation capacity of different surfactants, preferentially the same surfactant should be used to prepare the SLN from the different lipids. In general binary mixtures of surfactants are much more efficient in stabilisation than one surfactant alone. Therefore binary mixtures were employed. Of course, there is no "ideal surfactant mixture" yielding physically stable SLN with any lipid matrix. Therefore it was necessary to perform a screening with a number of binary mixtures to identify this surfactant mixture giving stable particles with all investigated lipids. This identified mixture was composed of 1.25% Tagat S and 0.25% cholic acid. Including variation of the concentration of the surfactant mixture, about 100 formulations were screened.

The type of surfactant and also the concentration might affect chemical stability of the lipid matrix (e.g. differences in the incorporation of the surfactant in the outer shell of the particles, different solubilising capacities for water in the lipid phase). Therefore in selected formulations Tagat S was replaced by Tween 80, the cholic acid was replaced by sodium dodecyl sulfate (SDS), in addition the concentration of cholic acid was varied (0.10% and 0.25%). An overview of the composition of the SLN formulations gives Table 2.

Due to potential reactions and the interface lipid/water the total surface area of the nanoparticles in the aqueous SLN dispersions will have an effect on the chemical stability. Therefore, to eliminate or at least to minimise the effect of total surface, all SLN prepared from the different lipids should be similar in size. This was an additional obstacle in addition to the necessity to use preferentially the same binary surfactant mixture at the same concentration for all systems. Localisation in the interface, spatial arrangement and reduction in interfacial tension will be different for a surfactant mixture depending on the nature of the lipid phase. Due to this the production via the hot emulsification step will lead necessarily to size differences in the obtained hot oil-in-water emulsion and subsequently the formed SLN. To minimise this, size can be affected by applying less or more than three homogenisation cycles. This was done in the production for formulations which were outside the envisaged size range of 0.1–0.3 μm. In general, lower cycle numbers lead to larger particles, increase in the cycle number further reduces the size. Therefore variation of the production parameters could compensate for differences in the dispersion properties of the different lipids when using the same binary surfactant mixture. Table 3 gives an overview of the size characterisation data of the formulations.

To investigate the influence of storage temperature the produced batches were divided and vials stored at 4–8 °C in the fridge, at room temperature and at 40 °C. Ideally the SLN dispersions should remain unchanged in particle size at all three storage conditions. This can only be achieved for each lipid when using the optimised chemical type and concentration of surfactant specifically for each lipid matrix. This is however hardly possible when the same mixture at the same concentration is used for all lipids. Despite this, most of the formulations remained stable under all three storage conditions over the two week period of this short-term study (e.g. formulation 9), others showed a size increase at one or two storage temperatures (e.g. formulation 13, increase at 40 °C storage temperature). Table 4 gives an example of a stable formulation and a less stable one, i.e. 9 and 13.

For the determination of the chemical stability of the lipid matrix, particle aggregation is considered to have little or

Table 2: Composition of SLN formulations (lipid content 5%)

Formulation	lipid	emulsifier	co-emulsifier
Rp. 1	Dynasan 112	Tween 80 1.0%	SDS 0.05%
Rp. 2	Dynasan 112	Tween 80 1.0%	Cholic acid 0.1%
Rp. 3	Dynasan 112	Tagat S 1.25%	Cholic acid 0.25%
Rp. 4	Dynasan 114	Tween 80 1.0%	SDS 0.05%
Rp. 5	Dynasan 114	Tween 80 1.0%	Cholic acid 0.1%
Rp. 6	Dynasan 114	Tagat S 1.25%	Cholic acid 0.25%
Rp. 7	Dynasan 116	Tagat S 1.25%	Cholic acid 0.25%
Rp. 8	Dynasan 118	Tagat S 1.25%	Cholic acid 0.25%
Rp. 9	Softisan 138	Tagat S 1.25%	Cholic acid 0.25%
Rp. 10	Softisan 142	Tagat S 1.25%	Cholic acid 0.25%
Rp. 11	Softisan 154	Tagat S 1.25%	Cholic acid 0.25%
Rp. 12	Compritol 888	Tagat S 1.25%	Cholic acid 0.25%
Rp. 13	Compritol 888	Tween 80 1.25%	Cholic acid 0.1%
Rp. 14	Imwitor 900	Tagat S 1.25%	Cholic acid 0.25%
Rp. 15	Precirol Ato 5	Tagat S 1.25%	Cholic acid 0.25%
Rp. 16	Witepsol S51	Tagat S 1.25%	Cholic acid 0.25%
Rp. 17	Witepsol S55	Tagat S 1.25%	Cholic acid 0.25%

The suspensions were prepared adding water to 100% (w/w)

Table 3: Particle size analysis of SLN formulations at the day of production

	Rp. 1	Rp. 2	Rp. 3	Rp. 4	Rp. 5	Rp. 6	Rp. 7	Rp. 8	Rp. 9	Rp. 10	Rp. 11	Rp. 12	Rp. 13	Rp. 14	Rp. 15	Rp. 16	Rp. 17
LD (μm)																	
d 50%	0.165	0.287	0.205	0.179	0.215	0.245	0.285	0.310	0.201	0.234	0.294	0.320	0.106	0.094	0.104	0.095	0.084
d 90%	0.377	0.496	0.419	0.376	0.399	0.443	0.560	0.483	0.408	0.455	0.553	1.595	0.239	0.160	0.209	0.161	0.141
d 95%	0.440	0.514	0.484	0.418	0.441	0.498	0.579	0.549	0.469	0.529	0.569	2.185	0.323	0.194	0.261	0.193	0.163
d 99%	0.562	0.679	0.536	0.492	0.519	0.603	0.864	0.641	0.580	0.595	0.675	3.782	0.472	0.303	0.343	0.296	0.250
PCS (nm)																	
mean	176	178	182	194	204	254	282	273	223	248	231	240	158	101	171	129	119
PI	0.186	0.184	0.200	0.226	0.212	0.325	0.302	0.231	0.361	0.290	0.142	0.392	0.335	0.326	0.224	0.216	0.217

laser diffractometry [LD] diameters 50% to 99% and PCS data, mean diameter and [PI] – polydispersity index

Table 4: Examples of particle size analysis of a stable formulation (Rp. 9) and a formulation not stable at 40 °C (Rp. 13) on the day of production and after two weeks of storage

LD (μm)	Rp. 9				Rp. 13			
	Production day	After 2 weeks			Production day	After 2 weeks		
		4 °C	25 °C	40 °C		4 °C	25 °C	40 °C
D 50%	0.201	0.145	0.146	0.158	0.106	0.106	0.104	0.136
D 90%	0.408	0.371	0.346	0.377	0.239	0.240	0.221	0.405
D 95%	0.469	0.441	0.421	0.450	0.323	0.328	0.305	87.87
D 99%	0.580	0.565	0.554	0.585	0.472	0.464	0.470	157.6

no effect as long as the particles stay as aggregates. There will be little change in surface area (and surface related potential instability) if the particles do not fuse to a large lipid particle with less surface area. A physical instability of SLN leading to a change in surface area is the formation of a gel. Gel formation takes place by SLN particles building up network and lipid bridges in between them (Freitas and Müller 1998). However, gel formation was not observed.

An important parameter for the physical stability of particle dispersions is the zeta potential. Measurements of the zeta potential in distilled water or water with very low conductivity gives information about the surface charge on the particles (Nernst potential). The height of the zeta potential is related to the height of the Nernst potential under these measuring conditions. The zeta potential measured in the original dispersion medium (surfactant mixture) is a direct measure for the physical stability of the dispersion. The height of the zeta potential under these conditions is a measure for the thickness of the diffuse layer. The higher the zeta potential, the thicker is the diffuse layer and the more protective is this layer, that means the more stable is the suspension. As a guideline a minimum potential is required to obtain a physically stable suspension (Lucks et al. 1990; Müller and Lucks 1996). However, this is only valid in case of electrostatic stabilisation. When a sterically stabilising surfactant is present in the surfactant mixture, even lower zeta potentials are sufficient for a stable suspension. Electrostatic stabilisation and steric stabilisation effects are additive.

Formulations 2, 3 and 6 to 17 have in most cases similar zeta potentials in water and in the original dispersion medium (surfactant mixture). The ethoxylated surfactant Tagat S on the surface leads to a shift of the slipping plane in the diffuse layer. This is well known from particles having adsorbed layers of poloxamer on the surface (Carstensen et al. 1991). Under these circumstances the slipping plane at which the zeta potential is measured is approximately in the same distance to the particle surface under both measuring conditions resulting in identical or very similar zeta potentials (Table 5).

Table 5: Zeta potentials data measured at the day of production in distilled water and in surfactant mixture and differences between them

Formulation	Zeta potential (mV) values		Difference (mV)
	in distilled water	in surfactant mixture	
Rp. 1	−35.2	−59.5	−24.3
Rp. 2	−26.8	−33.1	− 6.3
Rp. 3	−24.7	−28.4	− 3.7
Rp. 4	−32.5	−48.2	−15.7
Rp. 5	−24.1	−34.5	−10.4
Rp. 6	−22.3	−28.7	− 6.4
Rp. 7	−25.2	−27.2	− 2.0
Rp. 8	−25.7	−28.0	− 2.3
Rp. 9	−24.6	−28.7	− 4.1
Rp. 10	−21.8	−30.4	− 8.6
Rp. 11	−27.6	−26.1	− 1.5
Rp. 12	−28.1	−20.5	− 7.6
Rp. 13	−28.1	−29.4	− 1.3
Rp. 14	−24.1	−24.2	− 0.1
Rp. 15	−31.2	−30.1	− 1.1
Rp. 16	−24.6	−19.7	− 4.9
Rp. 17	−23.9	−21.8	− 2.1

All zeta potentials for the formulations 6 to 17 are in the range of approx. −20 to −30 mV, that means below the critical value for pure electrostatic stabilisation. However, due to the contribution of the steric stabiliser Tagat S most of the formulations are stable. Related to the zeta potential, formulation 17 should possess the lowest stability with −21.8 mV in the original dispersion medium.

Especially formulations 1 and 4 show distinctly higher zeta potentials when measured in the original dispersion medium compared to water. For example, formulation 1 has only −35 mV in water but −60 mV in the dispersion medium. This can be explained by increased adsorption of SDS onto the particle surface when present in a concentration of 0.05%. No decrease of the zeta potential occurred. Normally a decrease in zeta potential occurs due to "compression" of the diffuse layer with increasing electro-

Table 6: Examplary zeta potentials data, after two weeks of storage at different temperatures

Zeta potential (mV)		Rp. 4		Rp. 7		Rp. 14	
		in water	in surfactant mixture	in water	in surfactant mixture	in water	in surfactant mixture
Day 0		−32.5	−48.2	−25.2	−27.2	−24.1	−24.2
Day 14	5 °C	−32.6	−48.7	−25.1	−27.5	−24.1	−23.1
Day 14	25 °C	−34.2	−51.5	−23.8	−29.5	−24.5	−18.7
Day 14	40 °C	−33.8	−50.8	−23.6	−27.8	−23.4	−16.2

lyte content. In this case, with increasing SDS concentration the zeta potential increases also – an effect already described in the literature (Lucks et al. 1990). This effect is also present in some other formulations, e.g. 5, but less pronounced (Table 5, right column, difference between zeta potentials in water and original medium).

Zeta potentials were measured again after two weeks of storage for the samples stored at different temperatures. In general, there was no change for most formulations, values at all three temperatures were identical to the production day. Only in a few cases a deviation of a few mV occurred, Table 6 shows some selected examples.

Transformation of lipid bulk material into lipid nanoparticles leads to changes of the melting behaviour of the lipid accompanied by potential occurrence of lower melting α and β_i modifications. According to the Thomson equation the melting point decreases with decreasing particle size (Hunter 1986). In general, the onset temperature and the melting peak of the lipid nanoparticles are approximately 2–5 °C lower compared to the bulk material. Some lipids – as known from the literature – were not crystalline after production, e.g. Dynasan 112 SLN (Rp. 1–3) (Table 7). Production of nanoparticles also reduces the decrease of crystallinity (Müller 1991), for the investigated formulations a percentage of crystallinity between 50% and 98% was found (Table 7). The reduction in crystallinity is due to the partial formation of lower energy lipid modifications. In addition, surfactants distributed to the melted lipid phase during the production process can distort crystallisation resulting in a lower melting enthalpy. The effect of surfactants on lipid crystallisation can nicely be seen when comparing formulation 12 and 13. The Compritol SLN for formulation 12 are stabilised with 1.25% Tagat S and 0.25% cholic acid, the crystallinity index is 98%, that means they can be considered as fully crystalline. Replacing Tagat S by Tween 80 (identical concentration) and simultaneously decreasing the concentration of cholic acid to 0.10% results in a crystallinity of only 64% (Table 7, formulation 13). Figure 1 shows the corresponding DSC melting curves in comparison to the bulk material.

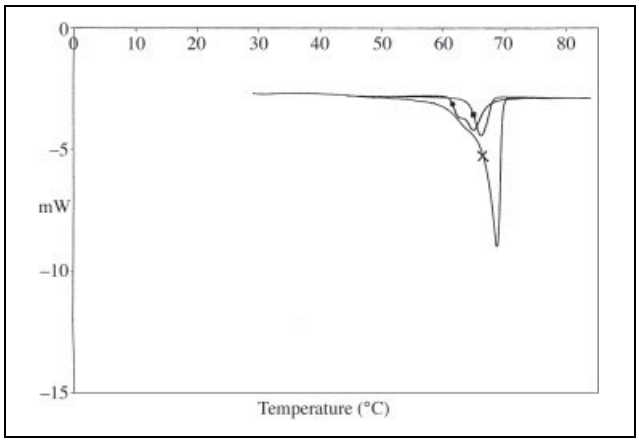

Fig. 1: Comparison of 2nd heating curves of pure Compritol (cross) with Rp. 12 (filled square) and Rp. 13 (filled circle)

Table 7: DSC data of lipid bulk material and SLN formulations

Formulation	Onset (°C)	Peak (°C)	Crystal (%)
Dynasan 112-bulk	43.12	45.35	100
Rp. 1	–	–	–
Rp. 2	–	–	–
Rp. 3	–	–	–
Dynasan 114-bulk	52.05	54.70	100
Rp. 4	–	–	–
Rp. 5	48.04	52.01	67.95
Rp. 6	46.64	51.97	71.57
Dynasan 116-bulk	53.28	60.41	100
Rp. 7	51.98	58.20	65.86
Dynasan 118-bulk	64.52	68.99	100
Rp. 8	61.76	67.14	64.78
Softisan 138-bulk	29.44	33.47	100
Rp. 9	25.98	30.31	97.22
Softisan 142-bulk	31.80	36.06	100
Rp. 10	26.90	36.67	69.48
Softisan 154-bulk	50.66	53.92	100
Rp. 11	49.03	53.08	50.26
Compritol 888-bulk	66.93	69.99	100
Rp. 12	65.05	68.57	98.0
Rp. 13	61.31	65.90	64.36
Imwitor 900-bulk	54.09	58.06	100
Rp. 14	51.2	55.97	92.08
Precirol Ato 5-bulk	49.90	55.79	100
Rp. 15	48.06	53.23	78.06
Witepsol S51-bulk	26.84	31.12	100
Rp. 16	–	–	–
Witepsol S55-bulk	21.8	21.31	100
Rp. 17	–	–	–

("–", i.e. lipid not yet crystallised)

The lipid crystallinity/presence of different modifications might also affect lipid stability. Therefore the formulations 12 and 13 being composed of the same lipid Compritol but having different degrees of crystallinity are a nice example to study this effect. Of course, it needs to be kept in mind that this different degree of crystallinity has been partially generated by using different surfactants when interpreting the data.

The first two stresses enforced onto SLN with potential effect on chemical stability of the lipid are the melting procedure of the lipid to produce the hot pre-emulsion and subsequently the high temperature at simultaneously high pressure of 500 bar during the homogenisation process. In addition to this the lipid itself is in a liquid state potentially facilitating chemical decomposition compared to lipid in its solid state, that means the produced SLN during long-term storage as aqueous suspension. Based on the knowledge of producing creams by phase inversion, the preparation of the pre-emulsion (initial heating) is not likely to have any effect on lipid stability. In addition, this process is very short and takes a maximum of 1–2 min. Based on the experiences from the preliminary study of

lipid stability during establishment of the method (Radomska et al. 1999), there will be little or no decomposition of the lipid within the first few weeks after production. Therefore – when analysing shortly after production (as done in this study) the chemical analysis of lipid stability provides information if at all or to which extent the production process itself (temperature, pressure) affects the lipid stability. Because production of the 17 test formulations and their characterisation including physical stability took more than two weeks, the GC analysis of the lipids was performed in week 3 and 4 after production. In contrast to the first study with only 3 lipids, in this broad study, the effect of production parameters as function of chemical nature of lipid and ratios of glycerides was systematically investigated.

Figure 2 shows exemplarily a GC graph of bulk material (Dynasan 116) and Fig. 3 the GC graph of the corresponding SLN formulation 7 obtained applying a constant temperature for GC analysis. The recovery for the SLN formulation 7 was 99.5%. Calculation of lipid stability of all other SLN formulations yielded a stable lipid content of 99–101% (detailed data in Table 8), that means no significant decomposition was found caused by the production process.

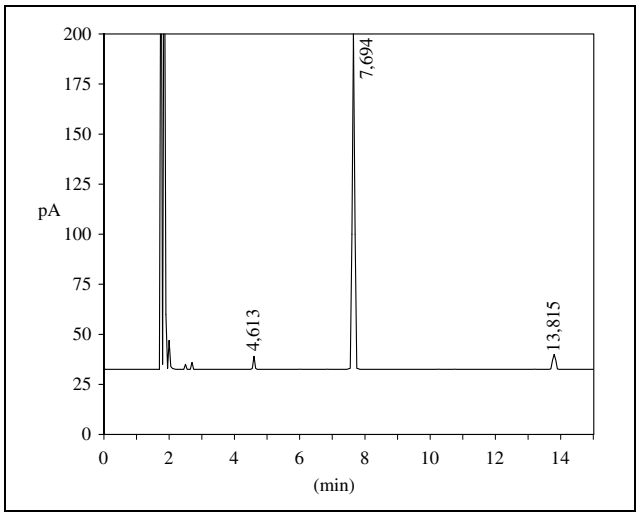

Fig. 2: GC of pure Dynasan 116 bulk material (= reference material for Fig. 3)

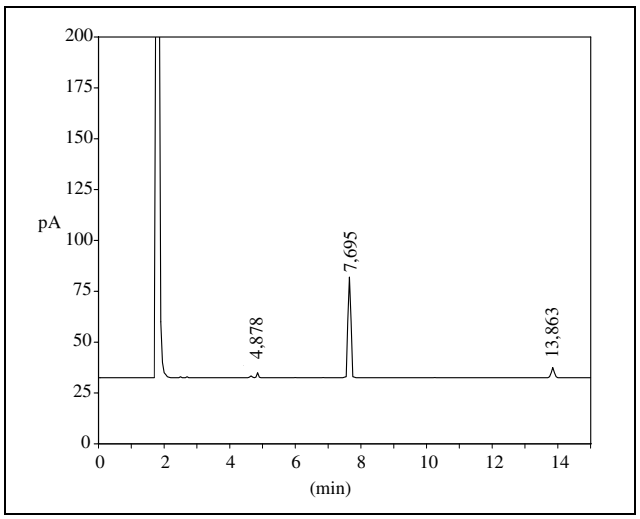

Fig. 3: GC of Dynasan 116 after extraction from aqueous SLN mixture stored at 25 °C

Table 8: Lipid content in SLN formulations after 4 weeks of the incubation at 25 °C

Formulation	Lipid content (%)
Rp. 1	100.1
Rp. 2	99.8
Rp. 3	99.0
Rp. 4	100.0
Rp. 5	99.6
Rp. 6	99.9
Rp. 7	99.5
Rp. 8	100.0
Rp. 9	99.7
Rp. 10	99.9
Rp. 11	99.9
Rp. 12	100.0
Rp. 13	100.0
Rp. 14	100.0
Rp. 15	99.7
Rp. 16	99.4
Rp. 17	99.2

Obviously the production process of SLN itself does not affect the chemical stability of the lipid excipient forming the particle matrix. The observed excipient stability is a pre-requisite to use SLN as a formulation for introduction to the pharmaceutical market. There were already products, which could not enter the market due to a lack of excipient stability (e.g. decomposition of the excipient lecithin in liposomes). The absence of any detectable amount of degradation in products is very promising because even minor amounts could have a catalytic effect on the degradation during long-term storage. The stability monitoring of the produced test formulations will be continued over a total period up to three years, which will provide information about the long-term stability of the lipid excipients and the factors affecting it.

3. Experimental

3.1. Materials

The lipids Dynasan 112, Dynasan 114, Dynasan 116, Dynasan 118, Softisan 138, Softisan 142 and Softisan 154 were a gift from Hüls AG (Witten, Germany). Compritol 888 and Imwitor 900 and Precirol Ato 5 were provided by Gattefosse (Frechen, Germany). Witepsol S51 and Witepsol S55 were a gift from Dynamit Nobel (Witten, Germany). The surfactant Tagat S was provided by Goldschmidt (Essen, Germany), all other surfactants were purchased from Sigma (Deisenhofen, Germany). All chemicals were used as received.

3.2. Methods

3.2.1. Preparation and characterization of the solid lipid nanoparticles

SLN were produced by the hot homogenisation technique (Müller et al. 1995, 1997) using a Micron LAB 40 high pressure homogeniser (APV Systems GmbH, Unna, Germany). The lipid was melted 5–10 °C above its melting point and dispersed in the hot aqueous surfactant solution under stirring using an Ultra Turrax (Janke und Kunkel GmbH and Co KG, Staufen, Germany). The obtained pre-emulsion was then high pressure homogenised applying 500 bar and typically three homogenisation cycles. If necessary, less cycles or one or two more cycles were applied to obtain particles being in the size range of approximately 0.1 to 0.3 µm (diameter 50%, laser diffractometer). After homogenisation the obtained hot oil-in-water emulsion was cooled down, the lipid was re-crystallised and formed solid lipid nanoparticles.

Particle size analysis was performed by photon correlation spectroscopy (PCS) and additionally by laser diffractometry (LD). For the PCS measurements a Malvern Zetasizer 4 was used (Malvern Instruments, Malvern, United Kingdom). PCS yields the mean diameter (z-average) of the bulk population of the particles and additionally a polydispersity index as measure for the width of the distribution. This polydispersity index (PI) ranges from 0 (monodisperse) to 0.500 (very broad distribution). Laser diffractometry (LD) was performed using a Coulter LS 230 (Coulter Electronics, Krefeld, Germany). LD gives a volume distribution weighing especially

large particles. It is therefore highly sensitive to detect larger particles resulting from particle aggregation during storage of the suspensions. As parameters to characterise the size distribution and physical stability, the diameters 50%, 90%, 95% and 99% were used. For example, diameter 90% means that 90% of the particles are below the given size (volume distribution).

It should be noted that due to the differences in the measuring principles between PCS and LD and the different measuring ranges (3 nm–3 μm for PCS, 40 nm up to a few hundred μm for LD), the LD diameter 50% is very often higher than the PCS mean diameter.

The zeta potential was measured using a Malvern Zetasizer 4 (Malvern Instruments, UK), measurements were performed in the large bore capillary applying a field strength of 20 V/cm. The electrophoretic mobility was converted to the zeta potential using the Helmholtz-Smoluchowski equation. Zeta potential measurements were performed in distilled water with a conductivity adjusted to 50 μS/cm by addition of sodium chloride solution. Additionally the zeta potential was measured in the original dispersion medium of the particles.

3.2.2. DSC study

Differential scanning calorimetry (DSC) measurements were performed using a Mettler Toledo System (Germany). Approximately 1–2 mg of lipid bulk material or equivalent SLN dispersion containing this amount of lipid were filled into 40 μL aluminium pans and sealed. Heating took place from 20 °C to 90 °C at a rate of 10 K/min, the cooling curve was run with the same rate. The crystallinity of the particles was quantified as so-called crystallinity index, that means the melting enthalpy of the lipid in the SLN dispersion was expressed as percentage of the melting enthalpy of the bulk lipid (Brittain and Fieses 2002). The bulk lipid was considered being fully crystalline, that means it has the index of 100%. Of course, this index is only a rough measure because the particles can crystallize partially in a different modification, peak separation is in most cases unfortunately not possible.

3.2.3. Gas-chromatography-assay

Chemical analysis of the lipid was performed by gas chromatography using a HP 5890 chromatograph (Hewlett-Packard, USA) as described previously (Radomska et al. 1999). Determination was performed via the corresponding methyl esters of the fatty acids of the lipids. Transmethylation of the lipids was made according the method described by Garces and Mancha (1993) for liquid lipids which was adopted to solid lipids. To separate the lipid from the SLN dispersions they were centrifuged at 17.000 rotations/min = 19.063 g for 30 min. 50 mg of the obtained lipid phase were mixed with methylating mixture containing methanol/toluene/dimethoxypropan/H_2SO_4 (39 : 20 : 5 : 2, by volume) in tubes with teflon caps. The quantity of methylating liquid was 3.3 ml, heptan was added to a total volume of 5 ml. This mixture was incubated in a water bath at 80 °C for 90 min. The tubes were cooled to room temperature and shaken, two phases formed. The upper phase contained the fatty acid methyl esters. The fatty acid composition was analysed using a silica capillary column with high polarity (Supelkowax™ 10, 30 m × 0.32 mm ID, 0.25 μm film thickness, Supelco, Bellefonte, USA). It was operated at constant temperature. The temperature of the injector and the detector (FID) were 200 and 220 °C, respectively. The carrier gas was helium at a flow rate 1.5 ml/min.

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