ELSEVIER

International Journal of Pharmaceutics 237 (2002) 119–128

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Lipase degradation of Dynasan 114 and 116 solid lipid nanoparticles (SLN)—effect of surfactants, storage time and crystallinity

Carsten Olbrich, Oliver Kayser, Rainer H. Müller *

Department of Pharmaceutics, Biopharmaceutics and Biotechnology, The Free University of Berlin, Kelchstrabe 31, D-12169 Berlin, Germany

Received 2 January 2001; received in revised form 21 January 2002; accepted 23 January 2002

Abstract

In vivo drug release from solid lipid nanoparticles (SLN) takes place by diffusion and degradation of the lipid matrix. SLN with different degree of crystallinity were prepared to study the effect of crystallinity on the degradation velocity. These SLN were produced by using glycerides with different length of fatty acid chains and known differences in crystallisation velocity (Dynasan 114 and 116), and using stabilisers interfering differently with the crystallisation process of the lipid matrix (cholic acid sodium salt (NaCh), Poloxamer 407 (Plx 407)). NaCh disturbs the crystallisation process, Poloxamer shows little interference. The particles were characterised by photon correlation spectroscopy (PCS) and differential scanning calorimetry (DSC), degradation velocity was determined directly after production and during storage up to 4 weeks under different storage conditions using an especially developed assay based on the NEFA Test kit. After production, SLN with a lower crystallinity matrix (Dynasan 114 and 116, NaCh) degraded faster than higher crystalline particles (all SLN with Plx 407), and showed a decrease in degradation velocity with increasing crystallinity during storage. Fast crystallising particles made from Dynasan 116 stabilised with the non-interfering Plx 407 showed no change in the degradation velocity during storage. SLN produced with a higher crystalline lipid in combination with the crystallisation-disturbing NaCh (Dynasan 116, NaCh) required a 'ripening time' to reach sufficient crystallinity.

Keywords: SLN; Nanoparticles; Pancreatic lipase; Crystallinity; Dynasan

1. Introduction

Nanoparticles made from solid lipids (SLN) are a colloidal carrier system for topical, oral and parenteral administration of mainly lipophilic drugs (Siekmann and Westesen, 1992; Müller and Lucks, 1996; Cavalli et al., 1996; Heiati et al., 1997; Gasco, 1998). The system consists of lipid nanoparticles which are solid at room temperature. Solid lipid nanoparticles (SLN) are dispersed in an aqueous surfactant solution and are biodegradable, easy to produce (even in large

* Corresponding author. Tel.: +49-30-8385096; fax: +49-30-8385016.

E-mail address: mpharma@zedat.fu-berlin.de (R.H. Müller).

0378-5173/02/$ - see front matter
PII: S0378-5173(02)00035-2

scale) and biocompatible (Müller et al., 1995). SLN are of broad interest and recently it has been published a summary of nearly 10 years of research on SLN reviewing over 100 publications concerning SLN (Müller et al., 2000). SLN can be used to improve the bioavailability of drugs, e.g. cyclosporine A (Penkler et al., 1999), to protect sensitive drugs from decomposition (Jenning et al., 2000) and as a controlled release system for lipophilic drugs (zur Mühlen et al., 1998). As a new application SLN have been successfully tested for their capacity to be used as vaccine adjuvants (Müller et al., 1999; Müller and Olbrich, 1999; Olbrich et al., 2000). SLN are well tolerated when phagocytosed by phagocytic cells (Müller et al., 1988; Müller and Olbrich, 1999) making them interesting for i.v. applications.

SLN possess properties of emulsions and polymeric particles (solid matrix). The solid matrix offers the possibility to improve the stability against coalescence and the reduced mobility of incorporated drug molecules is a prerequisite for protecting them against chemical degradation and for a controlled drug release. Drug release can take place either by diffusion or by degradation of the lipid matrix which occurs mainly by enzymes like lipases and only to a very little extent by hydrolytic processes (Olbrich et al., 1998a). The nature of the lipid matrix and the surfactants have been shown to influence the biodegradation of SLN (Olbrich et al., 1997; Olbrich and Müller, 1999). Using triglycerides with long chain fatty acids and/or sterically hindering surfactants like Poloxamer 407 or Poloxamine 908 a delayed degradation takes place (Olbrich et al., 2000). When using short chain fatty acid triglycerides and/or degradation promoting surfactants as bile salts (e.g. cholic acid sodium salt) leads to a relatively fast degradation. The influence of particle size on degradation is different for certain surfactants. Poloxamer 407 stabilised Dynasan 114 SLN are more sensitive against size effects than cholic acid sodium salt stabilised Dynasan 114 SLN (Olbrich et al., 2000). However, no studies were performed to evaluate the influence of the physical state and degree of crystallinity of the lipid matrix on SLN degradation velocity, being the aim of this study. The degree of crystallinity of the particles after production is a function of the nature of the lipid, surfactants and stabilisers used and also can change during storage time of the particles. To elucidate the degradation properties of the SLN formulations an established lipase/colipase assay (Müller et al., 1995; Olbrich and Müller, 1999) was used.

2. Materials and methods

2.1. Materials

As lipids trimyristin (Dynasan 114) and tripalmitin (Dynasan 116), gifts from Condea, Witten (Germany), have been used. The surfactant Poloxamer 407 (Pluronic F127) (from BASF AG Ludwigshafen/Rhein (Germany) was kindly provided as a gift. Cholic acid sodium salt, porcine pancreatic lipase (Type IV) 30 000 U/mg, colipase from porcine pancreas and calcium chloride dihydrate were purchased from Sigma Aldrich Chemicals (Deisenhofen, Germany), the NEFA C testkit from Wako Chemicals, Neuss, Germany.

2.2. Methods

2.2.1. SLN preparation and size measurement

SLN were produced by using the hot homogenisation technique. Dynasan 114 and 116 (5%) were melted at about 10 °C over the melting point and poured into a hot aqueous surfactant solution. The concentration of surfactants was 0.5% for all formulations. The melted lipid was dispersed in the hot surfactant solution by high speed stirring (10 000 rpm) using an Ultra Turrax (Jahnke und Kunkel, Germany) to yield a pre-emulsion. This pre-emulsion was then homogenised using an APV Gaulin LAB 40 homogeniser (APV Gaulin, Lübeck, Germany) at 500 bar applying three homogenisation cycles. Details of the production method are given in Müller et al. (1995).

The determination of the particle size was performed by photon correlation spectroscopy (PCS) using a Zetasizer 4 (Malvern Instruments, Malvern, UK). PCS yields the diameter of the bulk population (z-average) and a polydispersity

index (PI) to characterise the distribution ranging from 0.000 to 1.000 (monodisperse to very broad). The dispersions were divided into two fractions and stored at room temperature (20–25 °C) and at 4 °C.

2.2.2. DSC measurements

For the determination of the degree of crystallinity of the particle dispersion differential scanning calorimetry (DSC) was used (Mettler Toledo DSC 821e, Mettler Toledo, Kassel, Germany). The heating rate was 10 K/min from 25 to 85 °C. The rate of crystallinity was estimated by comparison of the melting enthalpy/g of the bulk material with the melting enthalpy/g of the dispersion (Siekmann and Westesen, 1994). From the solid bulk material about 5 mg and from the dispersions about 40 mg were accurately weighted into aluminium pans which were sealed. The apparatus was calibrated once a week after the instructions of the manufacturer using gallium and indium as standards.

2.2.3. Enzymatic degradation assay

Lipase and colipase were dissolved in distilled water at concentrations of 2000 U/ml lipase and 50 μg/ml colipase. Six hundred microliters of the lipase solution were mixed with 360 μl of colipase solution. This mixture was incubated at 37 °C for 15 min to form the lipase/colipase complex, necessary for the adsorption of the enzyme on the lipid particle surface. To this pre-incubated mixture, 228 μl of 0.01 M borate/boric acid buffer (pH 7.4), containing 0.02 mol calcium chloride were added. After addition of 12 μl of SLN dispersion, the final concentrations of lipase are 1000 U/ml and 15 μg/ml colipase. Samples of 10 μl were taken and analysed by using the NEFA (non-esterified-fatty acids) C testkit for their content of free fatty acids. This test kit (originally for the determination of free fatty acids in plasma and serum) was adjusted for the determination of free fatty acids, the degradation products of the lipase incubation (Olbrich et al., 1998b). Quantitatively a purple chinonimin colouring is formed, whose absorption maximum is at 550 nm. This coloured solution, the product of the enzymatic reaction of the NEFA C testkit is measured in a microtitre-plate photometer (Easy Reader EAR 400 AT, SLT Instruments, Grödig, Austria). The amount of free fatty acids was calculated by using oleic acid as standard. The maximum amount of free fatty acids which are detectable with this assay is 66% of the theoretical value, because the lipase/colipase complex can degrade the glycerides only to the monoglycerides (Winkler et al., 1990), that means when 66% of FFA (free fatty acids) are reached this corresponds to the maximum possible degradation in this test (= full degradation). On the basis of the saponification values, a 100% value for full degradation was determined (German Pharmacopoea, 9th ed.). In the enzymatic degradation assay the percentages of free fatty acids are expressed in relation to the 100% from saponification. Statistical analysis was performed using *t*-test analysis.

3. Results and discussion

The possibility of producing SLN dispersions from lipids, solid as bulk lipids at room temperature, which remain in the liquid state after production, even when stored at 4 °C (trilaurin-SLN) (Westesen and Bunjes, 1995) was shown previously. Trimyristin (Dynasan 114) dispersions remain in the liquid state after production when stored at room temperature and solidify after cooling to 4 °C (Bunjes et al., 1996). Because of this property Dynasan 114 dispersions have been chosen to examine the influence of the physical state of the lipid matrix on the enzymatic degradation.

As an example for polymorphic transitions in SLN matrices, Dynasan 116 SLN have been produced. Polymorphic transitions after crystallisation of triglyceride nanoparticles are slower for longer chain triglycerides (Dynasan 116) than for shorter chain triglycerides (Bunjes et al., 1996). For Dynasan 116, 118 and Cetylpalmitate it has been demonstrated the influence of surfactants on the degradation of SLN (Olbrich and Müller, 1999). Also for Dynasan 114 it can be expected that the type of surfactant is influencing SLN degradation. Physically stable dispersions of Dynasan 114 were produced using Poloxamer 407

(Pluronic F 127) and cholic acid sodium salt. These stabilisers demonstrated their pronounced ability to influence lipid degradation on Dynasan 116 and 118 SLN (Olbrich and Müller, 1999). Therefore, they were used to study the influence of stabilisers/surfactants, storage time, storage temperature and consequently degree of crystallinity on the degradation behaviour of Dynasan 114 and 116 SLN over a period of 4 weeks.

Fig. 1 shows the degradation of Dynasan 114 SLN stabilised with the two different surfactants. SLN stabilised with cholic acid sodium salt are degraded fastest and completely after 120 min of degradation in the lipase/colipase solution (= 66% FFA). Dynasan 114 SLN stabilised with Poloxamer 407 show a reduced degradation within 120 min compared with other surfactants like the lecithin Lipoid E80, Tween 80 or Poloxamer 188 and cholic acid sodium salt (Olbrich et al., 2000). The PCS diameter of Dynasan 116 SLN produced with cholic acid sodium salt is larger (260 nm; PI, 0.167) than SLN produced from Dynasan 114 using the same surfactant (186 nm; PI, 0.219). This is in agreement with data. For Dynasan 116 also cholic acid sodium salt stabilised SLN were degraded fastest and Poloxamer 407 as surfactant led to slowest degradation (Olbrich and Müller, 1999). The larger sized NaCh-stabilised particles are degraded fastest, i.e. this proves that the stabiliser is the determining factor and size differences of approximately 100–200 nm have little effect as already shown previously (Olbrich and Müller, 1999). Dynasan 114 SLN, produced with either NaCh or 407 as stabilisers have been stored at room temperature and at 4 °C over a period of 4 weeks. It was described that Dynasan 114 dispersions remain liquid when stored at room temperature whereas they solidify when cooled down to 4 °C (Bunjes et al., 1996). The degree of crystallinity of the formulations was determined using DSC (Fig. 2). Poloxamer 407 stabilised SLN crystallise faster and completely after 4 days of storage time at 4 °C. The NaCh formulation crystallises initially slightly faster (after 1 day) and after that slower, only to an extent of about 80% after 28 days of storage (Fig. 2). The surfactant cholic acid sodium salt seems to disturb the crystallisation process. It was also described for Dynasan 116 SLN stabilised with lecithin and sodium glycocholate to show retarded crystallisation (Siekmann and Westesen, 1994). The polymeric Poloxamer 407, which adsorbs only at the particle surface, seems not to influence the crystallisation process in such a pronounced manner like cholic acid sodium salt.

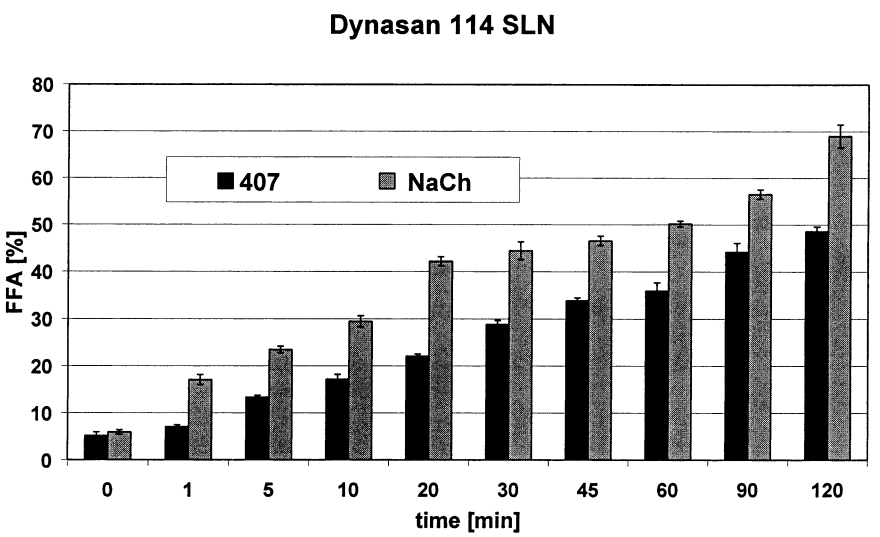

Fig. 1. FFA values versus time for the slowest (Dynasan 114, polxamer 407) and fastest degraded SLN formulations (Dynasan 114, cholic acid sodium salt (NaCh)). PCS data of the 407 SLN are: 456 nm (SD, 5.7), PI, 0.210 (SD, 0.04); PCS data of the NaCh SLN: 256 nm (SD, 7.2), PI, 0.195 (SD, 0.03).

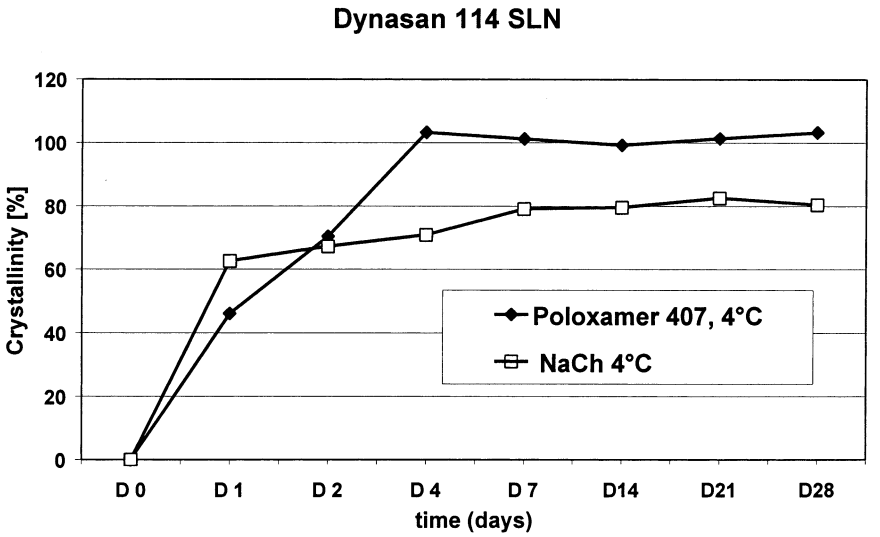

Fig. 2. Degree of crystallinity of two Dynasan 114 SLN formulations stored at 4 °C over a period of 28 days (NaCh: cholic acid sodium salt, 407: poloxamer 407 day 0: after production in melted hot state crystallinity is zero).

The crystallisation process in Dynasan 114 SLN can be clearly described. It was shown for phospholipid/bile salt stabilised Dynasan 114 SLN the transition from the α-polymorph to the thermodynamic stable β-polymorph to be completed within a few minutes (Bunjes et al., 1996). When performing the first DSC measurements 1 day after the production of the NaCh and the Poloxamer 407 stabilised SLN, they showed only one melting event from the β-polymorph for both surfactants (NaCh: onset 49.11 °C, peak 54.35 °C; Poloxamer 407: onset 54.05 °C, peak 55.57; lipid bulk: onset 55.76 °C, peak 58.87 °C). It could be confirmed that the Dynasan 114 nanoparticles stabilised with NaCh melt 3–5 °C lower than the corresponding bulk material but the melting endotherm is broader but still having a similar peak geometry. The PCS diameters of the Dynasan 114 SLN stored at 4 °C are increasing (Table 1). When crystallising the liquid particles initially spherical get an anisotropic shape, resulting in larger PCS diameters and also higher polydispersities (Bunjes et al., 1996). For the NaCh stabilised particles the sizes remain in a size range possessing similar degradation kinetics. That is, no effect of size on degradation velocity should be relevant (Olbrich et al., 2000). Also for the 407 formulations where the range of non-size-influenced degradation is more narrow (Olbrich et al., 2002 in press), the degradation takes place without being superimposed by size effects.

The degradation of Poloxamer 407 and NaCh stabilised D114 formulations is only influenced by the degree of crystallisation of the particle matrix in the case of the storage at 4 °C (Figs. 3 and 4).

The time course of FFA production is very similar for all Poloxamer 407 formulations independent on storage temperature and time, i.e. independent on the degree of crystallinity. The same is valid for NaCh SLN stored at room temperature. The FFA value at 20 min (D0) is not significantly different from the values of the other days ($P = 0.05$) (Fig. 3). There are differences in the degradation velocities of each NaCh-formulation, stored at 4 °C indicating an influence of the different degrees of crystallinity at 5 and 20 min (Fig. 4). After 5 min of incubation time, FFA values for the D1, D4 and D28 formulations are identical, only the D0 value is significantly different ($P = 0.05$). After 20 min of incubation the same is valid and after 60 min incubation time all formulations are degraded to an identical extent. The melting temperature of SLN is reduced to about 50 °C (onset) compared with the bulk ma-

terial (56 °C) so that no melting of the particles will occur when incubated at 37 °C in the incubation medium. The faster initial degradation is therefore, attributed to non-crystallised liquid lipid matrix.

For Dynasan 116 the kinetics of polymorphic transitions are slower than for Dynasan 114 bulk lipid and for both lipids they take place faster when formulated as nanoparticles (Bunjes et al., 1996). Dynasan 116 SLN stabilised with Polox-

Table 1
PCS diameter and polydispersities (PI) of Dynasan 114 SLN

Stabiliser	Storage temperature	Storage time (days)	Size (nm)	SD	PI	SD
NaCh	4 °C	0	186	2.2	0.219	0.09
		1	202	1.6	0.228	0.1
		4	205	2.6	0.255	0.09
		28	216	3.4	0.305	0.17
407	4 °C	0	245	2.1	0.189	0.05
		1	258	2.2	0.214	0.08
		4	299	1.2	0.231	0.04
		28	326	3.5	0.241	0.07
NaCh	RT	0	186	3.1	0.219	0.09
		1	187	1.5	0.203	0.14
		4	194	2.3	0.2	0.18
		28	198	1.8	0.194	0.27
407	RT	0	245	2.1	0.189	0.06
		1	248	1.8	0.19	0.09
		4	253	2.2	0.185	0.05
		28	272	2.5	0.193	0.07

Prepared with cholic acid sodium salt (NaCh) and Poloxamer 407 (407), stored at 4 °C and room temperature for 28 days. SD, standard deviataion.

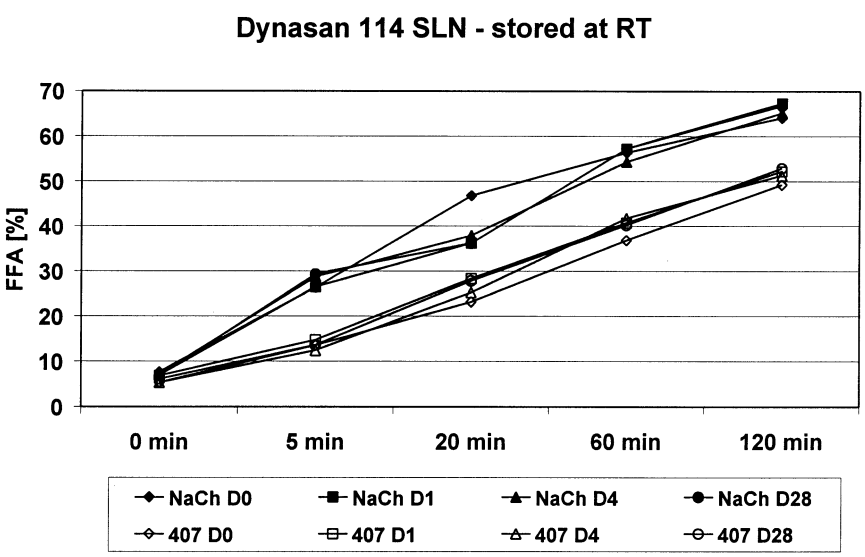

Fig. 3. Lipase/colipase degradation of Dynasan 114 SLN, stored at room temperature (RT) as a function of time (surfactants: cholic acid sodium salt: NaCh. Poloxamer 407:407) (days, D).

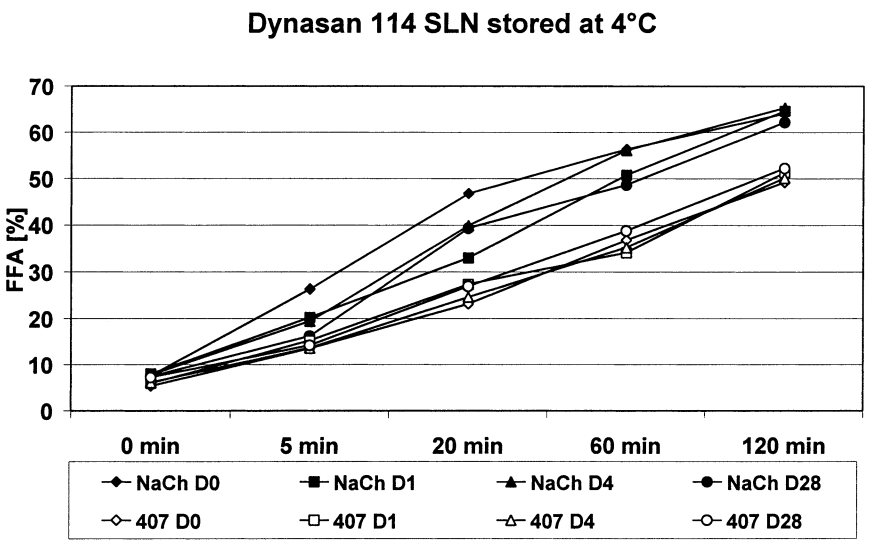

Fig. 4. Lipase/colipase degradation of Dynasan 114 SLN, stored at 4 °C as a function of time (surfactants: cholic acid sodium salt: NaCh. Poloxamer 407:407) (days, D).

amer 407 are in the β modification just after production (onset 56.45 °C, peak 61.58 °C). Compared with Dynasan 116 bulk material (onset 60.58 °C, peak 63.74 °C) there is a reduction in the melting peak of about 2 °C, by comparing the heat of fusion of β-modification the crystallinity is about 95%. This formulation could not be used for the study, because at day 1 a semisolid gel had formed. The cholic acid sodium salt stabilised Dynasan 116 SLN remained physically stable. After production they showed three modifications α, β′ and β (Fig. 5) which is in agreement with published experimental data (Siekmann and Westesen, 1994). Within 7 days of storage at 4 °C the α and β′ modification is disappearing and the thermodynamically stable β modification becomes the only modification (Bunjes et al., 1996). This was also observed with the Dynasan 116 NaCh SLN (Fig. 5). The transition is only a little faster in the samples stored at 4 °C than in the samples stored at room temperature (Table 2). Compared with bulk material (onset 60.58 °C, peak 63.74 °C) there is also a reduction in the melting event of about 2 °C. Because the heating rate of the DSC was 10 K/s details of the β′ modification could not be resolved for calculation when studying the SLN dispersions.

Cholic acid sodium salt is reducing the velocity of the polymorphic transitions in Dynasan 116 SLN. The degree of crystallinity was estimated by comparing the heat of fusion of the β modification of bulk material with that of SLN. Calculation was performed only when α and β′ signals were not detectable any more. This is the case for samples stored at room temperature after 14 days and for samples stored at 4 °C after 7 days. The degree of crystallinity is then 78.5% (room temperature) and 79% (4 °C). After 4 weeks of stor-

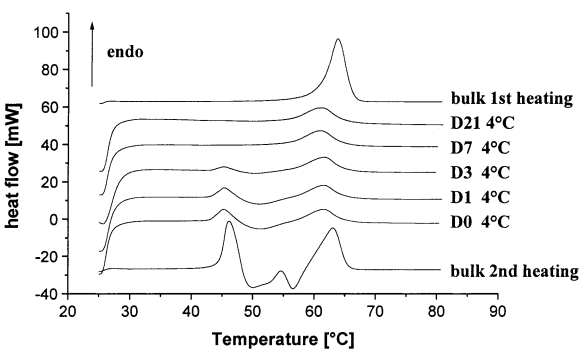

Fig. 5. DSC measurement of Dynasan 116 bulk (1st and 2nd heating curve) and NaCh stabilised Dynasan 116 SLN stored at 4 °C over a period up to 21 days (scan rate: 10 K/min; days, D).

Table 2
DSC data of α and β modification of Dynasan 116 SLN stabilised with NaCh

Storage temperature	Storage time	α-Modification			β-Modification			Crystallinity (%) (β-modification)
		Onset	Peak	J/g	Onset	Peak	J/g	
RT	Bulk	–	–	–	60.58	63.74	205.29	100
	D0	42.98	45.15	2.25	56.96	61.40	4.50	–
	D1	43.01	45.32	2.18	56.63	61.53	5.38	–
	D2	42.97	45.11	0.99	56.47	61.41	5.90	–
	D3	43.19	45.24	0.63	56.4	61.50	6.03	–
	D7	43.20	45.04	0.29	55.95	61.44	7.29	–
	D14	0.00	0.00	0.00	56.02	61.02	8.06	78.5
	D21	0.00	0.00	0.00	55.49	60.71	7.72	75.2
	D28	0.00	0.00	0.00	55.81	60.79	8.74	85.1
4 °C	Bulk	–	–	–	60.58	63.74	205.29	100
	D0	42.98	45.15	2.25	56.96	61.40	4.50	–
	D1	43.18	45.08	0.66	56.26	61.49	6.48	–
	D2	43.47	44.94	0.25	55.97	61.49	7.05	–
	D3	43.46	45.10	0.28	55.92	61.63	7.76	–
	D7	0.00	0.00	0.00	55.63	60.93	8.16	79.5
	D14	0.00	0.00	0.00	56.02	61.02	8.06	78.5
	D21	0.00	0.00	0.00	55.97	61.00	8.02	78.1
	D28	0.00	0.00	0.00	55.96	61.09	8.57	83.5

Calculation of the degree of crystallinity of the β modification by comparing heat of fusion of bulk with heat of fusion of SLN. SLN have been stored at room temperature and at 4 °C for 28 days (scan rate 10 K/min).

age the crystallinity is 85.1% (room temperature) and 83.5% (4 °C) (Table 2). The degree of crystallinity should increase with further storage, because it was found for Dynasan 116 SLN, prepared with phospholipids and sodium glycocholate to be 100% after 60 days (Siekmann and Westesen, 1994). This could also be obtained with Dynasan 116 SLN stabilised with cholic acid sodium salt.

As described for Dynasan 114 SLN, the particle size of Dynasan 116 SLN dispersions is increasing with storage time (from 260 to 330 nm after 28 days), because of the changes in the shape of the particles when the fats crystallise.

The degradation behaviour of Dynasan 116 SLN stored at 4 °C is different at different storage times (days) (Fig. 6). Regarding the degradation rates at 0, 1, 3 days, there is no difference. But at day 21 there is a clear retarded degradation after 20, 60 and 120 min of lipase/colipase incubation. The means ($n = 3$) of the amounts of free fatty acids after 5 min (for samples stored 3 and 21 days at 4 °C are not significantly different, determined by t-test with 0.05 significance level. The FFA levels after 20, 60 and 120 min are significantly different, also determined by t-test (Fig. 6). The degradation of the Dynasan 116 SLN stabilised with cholic acid sodium salt seem to be strongly influenced by the storage time and consecutively by the extent of crystallinity, respectively the degree of β-modification. Results from the degradation studies from days 0, 1 and 3 are very well in agreement with results of Dynasan 116 SLN obtained previously (Olbrich and Müller, 1999). The influence of the increased particle size on degradation within the 21 days can only be estimated from results obtained for Dynasan 114 SLN. With this lipid the degradation was not influenced by an increase in SLN-size from 182 (PI, 0.171) to 304 nm (PI, 0.301), that means also no size effect is assumed for Dynasan 116 Table 3.

4. Conclusion

The type of surfactant and storage time affect the crystallinity of SLN and consequently degradation velocity. SLN prepared with fast crystallising lipids (glycerides with longer chain fatty acids as Dynasan 116) and surfactants not disturbing the crystallisation process of the lipid (Poloxamer 407) do not show changes in the degradation velocity during storage time. This is a pre-requisite to use SLN in formulations for the market because they have to remain unchanged in degradation and related drug release properties.

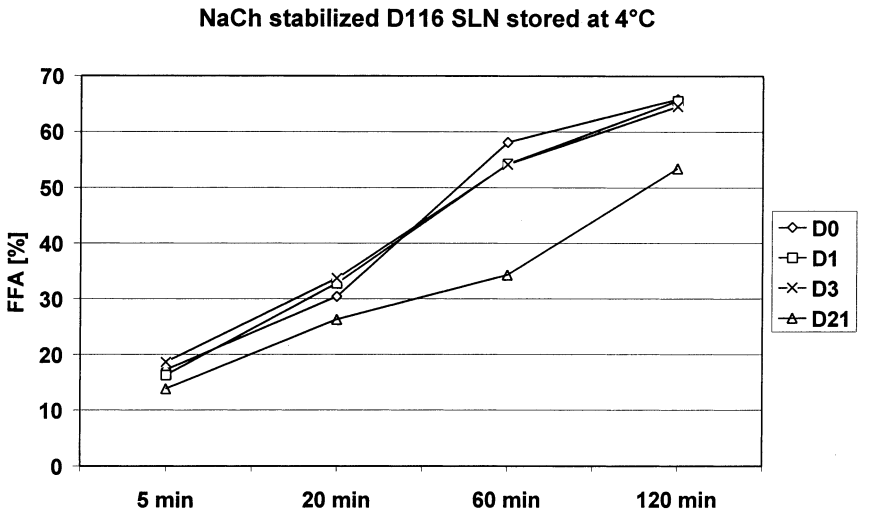

Fig. 6. Lipase/colipase degradation of Dynasan 116 SLN, stored at 4 °C as a function of time (surfactant: cholic acid sodium salt: NaCh) (days, D).

Table 3
PCS diameters and polydispersities (PI) of Dynasan 116 SLN prepared with cholic acid sodium salt (NaCh) and stored at 4 °C and at room temperature for 4 weeks

Storage time–temperature	Size (nm)	SD	PI	SD
D0–RT	260	1.914	0.167	0.016
D1–RT	263	2.108	0.187	0.015
D1–4 °C	291	5.325	0.185	0.009
D2–RT	278	5.514	0.172	0.016
D2–4 °C	301	4.219	0.209	0.008
D3–RT	285	4.86	0.186	0.011
D3–4 °C	310	3.917	0.218	0.018
D7–RT	305	1.097	0.217	0.005
D7–4 °C	313	1.234	0.218	0.018
D14–RT	322	1.401	0.233	0.006
D14–4 °C	322	1.401	0.233	0.006
D21–RT	329	2.888	0.232	0.011
D21–4 °C	332	6.198	0.204	0.016
D28–RT	331	1.873	0.233	0.01
D28–4 °C	330	10.96	0.235	0.018

SLN partially liquid and with a more pronounced α fraction due to disturbed crystallisation by the surfactant (e.g. Dynasan 114 SLN stabilised with NaCh) degrade faster and show a change in degradation behaviour with increasing storage time (e.g. progressing crystallisation when storing them, e.g. storage at 4 °C in Fig. 4). However, also such 'disturbing' surfactants might be used in combination with longer chain fatty acids (Dynasan 116) when applying a 'ripening process' of 3 weeks storage leading to a sufficiently high crystallinity and related slower degradation (Fig. 6) thus broadening the range of excipients which can be used for SLN production.

Further studies should include variation of the amount of lipase and also include other lipid digesting enzymes (esterases, different lipases) to assess the effect of enzyme concentration and type.

References

Bunjes, H., Westesen, K., Koch, M.H.J., 1996. Crystallization tendency and polymorphic transitions in triglyceride nanoparticles. Int. J. Pharm. 129, 159–173.

Cavalli, R., Marengo, E., Rodriguez, L., Gasco, M.R., 1996. Effects of some experimental factors on the production process of Solid Lipid Nanoparticles. Eur. J. Pharm. Biopharm. 43, 110–115.

Gasco, M.R., 1998. Pharmaceutical composition in form of solid lipid microparticles suitable to parenteral administration, WO9856362 A1.

Heiati, H., Tawashi, R., Shivers, R.R., Philips, N.C., 1997. Solid Lipid Nanoparticles as drug carriers. Incorporation and retention of the lipophillic prodrug 3′-azido-3′-deoxythymidine palmitate. Int. J. Pharm. 146, 123–131.

Jenning, V., Gysler, A., Schafer-Korting, M., Gohla, S.H., 2000. Vitamin A loaded solid lipid nanoparticles for topical use: occlusive properties and drug targeting to the upper skin. Eur. J. Pharm. Biopharm. 49, 211–218.

Müller, R.H., Lherm, C., Jaffray, P., Couvreur, P., 1988. Toxicity of cyanoacrylate particles in L929 fibroblast cell cultures—relation between toxicity and in vitro characterization parameters. Arch. Pharm. 321, 681–687.

Müller, R.H., Mehnert, W., Lucks, J.S., Schwarz, C., zur Mühlen, A., Weyhers, H., Freitas, C., Rühl, D., 1995. Solid Lipid Nanoparticles (SLN)—an alternative colloidal carrier system for controlled drug delivery. Eur. J. Pharm. Biopharm. 41, 62–69.

Müller, R.H., Lucks, J.S., Arzneistoffträger aus festen Lipidteilchen, Feste Lipidnanosphären (SLN), European Patent No. 0605497 (1996).

Müller, R.H., Grubhofer, N., Olbrich, C., 1999. Stability-, biocompatibility-optimized adjuvans (SBA) for improved humoral and cellular immune response. Patent application No. 199 23 256.3.

Müller, R.H., Olbrich, C., 1999. Solid Lipid Nanoparticles (SLN): phagocytic uptake, in vitro cytotoxity and in vitro biodegradation. Pharm. Ind. 61, 462–467.

Müller, R.H., Mäder, K., Gohla, S., 2000. Solid Lipid Nanoparticles (SLN) for controlled drug delivery—a review of the state of the art. Eur. J. Pharm. Biopharm. 50, 161–177.

Olbrich, C., Mehnert, W., Müller, R.H., 1997. Effect of surfactant and lipid composition on the in vitro degradation time of solid lipid nanoparticles (SLN). Intern. Symp. Control Rel. Bioact. Mater. 24, 921–922.

Olbrich, C., Mehnert, W., Müller, R.H., 1998a. In vitro degradation properties of Solid Lipid Nanoparticles SLN™. In: Proc. 2nd World Meeting APGI/APV, Paris, pp. 577–578.

Olbrich, C., Mehnert, W., Müller, R.,H., 1998b. Development of an in vitro degradation assay for Solid Lipid Nanoparticles. In: Proc. 2nd World Meeting APGI/APV, Paris, pp. 627–628.

Olbrich, C., Müller, R.H., 1999. Enzymatic degradation of SLN—effect of surfactant and surfactant mixtures. Int. J. Pharm. 180, 31–39.

Olbrich, C., Kayser, O., Müller, R.H., Grubhofer, N., 2000. Solid lipid nanoparticles (SLN) as vaccine adjuvant—study in sheep with a mycoplasma bovis antigen and stability testing. Intern. Symp. Control Rel. Bioact. Mater. 27, 8110.

Olbrich, C., Müller, R.H., Kayser, O., 2002. Enzymatic degradation of Dynasan 114 Solid Lipid Nanoparticles (SLN)—effect of surfactants and particle size. J. Nanoparticle Res. In press.

Penkler, L., Müller, R.H., Runge, S.A., Ravelli, V., 1999. Pharmaceutical cyclosporin formulation with improved biopharmaceutical properties, improved physical quality, greater stability and method for producing said formulation. PCT-application PCT/EP99/02892.

Siekmann, B., Westesen, K., 1992. Submicron sized parenteral carrier system based on solid lipids. Pharm. Pharmacol. Lett. 1, 123–126.

Siekmann, B., Westesen, K., 1994. Thermoanalysis of the recrystallization process of melt-homogenized glyceride nanoparticles. Colloids Surf., B: Biointerfaces 3, 159–175.

Westesen, K., Bunjes, H., 1995. Nanoparticles prepared from lipids solid at room temperature always possess a solid lipid matrix? Int. J. Pharm. 115, 129–131.

Winkler, F.K., D'Arcy, A., Hunziker, W., 1990. Structure of human pancreatic lipase. Nature 343, 771–774.

zur Mühlen, A., Schwarz, C., Mehnert, W., 1998. Solid lipid nanoparticles (SLN) for controlled drug delivery–drug release and release mechanism. Eur. J. Pharm. Biopharm. 45, 149–155.