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Enzymatic degradation of SLN—effect of surfactant and surfactant mixtures

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Abstract

Solid lipid nanoparticles (SLN) show different degradation velocities by the lipolytic enzyme pancreatic lipase as a function of their composition (lipid matrix, stabilizing surfactant). In combination with pancreatic colipase a degradation assay has been developed for studying the degradation behavior. As a measure to follow the degradation the formed free fatty acids have been analyzed using an enzymatic test. In the studies SLN degradation showed dependencies in relation to the length of the fatty acid chains in the triglycerides and the surfactants used for SLN production. The longer the fatty acid chains in the glycerides, the slower the degradation. The influence of surfactants can be degradation accelerating (e.g. cholic acid sodium salt) or a hindering, degradation slowing down effect due to steric stabilization (e.g. Poloxamer 407). As a second steric stabilizer, Tween 80 has been used and the results showed a less pronounced effect on hindering the degradation process than for Poloxamer 407. This result seems to be correlated to the number of ethyleneoxide chains in the molecule. The longer the ethyleneoxide chains are in the molecule, the more hindered is the anchoring of the lipase/colipase complex and consequently the degradation of the SLN. The result can be used to adjust degradation of SLN and consequently drug release in a controlled way.

Keywords: Solid lipid nanoparticles; Biodegradation; Pancreatic lipase; Colloidal drug carrier; Lipid

1. Introduction

Solid lipid nanoparticles (SLN) could be established as an alternative particulate carrier system by various research groups (Amselm et al., 1992; Siekmann and Westesen, 1992; Boltri et al., 1995; Sjostrom et al., 1995; Müller and Lucks, 1996; Bargoni et al., 1998; Heiati et al., 1998). They combine advantages of emulsions, liposomes and polymeric nanoparticles. Similar to emulsions and liposomes they are composed of physiologically

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Table 1
PCS diameters and polydispersity indices of cetylpalmitate, Dynasan 116 and 118 SLN batches ($n = 3$)

Lipid (5%)	Surfactant (0.5%)	Diameter (nm)	S.D. (%)	Polydispersity index
Cetylpalmitate	Cholic acid sodium salt	170	2.31	0.166
	Lipoid E80	431	2.00	0.273
	Poloxamer 407	303	3.02	0.200
	Tween 80	453	2.52	0.22
Dynasan 116	Cholic acid sodium salt	253	8.02	0.185
	Lipoid E80	350	1.03	0.235
	Poloxamer 407	388	7.21	0.298
	Tween 80	315	1.53	0.223
Dynasan 118	Cholic acid sodium salt	283	7.21	0.187
	Lipoid E80	385	2.14	0.232
	Poloxamer 407	422	6.43	0.321
	Tween 80	314	4.23	0.245

well tolerated excipients and can be produced on large industrial scale by high pressure homogenization (Müller et al., 1997; Hildebrand et al., 1998). Identical to polymeric nanoparticles their solid matrix protects incorporated active ingredients against chemical degradation and provides the highest flexibilities in the modulation of the drug release profiles. Using model drugs it could be shown, that in vitro drug release could be varied from minutes (burst release) (zur Mühlen et al., 1998) to up to 7 weeks (Mehnert et al., 1997).

Drug release in these in vitro studies took place by solid phase diffusion, the release media did not contain any enzymes. However, in vivo release will take place by diffusion and by matrix degradation. To optimize the release profiles for the in vivo situation, knowledge about the enzymatic degradation velocity of SLN is essential. Previously an SLN enzymatic degradation assay was established using a lipase/colipase complex (Olbrich, et al., 1997). It could be shown that the degradation velocity depends on the composition of the lipid matrix (Müller et al., 1996). In general, degradation velocity increased with decreasing length of the fatty acid chain length when using glycerides as lipid matrix (Olbrich et al., 1998a). In addition, the degradation of SLN based on waxes (e.g. cetylpalmitate) was found to be slower compared to glyceride matrices.

Table 2
PCS diameters and polydispersity indices of Dynasan 116 SLN batches stabilized with different ratios of cholic acid sodium salt to Poloxamer 407 ($n = 3$)

Ratio cholic acid sodium salt: Poloxamer 407	Diameter (nm)	S.D. (%)	Polydispersity index
100:0	253	8.02	0.185
75:25	338	12.85	0.223
50:50	353	19.30	0.211
40:60	357	3.21	0.251
30:70	378	4.89	0.275
25:75	364	11.37	0.284
20:80	385	7.89	0.306
10:90	370	5.24	0.259
0:100	388	7.21	0.298

Table 3
PCS diameters and polydispersity indices of Dynasan 118 SLN batches stabilized with different ratios of cholic acid sodium salt to Poloxamer 407 ($n = 3$)

Ratio cholic acid sodium salt: Poloxamer 407	Diameter (nm)	S.D. (%)	Polydispersity index
100:0	283	7.21	0.187
75:25	309	4.58	0.206
50:50	346	2.14	0.220
40:60	352	5.42	0.213
30:70	357	3.21	0.210
25:75	368	1.25	0.235
20:80	379	2.14	0.245
10:90	378	3.23	0.268
0:100	422	6.43	0.321

A prerequisite for the degradation of SLN after oral administration is the anchoring of the lipase/colipase complex onto the particle surface. Therefore it was expected that not only the composition of the lipid matrix, but also the nature of the stabilizing surfactant layer would be a determining factor for degradation. Compounds such as cholic acid sodium salt are known to promote the anchoring of the lipase colipase complex on surfaces (Borgström, 1975). Sterically stabilizing polymers such as the Poloxamer series are known to prevent or to hinder the absorption of large molecules such as proteins (Blunk et al., 1993). A similar effect was expected for the lipase/colipase. The aim was therefore to study the effects of different surfactants on the degradation velocity. To assess whether a desired degradation velocity can be achieved in a controlled way, mixtures of surfactants promoting degradation with surfactants slowing down the degradation rate were investigated.

2. Materials and methods

2.1. Materials

As lipids Cetylpalmitate from Gattefossé (Weil am Rhein, Germany) and Dynasan 116 and 118 (glycerol-tripalmitate and -tristearate) from Contensio GmbH (former Hüls AG, Witten, Germany) were kindly provided as gifts. Poloxamer 407 was donated by ICI (Essen, Germany) and Lipoid E80 from Lipoid KG (Ludwigshafen/Rhein, Germany). Cholic acid sodium salt, Tween 80, lipase (Type IV) 30000 U/mg, colipase from porcine pancreas and calcium chloride dihydrate were purchased from Sigma (Deisenhofen, Germany). The Nefa C testkit was purchased from Wako, Neuss, Germany.

2.2. Methods

2.2.1. SLN preparation and size measurement

SLN were produced by using the hot homogenization technique. The lipid was melted and poured into a hot aqueous surfactant solution. The concentration of surfactants were 0.5% for all surfactants. The melted lipid was dispersed in the hot surfactant solution by high speed stirring to yield a pre-emulsion, the obtained pre-emulsion was then homogenized using an APV Gaulin LAB 40 homogenizer at 500 bar applying three homogenization cycles. Details of the production method are given in (Müller et al., 1995).

Particle sizing was performed by photo correlation spectroscopy (PCS) using a Zetasizer 4 (Malvern Instruments, Malvern, UK). The system was used in the auto measuring mode. PCS yields the diameter of the bulk population (z-average) and a polydispersity index to characterize the distribution ranging from 0.000 to 0.500.

2.2.2. Enzymatic degradation assay

Lipase and colipase were dissolved in distilled water. Concentrations of the stock solutions were

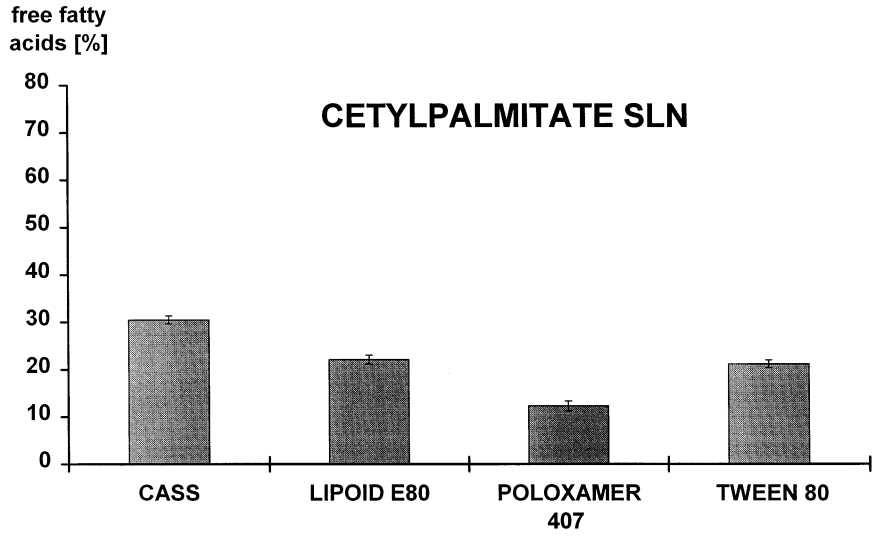

Fig. 1. Free fatty acids (FFAs) of cetylpalmitate SLN stabilized with different surfactants (cholic acid sodium salt (CASS), Lipoid E80, Poloxamer 407 and Tween 80) after 120 min of lipase/colipase incubation.

2000 U/ml lipase and 50 μg/ml colipase. The lipase solution (600 μl) was 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 onto the lipid particle surface. This is especially of importance where cholic acid has been used as a stabilizer. To this pre-incubated mixture, 228 μl of 0.01 M borate/boric acid buffer (pH 7.4), containing 0.02 mol calcium chloride was added. After the addition of 12 μl of SLN dispersion (5% lipid content) the final concentrations of lipase were 1000 U/ml and 15 μg/ml colipase. After certain time intervals samples of 10 μl were analyzed for

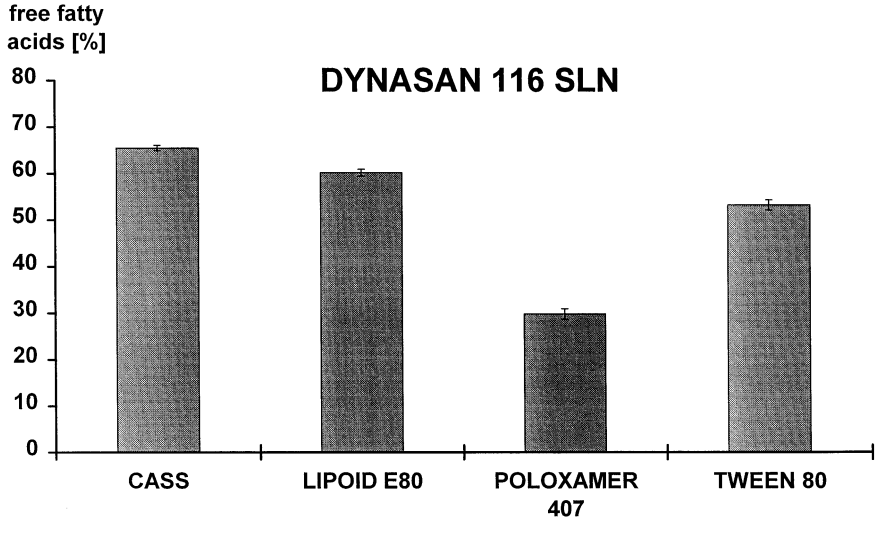

Fig. 2. Free fatty acids (FFAs) of Dynasan 116 SLN stabilized with different surfactants (cholic acid sodium salt (CASS), Lipoid E80, Poloxamer 407 and Tween 80) after 120 min of lipase/colipase incubation.

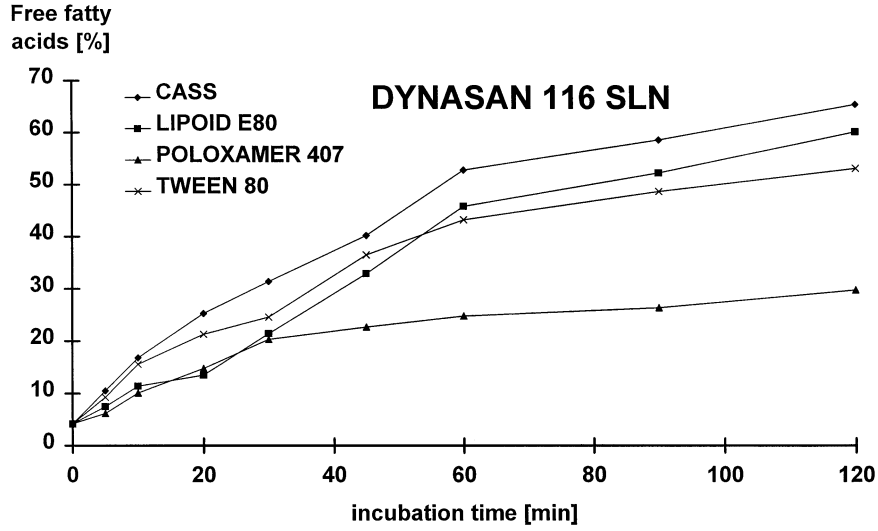

Fig. 3. Free fatty acids (FFAs) of Dynasan 116 SLN stabilized with different surfactants (cholic acid sodium salt (CASS), Lipoid E80, Poloxamer 407 and Tween 80) versus time (lipase/colipase incubation).

their free fatty acid content using the non-esterified-fatty acids (NEFA) C testkit. This enzymatic test kit, designed for the determination of free fatty acids in serum and plasma, has been adjusted for the determination of free fatty acids, the degradation products of the lipase incubation. As a measure of the fatty acid content, a chinonimin coloring is formed, whose absorption maximum is at 550 nm. This colored solution, the product of the enzymatic reaction of the NEFA C testkit is then transferred to a microtiterplate and measured with a microtiterplate reader (Easy Reader EAR 400 AT, SLT Instruments, Grödig, Austria). The amount of free fatty acids was calculated using oleic acid as standard. The maximum amount of free fatty acids which are detectable with this assay is 66% of the theoretical value, if all lipid is degraded to glycerol and fatty acids. On the basis of the saponification values, a 100% value for full degradation was determined. In the enzymatic degradation assay the percentages of free fatty acids were expressed in relation to the 100% from saponification. The lipase/colipase can degrade the glycerides only to the monoglyceride, that means when 66% of free fatty acids (FFA) are reached this corresponds to the maximum possible degradation in this test (= full degradation).

3. Results and discussion

To study the effect of the stabilizer on the degradation velocity, SLN were produced using cholic acid sodium salt, lecithin (Lipoid E80) and the two steric stabilizers Tween 80 and Poloxamer 407. These differ in the length of the sterically stabilizing polyethylene oxide chains, being 20 units in Tween 80 and 98 units in Poloxamer 407. In combination with the differences in the molecular weights (approximately 1840 and 11500, respectively) they provide sterically stabilizing layers of a different thickness and a potentially different effect on the anchoring of the lipase/colipase and consequently the degradation velocity. On polystyrene model carriers an adsorption layer thickness of approximately 2 nm was reported for Tween 80 but about 12 nm for Poloxamer 407 (photon correlation spectroscopy data (Müller, 1991)). It is not possible to measure the layer thickness on SLN by PCS (lack of stabilizer-free SLN as reference for PCS measurement). However, based on the polystyrene data a similar difference was expected for SLN. To assess whether the surfactant effect also depends on the nature of the lipid matrix, SLN were prepared with a wax as lipid matrix (cetylpalmitate) and

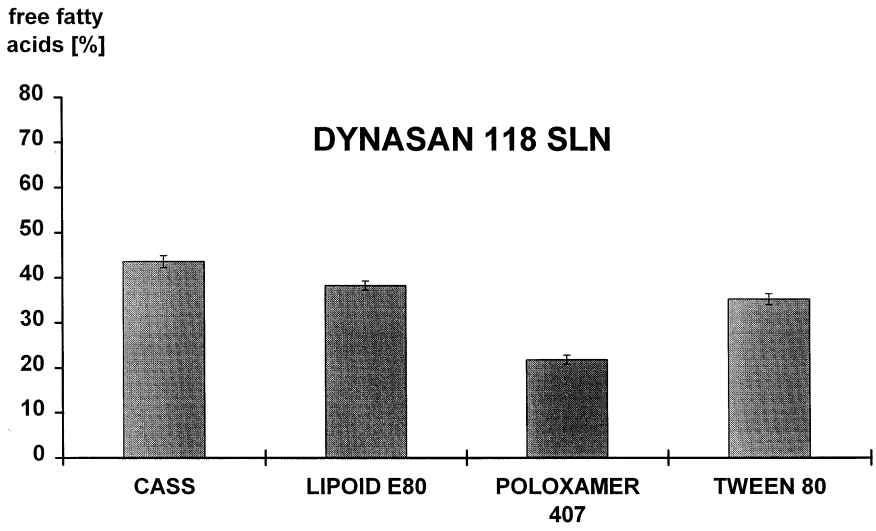

Fig. 4. Free fatty acids (FFAs) of Dynasan 118 SLN stabilized with different surfactants (cholic acid sodium salt (CASS), Lipoid E80, Poloxamer 407 and Tween 80) after 120 min of lipase/colipase incubation.

using glycerides (Dynasan 116 and Dynasan 118). Particle sizes and polydispersity indices measured by PCS are given in Tables 1–3. Particle size affects also degradation velocity (data not published), therefore it was favorable that most SLN were in the size range of 300–400 nm.

After 2 h of incubation with the lipase/colipase the cetylpalmitate SLN stabilized with cholic acid sodium salt showed the lowest per-

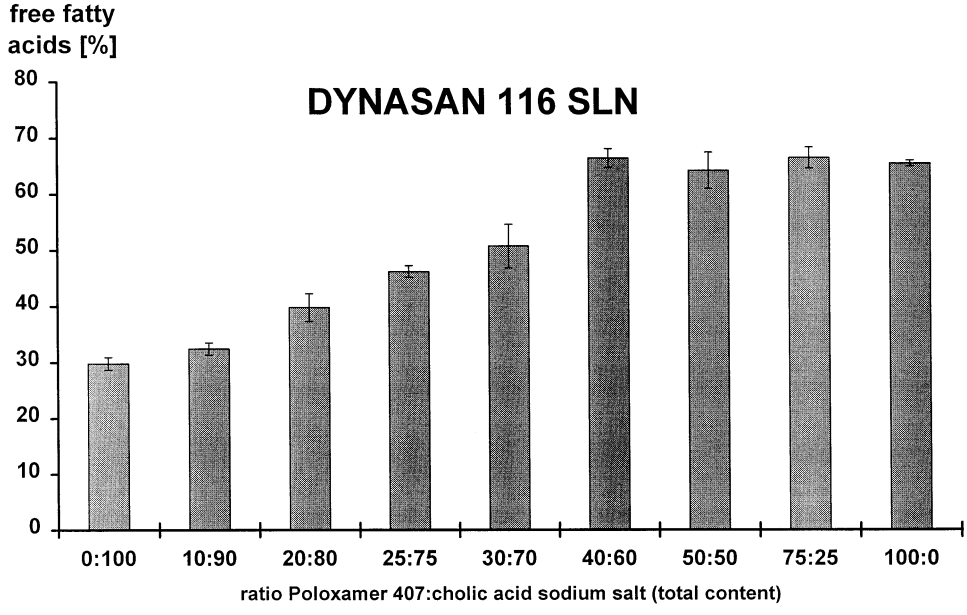

Fig. 5. Free fatty acids (FFAs) of Dynasan 116 SLN stabilized with different ratios of cholic acid sodium salt (CASS) and Poloxamer 407 (0:100–100:0) after 120 min of lipase/colipase incubation (total concentration of surfactants 0.5%).

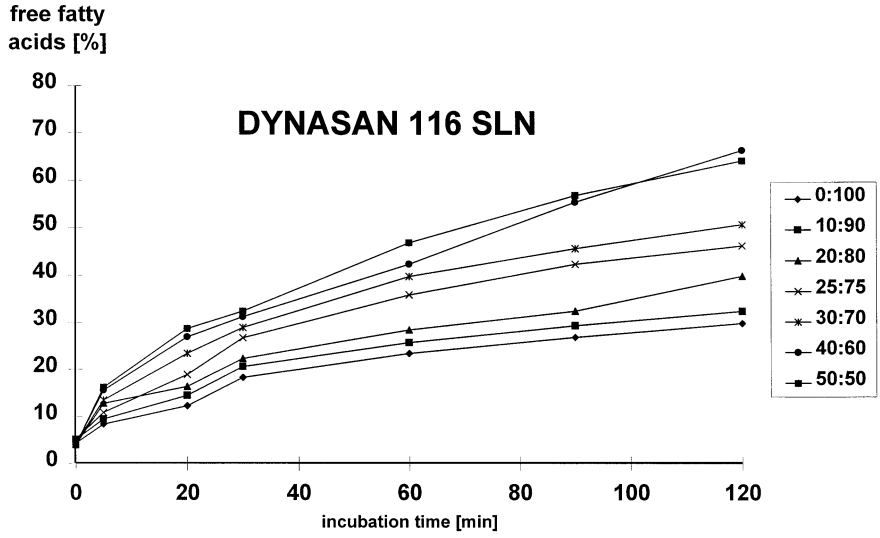

Fig. 6. Free fatty acids (FFAs) of Dynasan 116 SLN stabilized with different surfactants mixture ratios (cholic acid sodium salt (CASS) to Poloxamer 407, from 0:100 to 40:60, total surfactant content 0.5%) versus time (lipase/colipase incubation).

centage of degradation. Only about 30% of the theoretically obtainable FFAs were formed. The low rate of degradation can be explained by cetyl-palmitate being not an optimal substrate for the lipase/colipase. This enzyme system preferentially digests glycerides. Even with the anchoring and degradation promoting effect of cholic acid sodium salt, the degradation was not higher after 120 min of incubation time (Fig. 1). Lecithin, a natural compound in food, led to a slightly slower

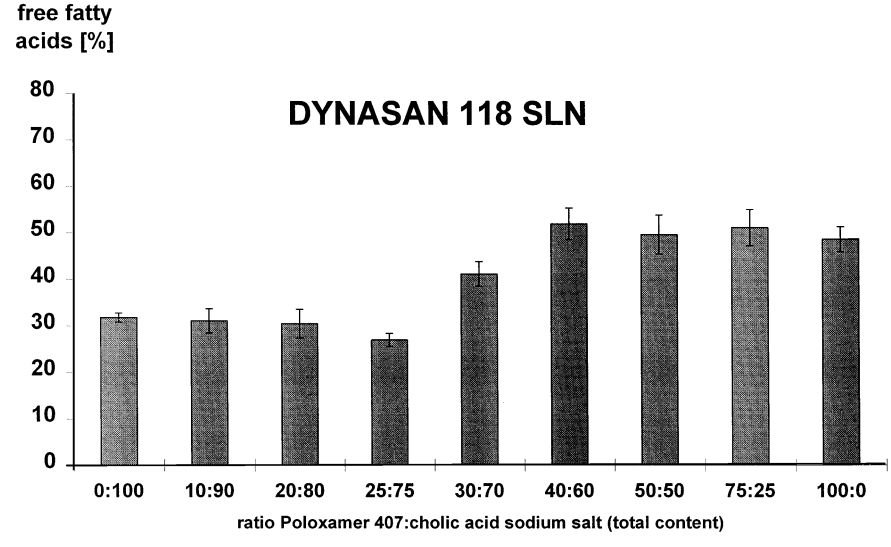

Fig. 7. Free fatty acids (FFAs) of Dynasan 118 SLN stabilized with different ratios of cholic acid sodium salt (CASS) and Poloxamer 407 (ratios 0:100 to 100:0) after 120 min of lipase/colipase incubation (total concentration of surfactants: 0.5%).

degradation of cetylpalmitate SLN (approximately 25%). Tween 80 stabilized SLN were degraded with a similar velocity, attributed to the limited sterically effect of this surfactant (short polyethyleneoxide chains). Least degradation was found for SLN stabilized with Poloxamer 407, indicating that the long polyethylene oxide chains hinder the an choring of the lipase/colipase complex. The slower degradation observed with Poloxamer 407 stabilized SLN cannot be attributed to a difference in size. Poloxamer 407 stabilized SLN are smaller than Tween 80 stabilized SLN and should therefore be degraded faster. These results support the explanation that the anchoring of the enzyme is sterically hindered.

The glyceride Dynasan 116 showed a distinctly higher degree of degradation compared to cetylpalmitate. About 66% of the theoretical value of FFAs (based on saponification value) were formed within 2 h for the cholic acid sodium salt stabilized SLN (Fig. 2). Considering that, with the enzymatic complex used, a maximum of 66% of FFAs can be formed (Section 2) the SLN are completely degraded to the monoglycerides. SLN stabilized with Lipoid E80 have a slower degradation, which is in agreement with the observations made with cetylpalmitate SLN. The observed higher percentages of degradation for Dynasan 116 SLN stabilized with lecithin reveal that Tween 80 has a slight effect in slowing down the degradation (61% compared to 45% FFA, respectively). Again, the most effective surfactant in slowing down degradation is Poloxamer 407, with less than 40% of FFA being formed in the degradation test (Fig. 2). These data are in agreement with the previous work showing the dependency of degradation velocity on the nature of the lipid matrix (Olbrich et al., 1998b). Degradation accelerating or slowing down effects of surfactants are significantly pronounced in cases where lipid matrices are used, which show a priori a fast degradation velocity. The differences in the degradation behavior are more obvious when looking at the time profiles. Fig. 3 shows the percentage of FFA formed as a function of incubation time. The cholic acid sodium salt stabilized Dynasan 116 SLN show $> 50\%$ degradation before the end of the test (i.e. after 60 min).

Fig. 4 shows the extent of degradation when using Dynasan 118 as the lipid matrix in combination with the four stabilizers. The stabilizers can be placed in the same order, the degradation velocity decreasing from cholic acid sodium salt, Lipoid E80 and Tween 80 to Poloxamer 407. When using Poloxamer 407 only approximately 20% of FFAs are formed within 2 h (almost 40% in the case of Dynasan 116 SLN). The data show that apart from the nature of the lipid matrix the stabilizer has a pronounced effect.

To assess the effect of surfactant mixtures, SLN were produced using different ratios of cholic acid sodium salt (degradation accelerating) to Poloxamer 407 (degradation hindering). Two different lipid matrices (Dynasan 116 and 118) were used to additionally assess the effect of the combination surfactant-nature of lipid matrix. For Dynasan 116 SLN a continuous increase in the degradation velocity was observed when adding cholic acid sodium salt stepwise up to 40% in the surfactant mixture (Fig. 5). At the ratio 40:60 the particles are fully degraded within 2 h, higher ratios of cholic acid sodium salt to Poloxamer 407 cannot anymore increase the degraded percentage because full degradation has been reached (66% FFAs formed). However, when looking at the degradation profiles percentage of FFA versus time (Fig. 6) it can be seen that increasing the cholic acid sodium salt content in the mixture above 40% leads to an almost full degradation at earlier times (approximately 50% at 90 min at only 40% cholic acid). Based on these data it can be summarized that for the lipid matrix Dynasan 116 the degradation velocity can be adjusted in a controlled way by using a surfactant mixture and varying the surfactant ratio. This opens the prospect for tailor-made drug release.

The SLN produced with the surfactant mixtures have sizes in a very narrow spectrum of approximately 300–400 nm, which minimizes the effect of size on the degradation velocity. In contrast to Dynasan 114 SLN, Dynasan 118 SLN does not show such a steady increase in the percentage degraded after 2 h, as observed with SLN made from Dynasan 116. Up to a ratio of 25:75 the cholic acid sodium salt cannot compensate or diminish the sterically stabilizing effect of the Poloxamer 407, the percentage degraded remains unchanged at approximately 30% FFA (Fig. 7). In this case, the increase in the degradation velocity takes place within the

ratios 25:75–40:60 and is relatively steep. Above 40–60—similar to Dynasan 116 SLN—further increase of the cholic acid sodium salt content in the surfactant mixture has no effect. It can also be summarized that in the case of Dynasan 118 as lipid matrix the degradation velocity can be modulated by varying the surfactant mixture ratio, but that this occurs in a relatively narrow window.

4. Conclusions

The data show that the degradation velocity is substantially affected by the stabilizer used for the preparation of the SLN. Some stabilizers as cholic acid sodium salt have degradation accelerating effects, others such as Poloxamer 407 distinctly slow down the degradation velocity. Degradation velocity can be modulated by changing the surfactant ratio. The very pronounced effects of the surfactants/surfactant mixtures on the degradation velocity allow the choice of lipid for SLN production (optimum drug incorporation) and a modulation of SLN degradation (and subsequently drug release) by a choice of the surfactant mixture. This opens the perspective of producing SLN with maximum drug loading capacity at simultaneously optimized drug release profiles.

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