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Journal of Controlled Release 71 (2001) 297–306

journal of controlled release

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Design of rolipram-loaded nanoparticles: comparison of two preparation methods

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Received 27 October 2000; accepted 29 January 2001

Abstract

The aim of the present work was to investigate the preparation of nanoparticles as a potential drug carrier and targeting system for the treatment of inflammatory bowel disease. Rolipram was chosen as the model drug to be incorporated within nanoparticles. Pressure homogenization–emulsification (PHE) with a microfluidizer or a modified spontaneous emulsification solvent diffusion method (SESD) were used in order to select the most appropriate preparation method. Poly(ε-caprolactone) has been used for all preparations. The drug loading has been optimized by varying the concentration of the drug and polymer in the organic phase, the surfactants (polyvinyl alcohol, sodium cholate) as well as the volume of the external aqueous phase. The rolipram encapsulation efficiency was high (>85%) with the PHE method in all cases, whereas with the SESD method encapsulation efficiencies were lower (<40%) when lower surfactant concentrations and reduced volume of aqueous phase were used. Release profiles were characterized by a substantial initial burst release with the PHE method (25–35%) as well as with the SESD method (70–90%). A more controlled release was obtained after 2 days of dissolution with the PHE method (70–90%), no further significant drug release was observed with the SESD method.

Keywords: Nanoparticles; Sodium cholate; Encapsulation; Inflammatory bowel disease; Poly[ε-caprolactone]

1. Introduction

Nowadays, the conventional treatment of inflammatory bowel disease requires the daily intake of anti-inflammatory drugs at high doses involving potential adverse effects. Therefore, one major strategy was to develop solid dosage forms releasing the drug in the colon in dependency of the pH or specific bacterial enzymes in the colon [1,2]. However, the efficiency of this strategy is reduced in many cases owing to diarrhea, an assured symptom of inflammatory bowel disease [3,4]. Since drug

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0168-3659/01/$ – see front matter
PII: S0168-3659(01)00230-9

carriers with a nominal size below 200 μm are affected less by this symptom [4], smaller drug carriers could display a prolonged intestinal transit time. Indeed, it has been reported that particles with a diameter in the lower micron or nanometre range show an accumulation in the inflammatory areas of the colon in the case of ulcerative colitis in rats [5].

In the inflamed tissue an increased adherence of particles with a nominal size of 100 nm, 1 μm, and 10 μm was observed. The optimal particle size ranged between 100 nm and 1 μm. In this range, the particle deposition in the inflamed tissue of the colon was 5–6.5-fold higher than in the healthy control. Particles showed an increased accumulation mainly in the areas of ulceration and the surrounding tissue. This may be due either to the increased secretion of sticky mucus leading to an attachment or to the uptake of particles into the macrophages which are present in the inflamed tissue in a highly increased number. Moreover, a prolonged retention of the particles in the inflamed regions up to 3 days was found [6].

Consequently, an increased residence time at the inflammation sites can be postulated for nanoparticles (NP) compared with existing drug delivery systems. This should allow a dose reduction and local drug delivery to the inflamed tissue. For instance, since dexamethasone-containing microparticles showed promising results in a preliminary in-vivo study with colitis-induced mice [7]. Thus, drugs which are usually not administered orally owing to their strong side effects may be therefore encapsulated within polymeric particles with the aim of oral administration.

In several different diseases, the proinflammatory cytokine tumor necrosis factor-α (TNF) forms a necessary element in the chain of pathophysiologic events leading to inflammation. Among the agents known to inhibit TNF production rather than to block its function, attention has focused on cAMP elevating phosphodiesterase inhibitors. Compared with the nonspecific phosphodiesterase inhibitor pentoxifylline, the specific type IV phosphodiesterase inhibitor rolipram is a 500-fold more potent inhibitor of TNF synthesis in human mononuclear cells [8]. Rolipram has initially been developed and studied clinically as an antidepressant drug [9]. Recently, the potential therapeutic use of rolipram in TNF-dependent diseases has been demonstrated in several animal models [10–12].

It was stated that ulcerous tissues contain high concentrations of positively charged proteins that increased the affinity to negatively charged substances [13]. For this reason a strong negative charge surface of NP might be suitable. Coating particles with anionic surfactants could be therefore a promising alternative. Owing to its interesting interfacial properties, its natural origin and consequently its biocompatibility, sodium cholate (SC) might be a suitable candidate. Surprisingly, only very little attention has been paid to its use for particle preparation so far [14]. The interest in SC consisted mainly in its ability to attract lipase and colipase to the particle surface in order to degrade solid lipid nanoparticles [15].

The aim of this work was the preparation and optimization of rolipram-containing NP. In order to develop NP with a strong negative surface charge, it was of high interest to optimize and characterize SC NP for their potential as a drug carrier system in inflammatory bowel disease. Comparing two different preparation methods should allow to develop an optimal carrier with a view to particle size, surface charge and release profile.

Due to the mainly lipophilic nature of rolipram, two emulsification techniques were directly compared: the pressure homogenization–emulsification (PHE) by using a microfluidizer and a modified spontaneous emulsification solvent diffusion method (SESD) based on nanoprecipitation. Since it is expected that nanoparticles could accumulate in the inflamed regions in the colon, the use of a biodegradable polymer was advised. Thus, NP were prepared with poly[ε-caprolactone] (PCL), a biocompatible and biodegradable polymer [16,17]. NP were compared in terms of size, polydispersity, surface potential, encapsulation efficiency and drug release. NP prepared with polyvinyl alcohol (PVA) under the same conditions were used as standard formulations due to its wide use as surfactant in the preparation of NP. Moreover, polyvinyl alcohol-coated NP were found to be very efficient in protecting NP from degradation during the passage through the gastrointestinal tract [18].

2. Materials and methods

2.1. Materials

The biodegradable polymer poly[ε-caprolactone] (M_w 10,000 Da) was purchased from Fluka (Steinheim, Germany). Polyvinyl alcohol (M_w 20,000 Da, 80% hydrolyzed) and sodium cholate (M_w 430.6 Da) chosen as surface active agents were respectively supplied by Sigma (Steinheim, Germany) and Fluka (Steinheim, Germany). Rolipram (solubility in distilled water: 473.8±29.1 μg/ml; $n=6$) was received as a gift from Schering AG (Berlin, Germany). All other chemical reagents were obtained from Sigma, Nacalai Tesque Inc. (Kyoto, Japan) and Prolabo (Strasbourg, France) and were of analytical grade.

2.2. Preparation methods

2.2.1. Oil/water emulsion pressure homogenization technique

The preparation of NP was achieved by adjusting the simple emulsion (o/w) technique, previously applied to the preparation of NP [19]. The adjustment was based on the use of a homogenizer in the one-step emulsification process, thus reducing considerably the size of the dispersed droplets. Briefly, rolipram (20 mg) was dissolved in 10 ml of methylene chloride containing 125 mg of the polymer (PCL) under magnetic stirring at 250 rev./min. This organic solution was thereafter poured into the PVA or SC aqueous solution (100 ml) and the emulsion was homogenized in a microfluidizer (AML 2, Guérin, Mauze, France) by cycling the emulsion in the compression cycle for 3 min at 200 bar. Thereafter, the solvent evaporation step was performed in a Büchi Rotavapor ⟨R⟩ (Büchi, Flawil, Switzerland) during 45 min reducing the pressure stepwise down to 10–30 mbar with a diaphragm pump (Vakuubrand, Wertheim, Germany). After evaporation of methylene chloride under reduced pressure, the polymer precipitated and NP were separated from the non-encapsulated drug and the free PVA or SC by dialysis against distilled water (membrane cut-off: 100,000 Da) for 12 h. The effect of various concentrations (0.1, 0.3, 1, 3 and 10%) of the two surfactants on the characteristics of the NP was evaluated.

2.2.2. Nanoprecipitation solvent extraction technique

The second preparation technique of NP was based on the nanoprecipitation method [20] and optimized as follows: PCL (125 mg) and rolipram (20 mg) were dissolved in 6 ml of acetone under magnetic stirring (250 rev./min). Ethanol (4 ml) was thereafter added stepwise to the organic solution. The organic solution was then added to an aqueous phase (100 ml) containing various concentrations of the two surfactants (0.1, 0.3, 1, 3 and 10%) stirred at 400 rev./min for 3 min. PVA NP were then isolated by centrifugation at 24,000×g at 4°C for 20 min. They were redispersed twice in distilled water in an ultrasonic bath and centrifuged before freezing in an ethanol solution prior to lyophilisation. SC NP were hardened by evaporation of the organic solvent mixture under reduced pressure to avoid caking during centrifugation. The removal of free drug and surfactant was done by an ultrafiltration system (Minitan, Millipore, Japan) cooled with an ice bath.

2.3. Analytical methods

2.3.1. Measurement of size and zeta potential

The NP were analyzed for their size distribution and their surface potential using either a Zetasizer II® (Malvern Instruments, UK) or a Photal laser particle analyser LPA 3100 (Otsuka Electronics, Japan). The results were normalized with respect to a polystyrene standard suspension (Malvern Instruments).

2.3.2. Determination of viscosity

The external aqueous phase was analyzed for its viscosity using a Haake Falling Ball Viscometer (Thermo Haake, Karlsruhe, Germany) according to the manual instructions. The temperature of the PVA and SC solutions was adjusted at 25°C.

2.3.3. Scanning electron microscopic studies

The external and internal morphology of NP were analyzed by scanning electron microscopy (SEM). The NP were fixed on supports, and coated with gold

under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. Samples were then observed with the scanning electron microscope (Cam Scan S2, Leica Cambridge Ltd., Cambridge, UK) at 20 kV.

2.3.4. Determination of rolipram loading

The amount of rolipram entrapped within NP was determined by measuring the amount of non-entrapped drug by a HPLC method in the supernatant recovered after centrifugation and washing of the NP. The results of this indirect assay were compared with those obtained by the direct assay performed as follows: freeze-dried NP (50 mg) were dissolved in methylene chloride (0.5 ml). Then, 30 ml of methanol were added and vortexed for 5 min. The resulting solution was centrifuged for 20 min at 10,000×*g*. One ml of supernatant was diluted in 10 ml of methanol and the concentration measured by HPLC. Since a good correlation was found for both methods and the indirect method was faster and easier, it was chosen as the appropriate one for the drug incorporation as well as the drug release tests.

In order to check the amount of drug adsorbed onto NP, blank NP were added to a rolipram standard solution (0.2 mg/ml). After stirring the colloidal dispersion for 30 min, NP were recovered by centrifugation and the supernatant was analyzed by HPLC for its drug content as follows: RP-18 column (LiChrospher® 100); eluent: acetonitrile:water 40:60; flow rate 1.0 ml/min. Rolipram was detected by UV absorbance at 280 nm, samples of 20 μl were injected into the column. This procedure allowed analysis of very dilute drug solutions (100 ng/ml) with removal of interfering substances, especially interference with possible detergents. The method was linear in the range of 100 ng/ml to 600 μg/ml. Free SC recovered from the supernatant was also quantifiable by the same HPLC method.

2.3.5. In vitro release profiles

Fifty mg of lyophilized drug-loaded NP were resuspended in a 100-ml flask containing artificial intestinal fluid and incubated into a bath at 37°C under gentle magnetic stirring at 250 rev./min. The artificial intestinal fluid was prepared according to USP 23: 6.8 g of monobasic potassium phosphate were dissolved in 650 ml of water and 190 ml 0.2 N sodium hydroxide. Ten grams pancreatin were added, mixed and the resulting solution was adjusted to a pH of 7.5 with 0.2 N sodium hydroxide. At appropriate intervals, 0.2-ml samples were withdrawn and filtrated through a 0.1-μm PTFE Millipore filter. The filtrate was assayed for drug release and replaced by 0.2 ml of fresh buffer. The amount of rolipram in the release medium was determined by the HPLC assay as described before.

3. Results and discussion

3.1. Oil/water emulsion pressure homogenization technique

As shown in Fig. 1, nanoparticles prepared by the PHE method had a submicron size and were relatively monodispersed with the two surfactants used for their preparation.

Since drug loss from the internal organic to the external aqueous phases should be kept to a minimum during the emulsification step, the stability of the emulsion is crucial. During the solvent evaporation process, there is a gradual decrease of the dispersion volume and consequently a subsequent increase of the viscosity of the dispersed droplets. This affects the droplets size equilibrium, potentially involving the coalescence and the agglomeration of the droplets during the early step of the solvent removal [21,22]. This problem has been reduced by adding surfactants into the continuous phase, providing a thin protective layer around the droplets and hence reducing their coalescence. While maintaining a constant volume for the external aqueous phase (100 ml), the amount of surfactant was varied. As reported in Fig. 2, the size of PCL NP decreased slightly when SC concentrations increased. Since NP were formed from the emulsion droplets after the solvent evaporation, their size is dependent upon both the size and the stability of the emulsion droplets. It could thus be concluded that the higher the SC concentration, the more SC molecules insert onto the surface of the droplets, involving an improvement in the protection of the droplets from coalescence and resulting consequently in smaller emulsion droplets than at lower SC concentrations.

The fitting by polynomial regression of oil/water

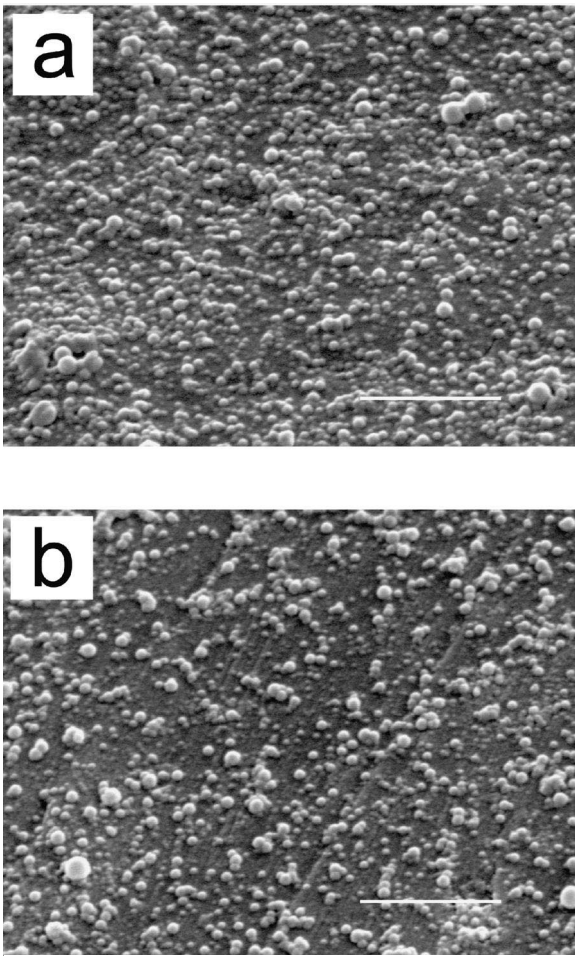

Fig. 1. Scanning electron microscopic images of NP prepared by PHE method using PVA (a) or SC (b) as surfactant. The scale bars represent 5 μm.

interface area against SC concentration showed a good correlation coefficient ($R^2 = 0.9996$). The calculations were done for the initial oil-in-water emulsion after homogenization and before the evaporation step, which is the dominant factor since the influence of the evaporation mostly consists of reduction of droplet size. The calculation of oil/water interface area was performed with the following equation:

$$I_{\text{oil/water}} = 4\pi \times \left[\frac{3 \times V_{\text{drop}}}{4\pi}\right]^{2/3} \times \frac{1000}{V_{\text{drop}}} \quad (1)$$

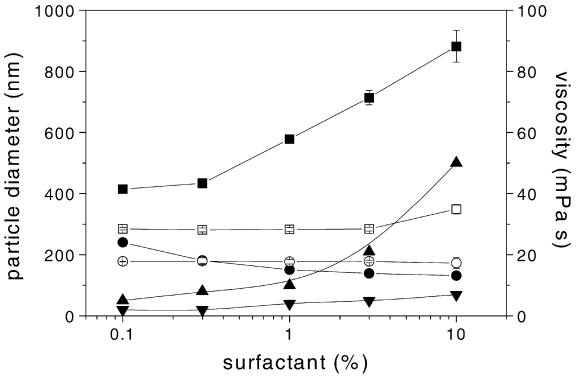

Fig. 2. Influence of the surfactant concentration on the particles diameter for NP prepared by both preparation methods and the two surfactants (■, PHE method with PVA; ●, PHE method with SC; □, SESD method with PVA; ○, SESD method with SC). The viscosity of the PVA (▲) or SC (▼) solutions is shown in mPa s. Data are shown as mean±S.E.

where V_{drop} was calculated as follows:

$$V_{\text{drop}} = \frac{4}{3}\pi \times r_{\text{part}} \times V_{CH_2Cl_2} \times \frac{\rho_{\text{PCL}}}{m_{\text{PCL}}} \quad (2)$$

where $I_{\text{oil/water}}$ is the total oil/water interface after the homogenization step in m^2; V_{drop} is the average volume of a single oil phase droplet after homogenization; r_{part} is the average particle radius; $V_{CH_2Cl_2}$ is the total initial oil phase volume (10 ml); ρ_{PCL} is the density of PCL (1.1523 g/ml) and m_{PCL} is the total initial polymer mass (125 mg).

The interface between emulsion droplet of the organic phase and the external aqueous phase correlates in this case with the surfactant concentration in the external phase. This might be due to the negligible influence of viscosity and also the low molecular structure of the surfactant, which is in favor of the mentioned insertion mechanism to the interface. The fitting allows the prediction of the particle size, however, its use is rather limited to this preparation method since there was no correlation for the SESD method.

In contrast, an increase in particle size was observed using higher PVA concentrations in the aqueous phase, which might be attributed to a reduction of the shear stress during the homogenization process resulting from a higher viscosity of the

aqueous phase and consequently a less favorable mixing efficiency and larger emulsion droplets. The viscosity measurement of the PVA solutions led to a curve which was shaped similar to that of the particle size (Fig. 2). The analysis of the emulsion stability by optical microscopy for 30 min showed no distinct differences concerning droplets' size for both surfactants. These influences on the NP diameter by the two different surfactants might be due to their different molecular structure. SC molecules are much smaller than the PVA chains resulting in a lower viscosity of the SC solution which reduced the shear forces and mixing efficiency. Moreover, PVA has an increased affinity to the aqueous phase and the PVA chains are preferentially directed towards the aqueous phase [20] similar to SC molecules which are also bound to the surface of the particles. Since after washing, the same zeta potential was observed, it was postulated that the SC molecules were strongly anchored in NP by their lipophilic part with the carboxylic head groups disposed towards the aqueous phase.

The change of zeta potential was minimal with increased PVA concentrations. This might be attributed to the uncharged properties of the PVA overlaying the particle surface. A distinct decrease of the zeta potential was observed with higher SC concentrations, probably owing to the SC insertion mechanism (Fig. 3).

The concentration of the two surfactants (SC and PVA) did not influence significantly the encapsulation efficiency (Table 1). With increased PVA and SC concentrations during NP preparation, the drug loss was slightly increased, probably due to the enhanced solubility of the drug within the aqueous phase.

Fig. 4 illustrates the in vitro release profiles obtained by representing the percentage of rolipram release with respect to the amount of encapsulated drug. The drug release was not significantly affected by the surfactant concentration (data not shown). Thus, the 1% formulations of both surfactants were compared in Fig. 4. In general, the drug release occurred in two phases: a first initial burst release was followed by a sustained release of the drug over 2 days resulting from the diffusion of the drug through the polymer. The results also showed that a higher and faster rolipram release was observed for

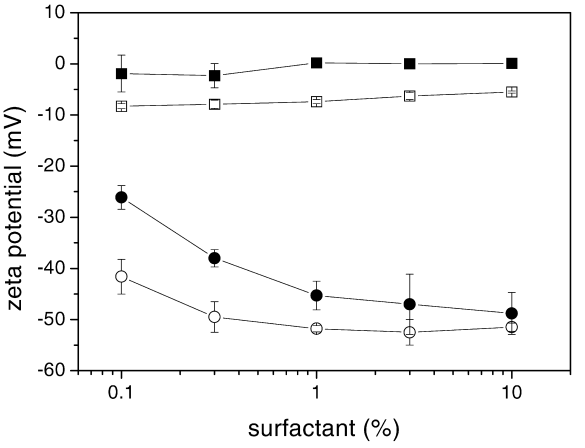

Fig. 3. Influence of the surfactant concentration on the NP zeta potential (■, PHE method with PVA; ●, PHE method with SC; □, SESD method with PVA; ○, SESD method with SC). Data are shown as mean±S.E.

SC NP, which might be due to a smaller particle diameter and consequently a larger surface area. Coffin and McGinity [23] stated that PCL NP are affected by polymer degradation after 50 to 100 days depending on the presence of anionic or nonionic surfactants, respectively. However, pancreatin, the enzyme of the artificial intestinal medium (USP 23) seems to have a noticeable effect on the drug release. Indeed, when the dissolution was carried out in phosphate buffer (pH 7.4) the maximum percentage of rolipram dissolved was only 80% (90% in artificial intestinal fluid). Furthermore, the maximum was reached after 3 days in artificial intestinal medium and 7 days in phosphate buffer. This confirmed the enzymatic contribution in the degradation of PCL [24].

This led us to hypothesize that the release of the drug results from both the diffusion out of the polymeric NP and from the erosion of the polymer.

3.2. Spontaneous emulsification solvent diffusion method

Similar to NP prepared by homogenization, NP prepared by the SESD method have a diameter in the nanometre range including a relatively low polydispersity (0.07–0.21). They showed a monomodal particle size distribution with both SC and PVA

Table 1
Influence of the surfactant concentration (% w/v) on the encapsulation efficiency as a function of the two preparation methods (PHE, SESD) and the two surfactants (organic phase volume: 10 ml; $n=3$)

Surfactant (%)	Encapsulation efficiency (%)			
	PHE/PVA	PHE/SC	SESD/PVA	SESD/SC
0.1	92.1±3.3	93.3±4.7	2.3±1.3	7.6±4.1
0.3	92.8±2.8	91.5±3.8	6.4±0.7	8.5±2.6
1.0	92.3±3.0	91.8±3.7	10.9±5.5	13.2±4.9
3.0	91.5±4.1	91.8±2.3	10.9±6.4	14.3±1.4
10.0	88.9±3.6	90.2±4.9	20.9±6.1	12.3±3.6

surfactants. As observed with photon correlation spectroscopy and SEM, NP did not appear agglomerated for all PVA concentrations tested. In addition, the redispersibility of PVA NP after centrifugation was easier with higher surfactant concentrations which might result from the higher amount of PVA present on the NP surface as well as the subsequently increased thicker hydrophilic and non-charged layer around the particles. On the contrary, when SC was used as surfactant the centrifugation is a critical step because of irreversible caking of the NP. This difference in behavior between the PVA and SC NP can be explained by their surface properties. Indeed, a zeta potential close to neutrality is in favor of a good redispersibility. Therefore, ultrafiltration was used for SC NP purification in the first series of experiments. The zeta potential showed a slight increase for NP prepared with higher PVA concentration which might be due to the same coverage of the particle surface by the overlaying PVA molecules postulated for the oil/water emulsion. The zeta potential of the SC NP decreased with higher surfactant concentration. However, above a concentration of 1% no further decrease of surface potential was observed. This might be explained by a saturation process of SC at the interface. In contrast to the PHE method, a constant interface area was obtained and a maximum of surfactant amount present at the interface was reached. The further increase of total SC concentration determined by HPLC did not contribute apparently to its increased deposition at the interface.

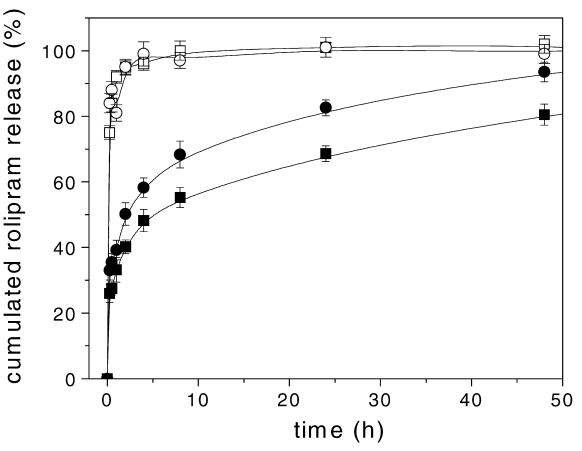

Fig. 4. Release profiles of rolipram NP in simulated intestinal fluid (pH 7.5; USP 23) at 37°C during 50 h (■, PHE method with PVA 1%; ●, PHE method with SC 1%; □, SESD method with PVA 0.1%; ○, SESD method with SC 0.01%). Data are shown as mean±S.E.

As shown in Fig. 2, no difference in NP diameter was observed until a PVA concentration of 3%. However, above 3%, larger particles were observed, resulting probably as mentioned before from a higher viscosity of the external aqueous phase and consequently larger droplets within the emulsion. For the preparation of NP with SC no significant size changes were observed. Compared with the PHE method, the concentration of surfactant had no significant impact on the size of the particles prepared either with SC or PVA by the SESD method. Since NP purification by ultracentrifugation is faster and easier than particle separation by ultrafiltration or dialysis, the first purification method is more suitable but bears the risk of irreversible caking of the NP. In order to prepare redispersable NP with a distinct negative zeta potential, various mixtures of the two surfactants (1% w/v) were tested. The particle size decreased with higher amounts of SC (Fig. 5). The redispersibility diminished stepwise from PVA:SC 100:0 down to PVA:SC 30:70 in the aqueous phase. When the PVA was below 30% in the

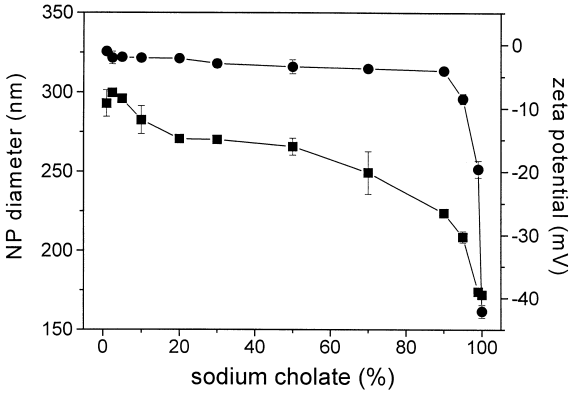

Fig. 5. Influence of the surfactant ratio of SC and PVA in a 1.0% surfactant aqueous phase using SESD method; NP diameter and zeta potential are shown as a function of SC ratio in percent (■, NP diameter; ●, zeta potential). Data are shown as mean±S.E.

surfactant mixture, irreversible caking occurred after the centrifugation step.

Up to a PVA:SC 10:90 ratio, the effect of PVA was predominant on the zeta potential (between 0 and −5 mV). Indeed, this is comparable to the values found using only PVA (Fig. 3), where the zeta potential values were very stable for a PVA range between 0 and 10% (w/v). Below this 10:90 ratio, the zeta potential was significantly decreased. This is probably due to the now dominant effect of SC with regards to PVA. It is assumed that the PVA layer did not cover completely the SC molecules. Consequently, owing to the accessible carboxylic groups, the zeta potential decreased sharply to much lower values (about −40 mV) which are comparable to the strong negative values observed when pure SC solution is used as external aqueous phase.

The encapsulation efficiency of NP prepared with the SESD method was lower than that obtained with the PHE one (Table 1). This could be explained by the extraction of the drug into the external aqueous phase during the solvent diffusion. Indeed, the drug has a good solubility in the mixture acetone/ethanol which enhanced significantly its diffusion towards the aqueous phase during particle solidification. In order to avoid the observed drug transport to the aqueous phase, the acetone/ethanol volume was reduced down to 5 ml. Below 5 ml of organic phase volume, the viscosity was increased preventing the diffusion of the drug to the aqueous phase. Moreover, the solubility of the drug into the aqueous phase was decreased as well.

The encapsulation efficiency was also affected by the surfactant concentration which increased the solubility of the drug in water. Consequently, the decrease of surfactant concentration in the aqueous phase involved an increase of encapsulation efficiency (Fig. 6). Below optimal surfactant concentrations (0.1% for PVA and 0.01% for SC) large uncontrolled precipitates were obtained reducing significantly the entrapment efficiency and increasing the size polydispersity. An increased NP diameter during this process resulted subsequently from the reduced availability of surfactant, stabilizing the interface during the particle solidification. Lower surfactant concentrations influenced the zeta potential as well. It was observed that more negative zeta potential values were obtained by decreasing the amount of PVA. On the other hand, less negative zeta potential values were determined by decreasing the amount of SC, due to the decrease of charge density by the carboxylic groups onto the surface of the NP. Furthermore, owing to the nonionic properties of rolipram it was not possible to change the drug entrapment by varying the pH of the aqueous phase [25].

A comparison of the release profiles led to slight differences concerning the two surfactants and their influence on the release behavior. However, com-

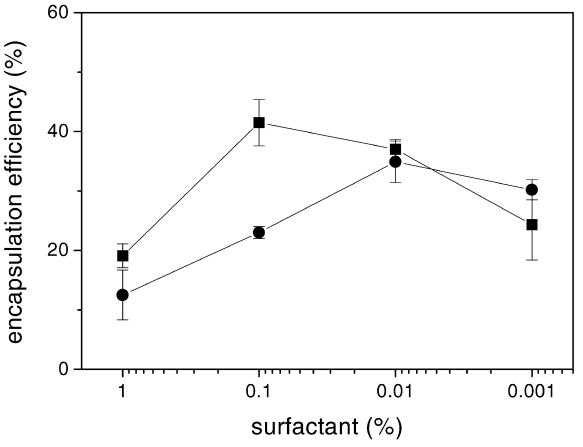

Fig. 6. Influence of the reduced surfactant concentration on the encapsulation efficiency (■, SESD method with PVA; ●, SESD method with SC; organic phase volume: 5 ml). Data are shown as mean±S.E.

pared to the NP prepared by PHE, quite different release kinetics were observed. The drug release showed a significant initial burst effect, involving the release of almost the whole encapsulated drug amount within the first 30 min (Fig. 4). Suchira et al. [26] described this burst release as an immediate dissolution of the adsorbed drug onto the particle surface. However, in our study, no adsorption of the drug onto the NP surface was detected. Consequently, the significant burst effect observed resulted from the low encapsulation efficiency of the NP formulations involving a lower total drug content within the NP. As reported from Polakovic et al. [27] the intensity of the burst release is mainly influenced by the amount of initial drug content in the NP. The authors mentioned that in certain cases the drug release seemed to be controlled rather by the crystal dissolution than by the rate of diffusion above a certain drug concentration in the particle matrix. However, below this threshold concentration the rate of diffusion is the important factor leading to an increased burst effect.

Although NP accumulation in the inflamed tissue allows a higher concentration of the carrier [6], a distinct burst effect may prevent their use as specific colon drug delivery system. An early drug release, such as the one due to the initial burst, may lead to systemic adverse effects. NP with higher drug loading and a smaller burst effect are certainly more desirable. Consequently, the use of the PHE method is to be preferred for this application. However, it has to be kept in mind that the burst effect is also due to the large increase of contact area between NP and surrounding medium (50 mg of NP in 100 ml of dissolution medium). Such conditions are generally not met in-vivo which means that the in-vivo burst effect may be much lower.

SC proved its potential as a biocompatible anionic surfactant for the preparation of NP. The NP were of low polydispersity and of strong negative surface charge. Moreover, during the release experiments in the artificial intestinal medium, SC NP showed a burst effect similar to the PVA NP but a faster drug release. Indeed, more than 80% of the incorporated drug was released within the first 2 days. This time period has been reported to be the approximate retention time in the inflamed tissue [6]. The efficiency of such a carrier for drug delivery in the inflamed tissue has to be proven in vivo in further experiments.

4. Conclusions

The present nanoparticulate formulations can be expected to be used as colloidal drug carriers of rolipram when prepared by an o/w homogenization emulsification or nanoprecipitation solvent extraction method. These NP were found to be attractive for peroral or transmucosal applications because of their monodispersed submicron-sized structure. Their biodegradability should prevent any complication in the case of long-term deposition of the NP inside the ulcerated tissue during gut passage. Moreover, the preliminary drug release tested in vitro with the PCL NP proved that NP prepared by the PHE method had the properties of a sustained release form. Nevertheless, the distinct burst release may question the problem of an early drug release prior to the deposition in the colon.

Acknowledgements

Alf Lamprecht acknowledges the ‘Monbusho Research Fellowship for Young Foreign Researchers’ grant from the Japanese Ministry of Education, Science and Culture.

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