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Pellets as carriers of solid lipid nanoparticles (SLN) for oral administration of drugs

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Dedicated to Prof. Dr. G. Zessin, Halle (Saale), on the occasion of his 65th birthday

In the present study, a method for the preparation of pellets with solid lipid nanoparticles is reported. Solid lipid nanoparticles (SLN) were prepared by high pressure homogenization of a coarse emulsion. The nanoparticles were characterized with regard to particle size and size distribution (laser diffraction and photon correlation spectroscopy) and zeta potential (laser Doppler anemometry). Lactose, microcrystalline cellulose and water were used in the production of pellets. The aqueous phase of the dispersion of nanoparticles was used as the aqueous phase in the processing of the pellets by extrusion and spheronisation. Pellets were characterized for size and size distribution (sieving) and surface defects (visual inspection). An USP XXIII apparatus was used to determine the release of the nanoparticles from the pellets and the concentration of the nanoparticles was assessed by laser diffractometry and the obscuration obtained compared to the obscuration of the standard (diluted solid dispersion). The batches of solid lipid nanoparticles produced were monodisperse, with a narrow size distribution and a zeta potential of −20 mV. The solid lipid nanoparticles were stable for at least 2 months, at 4 °C. More than 90% of the spherical pellets produced were in the range between 1.00 and 1.25 mm in diameter and presented a smooth surface. The pellets, once in contact with an aqueous environment disintegrated within 5 min, releasing the nanoparticles. The properties of the nanoparticles were not affected by incorporation in pellets and their release from the pellets was almost complete (between 80.5 and 96.0%).

1. Introduction

The development of nanoparticulate drug delivery systems has attracted much attention for the last two decades. Reasons for that interest are the possibility of providing protection for the drug from enzymes, targeting the drug to the site of action or reducing the side effects of drugs [1].

Solid lipid nanoparticles (SLNs), one class of nanoparticles, can be prepared by high pressure homogenization of lipids [2]. They combine the advantages of fat emulsions due to the low toxicity of the lipidic phase (e.g. parenteral nutrition) and conventional nanoparticles (e.g. solid matrix for controlled release of drugs) [3]. Solid lipid nanoparticle dispersions have been lyophylized, spray-dried or sterilized reflecting the high stability of these systems [4].

Nanoparticles are more often administered to patients by the intravenous route. The inconvenience for the patient and the need of medical staff justify the attempt to find an alternative route of administration, such as the oral route. The adhesive properties of nanoparticles are known to increase bioavailability and reduce or minimize erratic absorption [5]. There is evidence that absorption of nanoparticles occurs through the mucosa of the intestine by several mechanisms, namely through the Peyer's patches [6, 7], by intracellular uptake [8] or by the paracellular pathway [9]. Solid lipid nanoparticles are regarded as a candidate vehicle to improve the distribution of drugs in the gastrointestinal tract (GIT) and to control the release of the drug from the lipid matrix.

Pellets, due to their high stability (both physical and microbiological), high sphericity and mechanical strength (important properties required for further processing), seem to be suitable carriers for the nanoparticles [10]. Therefore, the resultant hybrid preparation appears to be an adequate oral dosage form. Pellets can be designed to be administered at different sites and to control the release of the nanoparticles both in time (as already achieved with drugs), and site of release of the SLNs, at different positions of GIT, tailored to specific needs. Furthermore, the administration of a few dozens of pellets each time, increases the dispersion of the nanoparticles in the GIT, reducing gastric emptying, intestinal transit and inter-subject variability.

Different technologies are available for the production of pellets, for instance extrusion and spheronisation of wet masses [11, 12]. The pellets produced can be used as such, filled in gelatin hard capsules or serve as starting material for the production of tablets [13, 14].

The aim of the present study was to prepare solid lipid nanoparticles loaded pellets. The pellets are intended to disintegrate in an aqueous environment, releasing the nanoparticles, which may, in turn, release the drug, adhere to the surface of the gastrointestinal mucosa or be absorbed.

2. Investigations, results and discussion

The solid lipid nanoparticles produced (Table 1) have shown identical properties (size, size distribution and zeta potential) to those described in the literature and produced under similar conditions [4]. Particles have shown a narrow size distribution, monomodal, with values for D_{50}, D_{90} and D_{95}, by volume, in the ranges of 310–330 nm, 320–330 nm and 320–340 nm, respectively. The zeta po-

Table 1: Percentage of the different components in the pellet formulations

	Microcrystalline cellulose	Lactose	Lipid dispersion	Water
Formulation A[a]	4.20	79.1	1.70	15.0
Formulation B[b]	8.30	75.0	1.70	15.0

[a] 5 : 95 : 20 parts of microcrystalline cellulose : lactose : solid lipid nanoparticles dispersion
[b] 10 : 90 : 20 parts of microcrystalline cellulose : lactose : solid lipid nanoparticles dispersion

tential of the nanoparticles was −20 mV. These results were in good agreement with the values previously reported [4]. The incorporation of prednisolone in the solid lipid nanoparticles, a model drug used in previous experiments [4, 15], has changed the surface properties of the nanoparticles, as ascertained by a change in the zeta potential from −36 mV (blank SLNs) to −20 mV.

A key problem in this study was the development of a methodology to compare the number, or concentration, of the nanoparticles prior and after disintegration of the pellets in the releasing media. The construction of a calibration curve from diluted dispersions, obtained from the stock dispersion used for the production of the pellets, overcame the problem. The high correlation coefficient ($r^2 = 0.9981$) between volumes, which are proportional to the number of particles, and the low values for the residues have demonstrated the validity of the method to estimate particle characterization from the obscuration produced by laser diffractometry (Fig.).

The visual inspection of the different types of pellets produced, showed a smooth surface with 90% or more within the range between 1.00 and 1.25 mm and a narrow size distribution. Preparations of intact and cut pellets were observed under a scanning electronic microscope revealing the presence of some individualized SLNs on the surface.

The factors studied (such as pH or ionic strength) tried to mimic, as far as possible, the conditions that the nanoparticles would find *in vivo*. Initially, the influence of the amounts of pellets in the release and recovery of SLNs, was assessed. As expected, different amounts of pellets in the dissolution vessel released different amounts of nanoparticles. Table 2 compares the results obtained with four different loads of pellets (tested in separate dissolution vessels) with two standard amounts of SLNs (in two other dissolution vessels). To obviate to the lack of microcrystalline cellulose and lactose in the standard samples, amounts equivalent to those used in formulations 'A' and 'B' were added to the disintegration vessels. From the calibration curve (Fig.), the expected values could be calculated and compared to the observed ones, showing that some nanoparticles were adsorbed either to the vessel walls, by the lactose or microcrystalline cellulose non-dissolved particles or to the filter or syringe. Excluding the percentage of the particles adsorbed to the vessel's walls, filter or syringe (see Table 2, 'corrected yield' column), about 12% of the nanoparticles remained adsorbed to the lactose or microcrystalline cellulose particles. The percentage of SLNs retained by the non-dissolved solids was constant and therefore independent of the amount of solids present or pellets included in the releasing media.

Table 2: Effect of the amount of pellets in the dissolution vessels on the release of the nanoparticles in the medium

	Obscuration (%)		Theoretical yield (%)	Corrected yield[a] (%)
	Expected	Observed		
8.083 g	7.75	6.32	81.5	87.0
5.321 g	7.35	5.85	79.6	85.0
2.588 g	6.85	5.75	82.7	88.3
1.284 g	6.76	5.50	81.2	86.7
1200 µl (standard)	7.47	7.00	93.7	–

[a] Variation of the yield of the samples to the standard

Results represent the mean values of 3 sets of experiments. Formulation of pellets: 5 parts of cellulose microcrystalline : 95 parts of lactose : 20 parts of SLN dispersion (Formulation A). Fraction of pellets used: 1.00–1.25 mm

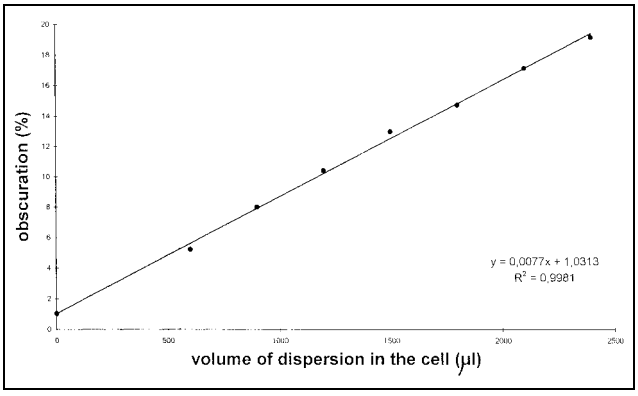

Fig.: Calibration curve of obscuration *versus* volume of dispersion in the cell

Pellets were designed to immediately release the SLNs, once they were placed in an aqueous environment. This was achieved in formulations 'A' and 'B' which contained, respectively, 5 and 10 parts of microcrystalline cellulose and disintegrated within 5 min. An increase of the amount of microcrystalline cellulose in the formulation led to an increase in disintegration time of the pellets. Those formulated with microcrystalline cellulose contents higher than 15 parts did not disintegrate within the specified time of 2 h and, consequently, the SLNs were not released (data not shown). Different amounts of each formulation 'A' or 'B' were placed in aqueous media (Table 3) and once again, the expected (i.e. theoretical) and the observed obscuration (i.e. experimental) were compared. The obscuration produced by one dilution of the standards were also considered. The variations to the standard were small and adsorption of the nanoparticles to the walls of the dissolution vessel to the filter may account for this. Variations due to fusion of nanoparticles can be excluded, since the size and the size distribution of the dispersions obtained after the release of the nanoparticles from the pellets were identical to those of the nanoparticles prior to pelletization. Comparison of the results to the standards (Table 3, column 'corrected yield') reflected the variations due to the presence of the microcrystalline cellulose and lactose in the aqueous dispersion. Results suggest that a small percentage of nanoparticles was adsorbed to the microcrystalline cellulose or lactose.

Modifications of pH are usually included in disintegration and dissolution studies. Since the release of the SLNs

Table 3: Effect of the amount of cellulose microcrystalline and lactose in the pellets on the release of the nanoparticles in the medium

		Obscuration		Theoretical yield (%)	Corrected yield (%)
		Expected	Observed		
Formulation A[b]	4.017 g	7.16	6.57	91.8	98.0
	3.949 g	7.01	6.05	86.3	92.1
	2.072 g	6.87	5.90	85.9	91.7
Formulation B[c]	4.965 g	7.29	6.31	86.6	92.4
	4.676 g	7.25	6.24	86.0	91.4
	2.531 g	6.94	6.53	93.0	99.3
SLN standard	1200 µl	7.47	7.00	93.7	–

[a] Variation of the yield of the samples to the standard

[b] 5 : 95 : 20 parts of microcrystalline cellulose : lactose : solid lipid nanoparticles dispersion

[c] 10 : 90 : 20 parts of microcrystalline cellulose : lactose : solid lipid nanoparticles dispersion

Results represent the mean values of 3 sets of experiments. Fraction of pellets used: 1.00–1.25 mm

from the pellets was not pH-dependent, major alterations were not expected. However, results (Table 4) have shown an increase in the number of the nanoparticles collected as the pH values increased. The observation suggests that the nanoparticles were either degraded at low pH values or that adsorption of the particles to the other ingredients of the formulation or to the equipment, was pH dependent.

Table 5 presents the results obtained in experiments in which the ionic strength of the medium increased by addition of sodium chloride. The decrease of the number of nanoparticles recovered from the dispersion suggests that the adsorption of the nanoparticles to the vessel surface or other materials increases with an increase in ionic strength. Another possible explanation could be the agglomeration between nanoparticles. However, a shift to larger diameters was not observed when the filtered dispersion was quantified suggesting that the size of the nanoparticles was little or not affected at all.

This study has not only proved the possibility of obtaining a solid dosage form from a liquid dispersion of nanoparticles, but has also shown an improved stability of the SLNs (physical, chemical and microbiological), as observed several weeks after production. Other equally important advantages of this design include easier handling throughout the processing phase and as a final product (especially if the pellets are encapsulated into hard gelatin capsules) and higher production outputs.

In conclusion, pellets of adequate size (1.00–1.25 mm in diameter) for further processing, narrow size distribution and smooth surface could be produced. These, once in contact with an aqueous environment, disintegrated quickly releasing the SLNs. These nanoparticles can, in turn, release their content (such as a conventional drug or a peptide) to the gastrointestinal tract for subsequent absorption.

Adsorption of the nanoparticles to the glassware or to the filter's membrane may have contributed to the decrease of the number of nanoparticles detected by comparison to the expected values. The theoretical yield was normalized to the standard, to abolish the effects due to the adsorption mentioned above and the corrected yield thus obtained shows that the release is higher than observed, suggesting that *in vivo* a high number of nanoparticles will be available to the patient. The ionic strength, the pH and, to a much lower extent, the presence of other materials in the media affected the recovery of the nanoparticles from the pellets.

Table 4: Effect of pH on the release of the nanoparticles in the aqueous medium

pH	Content	Obscuration (%)		Theoretical yield (%)	Corrected yield[a] (%)
		Expected	Observed		
1.5	5.621 g	7.39	5.95	80.5	91.1
	1200 µl (standard)	7.47	6.60	88.4	–
6.8	5.498 g	7.37	6.37	86.5	95.3
	1200 µl (standard)	7.47	6.78	90.8	–
7.4	4.250 g	7.19	6.29	87.5	97.9
	1200 µl (standard)	7.47	6.68	89.4	–

[a] Variation of the yield of the samples to the standard

Results represent the mean values of 3 sets of experiments. Formulation of pellets: 5 parts of cellulose microcrystalline : 95 parts of lactose : 20 parts of SLN dispersion (Formulation A)

Table 5: Effect of ionic strength on the release of the nanoparticles in the medium

Conc. of NaCl (%)	Content	Obscuration (%)		Theoretical yield (%)	Corrected yield[a] (%)
		Expected	Observed		
0.45	4.693 g	7.25	7.00	95.6	99.6
	1200 µl (standard)	7.47	7.17	96.0	–
0.90	4.703 g	7.26	6.15	84.7	91.7
	1200 µl (standard)	7.47	6.90	92.4	–
1.35	4.768 g	7.22	5.87	81.3	93.3
	1200 µl (standard)	7.47	6.56	87.1	–

[a] Variation of the yield of the samples to the standard

Results represent the mean values of 3 sets of experiments. Formulation of pellets: 5 parts of cellulose microcrystalline : 95 parts of lactose : 20 parts of SLN dispersion (Formulation A). Fraction of pellets used: 1.00–1.25 mm

3. Experimental

3.1. Materials

Solid lipid nanoparticles: Glycerol behenate (Compritol ATO 888, melting range 69–74 °C, Gattefossé, Germany) was the lipidic phase. The model drug, prednisolone, was supplied by Ferring AG (Germany). The pluronic F68 (Poloxamer 188, BASF AG, Germany) was the surfactant and bidistilled water the aqueous phase.

Pellets: Microcrystalline cellulose (Avicel PH 101, FMC Corp., USA), lactose monohydrate (Meggle GmbH, Germany) and deionized water.

3.2. Methods

3.2.1. Preparation and characterization of the solid lipid nanoparticles

Prednisolone (0.5%) was added to the melted glycerol behenate (90 °C) until it was completely dissolved. Simultaneously, the water was heated up to the same temperature and the pluronic F68 was dissolved. The melted lipidic solution was dispersed in the aqueous surfactant solution at 90 °C. A coarse emulsion was produced by stirring the mixture with an Ultra-Turrax T25 (Janke and Künkel GmbH, Germany) for 10 min at 20000 rpm. The coarse pre-emulsion was homogenized at 90 °C in a high pressure homogenizer (Micron LAB 40, APV-Gaulin, Germany) at 600 bar with 5 cycles. The homogenized product obtained was an o/w emulsion of melted lipid in the aqueous surfactant solution. The oil droplets solidified when the emulsion (1000 ml) was cooled down to room temperature, with the formation of solid lipid nanoparticles dispersed in the aqueous phase. All glassware in contact with the dispersion was siliconised with dichlorodimethylsilane (Merck, Germany).

The sizing of the particles was carried out by laser diffractometry (MasterSizer E, Malvern Instruments, UK) and Photon Correlation Spectroscopy (Malvern ZetaSizer IV, Malvern Instruments, UK). From the laser diffractometry data, the diameters 50%, 90% and 95% (D_{50}, D_{90} and D_{95}), by volume, were used to characterize the nanoparticles. The zeta potential was determined using the Zetasizer IV in water with the conductivity adjusted to 50 µS by addition of NaCl (electric field strength 20 V · cm^{-1}). The electrophoretic mobility measured was converted into zeta potential (mV) using the Helmholtz-Smoluchowski equation [16].

3.2.2. Preparation and characterization of the pellets

Lactose and microcrystalline cellulose were mixed in a sigma blade mixer (Erweka, Germany) in two different formulations: A (5 : 95 parts of microcrystalline cellulose/lactose) and B (10 : 90 parts of microcrystalline cellulose/lactose) for 10 min. Afterwards, 20 parts of the solid lipid nanoparticles dispersion containing 10% lipid (w/w) were added to the mixture and a wet mass was produced after another period of 10 min (Table 1). This wet mass (100 g) was then extruded (through a screen with 1 mm diameter perfurations, 20 rpm, Caleva model 10, Caleva Process Solutions, Ltd, UK) and the extrudates spheronised (1000 rpm, Caleva model 120, Caleva Process Solutions, Ltd, UK). The wet pellets were dried at room temperature for 48 h. Size and size distribution of the pellets was assessed by sieving.

3.2.3. Quantification of the release of the nanoparticles from the pellets

An USP XXIII apparatus (paddle) was used to study the release of the nanoparticles from the pellets. Deionised water or aqueous solutions (900 ml) were used taking into account the standard conditions for dissolution tests. Briefly, three different pHs of the media were obtained with HCl (pH 1.5) or a sodium phosphate buffer (EP; pH 6.8 and 7.4). The ionic strength of the media was adjusted with NaCl in increasing concentrations (0.45 up to 1.35%, w/v) to reflect to some extent the environment that the nanoparticles would find *in vivo*. After 2 h, large samples (200 ml) of the media were taken (200 ml) and filtered through a 8 µm filter (Sartorius, Germany) to remove non-dissolved large particles, such as microcrystalline

cellulose particles. This lag time was chosen to allow for complete disintegration of the pellets and dissolution of lactose in the media. The concentration of particles in this filtered dispersion (observed) was compared to the concentration of particles present in the initial dispersion (expected). The theoretical release yield was the ratio between the observed to the expected values (presented as %). The corrected yield (%) was derived from the ratio of the theoretical yield of the samples to the standards. Different concentrations of nanoparticles resulted in different obscurations, by laser diffractometry and a comparison was possible since a linear relationship was observed (Fig.). Since different amounts of pellets of the same formulation had known amounts of solid lipid dispersion, it was possible to predict the obscuration caused by the nanoparticles in the sample from the calibration curve. All the preparations of pellets were produced from the same solid lipid nanoparticles stock dispersion and experiments were run in triplicate.

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