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Effect of PEG2000 on drug delivery characterization from solid lipid nanoparticles

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The purpose of this work is to develop a PEG2000-modified solid lipid nanoparticles (SLN) intended to encapsulate a drug within their matrix and to study their characteristics. In the present report, drug-loaded SLN were prepared by a novel solvent diffusion method in an aqueous system. Monostearin and PEG2000 were used as carrier material and modifying agent, respectively. The model drug salbutamol sulphate was incorporated to study the characterization of entrapment efficiency, size, zeta potential (charge) and drug delivery characterization. In the test solution of pH 7.2 phosphate buffer, drug-release behavior from SLN suspension exhibited a biphasic pattern. With the monoastearin-based SLN, a distinctly prolonged release over a monitored period of 14 days was observed after a burst drug release in the first 8 hours. Over the monitored period of prolonged release, there was delayed release in the first 5 days with nearly 2.51% of the drug released each day, following which a slightly higher release rate (8.14% per day) appeared in the last 9 days. In contrast, the drug release rate from PEG2000-modified SLN was faster. Nevertheless, further work is required in order to optimize the release behavior of various entrapped drugs. These results also demonstrate that modification with PEG2000 can accelerate release of hydrophilic small molecule drugs from SLN.

1. Introduction

Microspheres using biodegradable material are currently being considered as a drug delivery system for long-term release. Solid lipid nanoparticles (SLN) are another alternative colloidal carrier system for controlled drug delivery (Müller et al. 2000). Some characteristics of SLN, such as their high bioavailability (Cavalli et al. 2002) and targeting effect (Wang et al. 2002), are being given more attention in recent years.

We observed prolonged release of hydrophobic drugs and hydrophilic biomolecules from SLN, prepared by a solvent diffusion method in an aqueous system, in our earlier research (Hu et al. 2002, 2004). Usually, a hydrophilic small molecule drug is difficult to incorporate into SLN, as this consists of hydrophobic material. On the other hand, prolonged release over a long time is not achievable due to the SLN being rapidly attacked by the mononuclear phagocyte system *in vivo* and then subsequently degraded in the cell.

Polyethylene glycol (PEG), a polymer extensively investigated for surface modification of particles in a formulation, possesses the main requisites for pharmaceutical application: water solubility, biocompatibility and low cost of preparation (Morita et al. 2001). Indeed, owing to multifunctional character, it allows a variety of modifications following simple physical or chemical procedures.

Many studies have focused on modifying drug release using PEG incorporated into microspheres (Cavalloaro et al. 2002; Garcia-Fuentes et al. 2002). However, the effect of PEG on drug release from matrix-type SLN has not been studied. The aim of the present work was to develop matrix-modified SLN intended to encapsulate drug a within their matrix and to study their physicochemical properties, especially for enhancing drug release from SLN and liberating it from the reticuloendothelial system.

2. Investigations, results and discussion

2.1. Solvent diffusion method in an aqueous system

The size and the polydispersity indices of PEG 2000-free SLN prepared by the solvent diffusion method in an aqueous system are shown in Table 1. The volume mean diameters exhibited similar size distributions at the different preparation temperatures, with a volume mean diameter of 328 nm with a polydispersity index of 0.318 at low temperature (0 °C).

From our previous research on preparation of SLN by the solvent diffusion method, the PVA molecules in the aqueous phase are adsorbed around the emulsion droplets, which are formed by the diffusion of organic solvent (containing lipid) to the aqueous phase, resulting in spontaneous droplet formation in the submicron range. In the present research, we used a similar method to separate the SLN from the usual dispersed aqueous medium (containing PVA molecules). After preparation, the SLN suspension was adjusted to become acidic. Under the usual con-

Table 1: Size of PEG2000-free solid lipid nanoparticles (SLN) at different preparation temperatures, determined by Zetasizer (3000HS, Malvern Instruments, UK)

Dosage forms	Temp. (°C)	Number average (nm)	Volume average (nm)	Polydispersity index
Drug free	25	297.3 × 244.0 (100.0%)	250.2 × 171.0 (100.0%)	0.148
	0	101.2 × 40.0 (85.4%)	115.9 × 69.5 (22.3%)	0.290
		311.3 × 111.6 (14.6%)	320.6 × 135.2 (77.7%)	
Drug loaded	25	99.9 × 37.4 (85.6%)	101.5 × 39.6 (14.8%)	0.266
		319.4 × 161.0 (14.4%)	327.5 × 162.4 (85.2%)	
	0	107.9 × 49.3 (61.1%)	109.7 × 51.1 (4.6%)	0.318
		343.1 × 122.7 (38.9%)	344.0 × 122.1 (95.4%)	

Table 2: Zeta potential of drug-free solid lipid nanoparticles (SLN) and drug-loaded SLN in the different dispersed aqueous phases (mV, prepared at 0 °C)

Dispersed aqueous phase	Drug-free SLN		Drug-loaded SLN	
	Zeta potential (mV)	width (mV)	Zeta potential (mV)	width (mV)
Usual aqueous phase (pH 5.7)	−20.0	1.2	−22.9	0.9
Acidic aqueous phase (pH 1.0)	0.8	1.5	1.0	0.6

ditions of the dispersed aqueous medium at pH 5.7 and containing 1% PVA (prepared from 1% PVA in distilled water), coacervation and finally precipitation of lipid did not take place. At the lower pH, the zeta potential of the system is more nearly zero (as shown in Table 2), producing aggregation of the SLN, and then separation of SLN from the suspension is easily achieved by centrifugation.

The sizes and zeta potentials of SLN prepared with different percentages of PEG 2000 in the organic phase during preparation are listed in Table 3. The particle size exhibited a slight enhancement with an increased proportion of PEG in the system, but no further change of zeta potential.

2.2. Efficiency of drug encapsulation

The effect of different temperatures of the dispersed aqueous phase on drug encapsulation efficiency of SLN is shown in Fig. 1. The encapsulation efficiencies of SLN depended on the temperature of the dispersed aqueous phase and were in the rank order of low temperature (0 °C), followed by room temperature (25 °C). In addition, the amount of salbutamol sulphate incorporated into SLN is also correlated with the percentage of PEG2000 (w/w) in the organic phase during the preparation and decreased with an increasing proportion of PEG2000 in the organic phase.

The solubility of a drug in water is an important factor determining the amount of the drug incorporated into a drug delivery system, given preparation by the solvent diffusion method in an aqueous system. Salbutamol sulphate may cause difficulties in encapsulation, because it is a hydrophilic low-molecular-weight substance and thus, cannot dissolve completely in organic solvents (i.e. anhydrous ethanol, acetone). It must be dissolved in a little distilled water first. The existence of water in the organic phase may affect the diffusion process of the organic phase (containing the hydrophilic low-molecular-weight drug and lipid) to the aqueous phase and thus some of the drug to be incorporated into SLN can easily be lost. The phenomenon of decreasing drug encapsulation efficiency, as a result of increasing the proportion of PEG2000 in the organic phase, was due to a similar mechanism process, produced by the hydrophilicity of PEG2000, as shown in Fig. 1.

The low temperature condition of the dispersed aqueous phase was produced by an ice-water bath, and the nanoparticles were prepared under this condition in order to

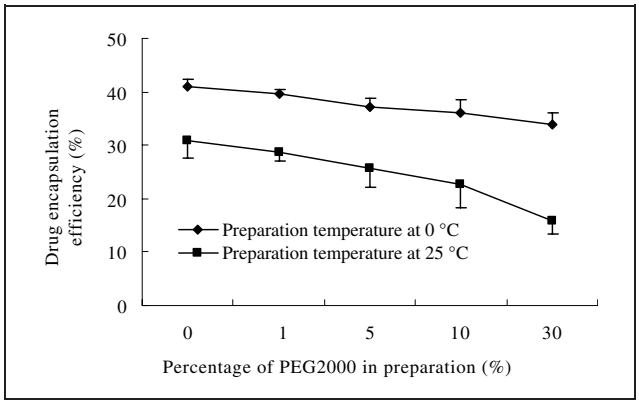

Fig. 1: Effect of percentage of PEG2000 in organic phase on drug encapsulation efficiency of solid lipid nanoparticles (SLN)

Table 3: Effect of different percentages of PEG2000 (w/w) in organic phase on average diameter, and zeta potential of solid lipid nanoparticles (SLN) (prepared at 0 °C)

PEG 2000 in organic phase (%)	Number average (nm)			Volume average (nm)				Zeta potential (mv)
	Area	Mean	Width	Area	Mean	Width	average (nm)	
0	61.1	107.9	49.3	4.6	109.7	51.1	328.2	−19.6 × 1.9
	38.9	343.1	122.7	95.4	344.0	122.1		
1	100.0	381.1	115.7	100.0	374.1	149.8	374.1	−21.8 × 1.4
5	100.0	407.8	152.5	100.0	391.1	149.8	391.1	−20.2 × 1.0
10	83.7	114.0	60.7	24.1	142.6	79.0	354.4	−20.3 × 2.1
	16.3	416.5	147.1	75.9	421.7	144.7		
30	71.8	138.0	37.0	8.7	141.0	38.0	405.9	−19.6 × 0.7
	28.2	424.4	98.0	91.8	428.8	97.1		

improve the encapsulation efficiency of salbutamol sulfate. In the solvent diffusion method in an aqueous system, the diffusion rate of the water miscible organic solvent to the aqueous phase was very rapid. The encapsulation efficiency of salbutamol sulphate in SLN was increased to 41% as the temperature of the dispersed aqueous phase was decreased to 0 °C, which is attributed to the rapid deposition of lipid in droplet form during stirring and decreased leakage of salbutamol sulfate into the outer aqueous phase. It is suggested that rapid deposition at the interface between the droplets and the aqueous medium would prevent the leakage of salbutamol sulphate, leading to an improvement in the drug encapsulation efficiency, with decreasing temperature of the dispersed aqueous phase.

The average volume diameter of PEG2000-modified SLN loaded with salbutamol sulphate increased from 328 to 405 nm, when the percentage of PEG2000 in the resultant organic preparation solution went from 0% to 30% (w/w). Since PEG2000 is a significant hydrophilic component of the carrier system, this modification also led to a reduction in entrapment efficiency, varying from 41% to 33%. In this research, when SLN was modified with PEG2000, hydrophilic channels appeared in the colloidal drug carriers, which would increase the leakage of salbutamol sulphate into the outer aqueous phase. It is also suggested that the leakage of salbutamol sulphate is positively correlated with the proportion of PEG 2000 in the carrier system.

2.3. In vitro release behavior of salbutamol sulphate from SLN

With regard to the possibility of prolonged release of SLN, the release behavior of salbutamol sulphate in SLN was investigated in a pH 7.2 phosphate buffer, in order to simulate the behavior of SLN exposed *in vivo*. Salbutamol sulfate-loaded SLN prepared in low temperature conditions were used as a sample for the release test.

Drug-release from a monostearin SLN suspension exhibited a biphasic pattern with an initial burst followed by a prolonged release over 14 days. At first, burst drug release from SLN was observed and 12.2% of loaded salbutamol sulphate was released in the first 12 h. In the next 5 days, a distinctly prolonged release was observed and nearly 2.51% of drug was released per day. Finally, prolonged release was continuously observed and the release rate was enhanced to 8.14% in each day, over a monitored period of 9 days (Fig. 2). In contrast, drug release from PEG2000-modified SLN was faster. After modification of the SLN matrix with PEG2000, e.g. when 30% (w/w) of PEG2000 was present in the organic phase, nearly 100% of the drug was released from SLN in only 5 days, following the similar biphasic pattern as with monostearin SLN.

Drug release rate was increased with a greater proportion of PEG2000 in the organic phase in preparation and showed a positive correlation. At the same time, the amount of burst release was also increased. When 30% (w/w) of PEG2000 was present in the organic phase, the drug release profile from the SLN was nearly zero order after the burst release of drug. These results demonstrate that modification with PEG2000 can accelerate release of hydrophilic drugs from SLN. Being a hydrophilic molecule, when PEG2000 in present in the lipid matrix of SLN, many hydrophilic channels are formed in the colloidal drug carrier, which would increase the release of the also hydrophilic salbutamol sulphate into the outer aqueous phase.

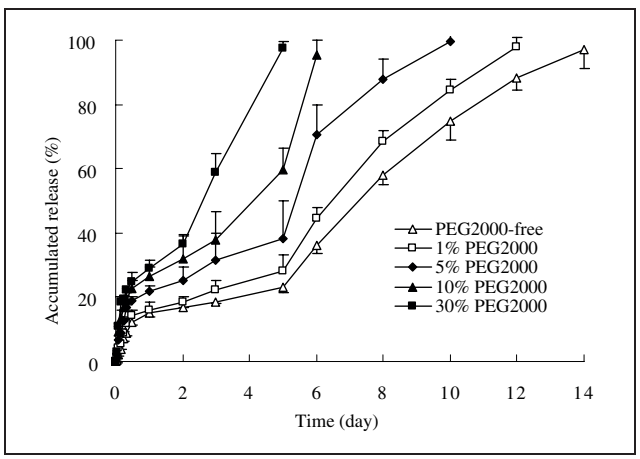

Fig. 2: Release profiles of salbutamol sulphate from SLN in pH 7.2 phosphate buffer

3. Experimental

3.1. Materials

Monostearin (Shanghai Chemical Reagent Co., Ltd, China) was used as the lipid component of SLN. Salbutamol sulphate was chosen as a model drug (Yancheng Pharmaceutical Co., Ltd, China). Polyethylene glycol (PEG 2000, Shanghai Chemical Reagent Co., Ltd, China) was used as the modifying agent for SLN. Polyvinyl alcohol (PVA 04-86, Beijing Chemicals Co., Ltd, China) was used as a dispersing agent in water phase. Ethanol, acetone and other chemicals were of analytical reagent grade.

3.2. Preparation of solid lipid nanoparticles

The weighed monostearin and weighed PEG 2000 were completely dissolved in a mixture of acetone (12 ml) and ethanol (12 ml) in a water bath at 50 °C. Then the drug solution was added (salbutamol sulphate 8.0 mg, the percentage of drug in SLN being 2% (w/w), previously dissolved in 0.5 ml distilled water) to the above organic phase. The resultant organic solution was poured into 240 ml of an aqueous phase containing 1% PVA (w/v) under mechanical agitation (DC-40, Hangzhou Electrical Engineering Instruments, China) at 400 rpm at different temperatures for 5 min. The SLN nanosuspension was quickly produced. The pH value of the SLN nanosuspension was adjusted to 1.0 with 2.0 M hydrochloric acid and the SLN were quickly produced in an aggregated state (original suspension). The entire dispersed system was then centrifuged (20,000 rpm for 30 min, SIGMA Labrorzentrifugen GmbH, Germany) to give the original precipitate. The original precipitate was re-suspended in distilled water (re-suspended suspension, lipid concentration 1.0 mg/ml) by probe-type ultrasonic treatment with 20 times (active every 1 second for a 2 second duration) in ice-bath (400W, JY92-II, Scientz Biotechnology Co., Ltd, China).

3.3. Measurement of physicochemical properties of solid lipid nanoparticles

The mean diameter of SLN was determined with a Zetasizer (3000HS, Malvern Instruments, UK) after dilution of the suspension 20 times with distilled water. The zeta potential of SLN in suspension was determined with a Zetasizer (3000 HS, Malvern Instruments, UK) after being diluted 20 times with the original dispersion medium used for preparation. For example, the measurement of the zeta potential data of SLN prepared in an aqueous system containing 1% PVA after adjusting the pH to 1.0, was performed using the prepared original SLN suspension diluted with the aqueous system of pH 1.0 containing 1% PVA. After dilution, the pH value of the system is unchanged. The zeta potential in the usual aqueous phase (pH 5.7) was determined with the re-suspended suspension, diluted with an aqueous system at pH 5.7 containing 1% PVA, at the same solid content of SLN as used for determination in acidic aqueous phase.

The original suspension was placed in an Ultrafree tube with a cutoff of 10,000 Da (Ultrafree, MC Millipore, Bedford, USA) and centrifuged for 5 min at 14,000 × g (SIGMA Labrorzentrifugen GmbH, Germany). The drug content in the filtrate was measured spectrophotometrically at 276 nm by an HPLC method (pump, Agilent G1310A; column, Hypersil ODS, 250 × 4.0mm^2, 5 μm; detector, Agilent G1314A Variable Wavelength Detector; mobile phase, 0.07 M phosphoric acid-triethylamine-methanol (85/0.4/15, v/v/v), pH value of the 0.07 M phosphoric acid-triethylamine solu-

tion adjusted to 3.7 with phosphoric acid before use; flow rate, 1.0 ml/min). The percentage drug entrapment efficiency in the SLN was calculated from Eq. (1).

$$\text{Drug entrapment efficiency} = (\text{weight of drug added to system} - \text{analysed weight of drug in ultrafiltrates}) \times 100/\text{weight of drug added to system} \quad (1)$$

3.4. In vitro release from SLN

The drug release profiles from SLN were investigated *in vitro*. The original precipitate of SLN was re-suspended with 20 ml pH 7.2 phosphate buffer (composed of 0.2 M sodium hydroxide and 0.2 M monopotassium phosphate, 3 : 1, v/v, if necessary, adjusted with 2 M hydrochloric acid or 2 M sodium hydroxide to pH 7.2) in an appropriate 50 ml glass test-tube, subjected to probe-type ultrasonic treatment with 4 times (active every 1 second for a 2 second duration) in an ice-bath (400 W, JY92-II, Scientz Biotechnology Co., Ltd., China) and then shaken horizontally (Incubator Shaker HZ-8812S, Hualida Laboratory Equipment Company, China) at 37 °C with 60 strokes per min.

Suspension (0.5 ml) was withdrawn from the system at each time interval and placed in an Ultrafree tube with a cutoff of 10,000 Da (Ultrafree, MC Millipore, Bedford, USA), then centrifuged for 5 min at 14,000 × g. The filtrate was determined using the HPLC method as described above.

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