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A novel preparation of solid lipid nanoparticles with cyclosporin A for prolonged drug release

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Solid lipid nanoparticles were prepared by a novel solvent diffusion method in an aqueous system. The lipophilic model drug cyclosporin A was incorporated into SLN to study encapsulation efficiency, zeta potential (charge) and drug delivery. Stearylamine and cyclosporin A were dissolved in ethanol and acetone and the resultant organic solution was dropped into water at 60 °C. The drug-loaded SLN suspension quickly formed with an azury color. After burst drug release with 18% of the drug over the first 12 hours, a distinctly prolonged release over a monitored period of 16 days was observed, with nearly 4% of the drug being released each day. These results demonstrate the suitability of SLN produced with the proposed method as a prolonged release formulation for lipophilic drugs.

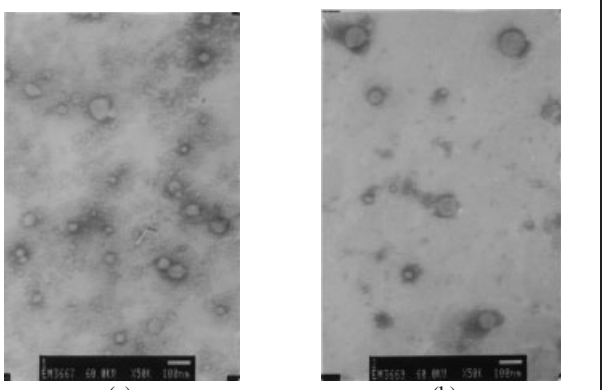
1. Introduction

In recent years, solid lipid nanoparticles (SLN) were more and more used as controlled released systems for drugs (zur Mühlen 1998a). However, there is still little data available on the mechanism of drug release, especially the burst release problem observed with these systems.
Drug release from SLN depends on the preparation method and the nanoparticles composition. In SLN produced with high pressure homogenization (Müller et al. 1996) or microemulsions (Gasco et al. 1993) on large industrial scale, the high surfactant concentration and/or the high temperature are the main reasons to induce burst release (zur Mühlen et al. 1998b). This investigation is the continuation of previous research on a novel solvent diffusion method (Hu et al. 2002). Cyclosporin A was used as a lipophilic model drug and the release behavior of the drug-loaded nanoparticles was observed.

2. Investigations, results and discussion

2.1. Preparation of cyclosporin A loaded solid lipid nanoparticles

Stearylamine was used as the lipid material to form cationic SLN for the purpose of improving cell membrane permeability. Cationic nanoparticles have the potential for gene delivery and have been shown to be less rapidly cleared from the circulation than negatively charged parti-



(a) (b)

Fig. 1: Transmission electron microscopy (TEM) of solid lipid nanoparticles with cyclosporin A by the solvent diffusion method in an aqueous system. The bar on the photograph means 100 nm. (a) Drug-free SLN prepared in aqueous phase. (b) Drug loaded SLN prepared in aqueous phase

Table 1: Size and Zeta potential of solid lipid nanoparticles by the solvent diffusion method in an aqueous system

Dosage forms	Number average (nm)			Volume average (nm)			Mean volume	Polydispersity	Zeta potential
	Area	Mean	Width	Area	Mean	Width	average (nm)		(mV)
Drug free	98.6	93.1	53.4	54.2	135.9	143.4	255.9	0.268	49.2 ± 1.6
	1.4	383.3	144.8	45.8	397.9	187.2			
Drug loading	98.2	75.3	29.3	44.3	76.8	31.3	209.8	0.534	42.3 ± 1.6
	1.8	309.6	126.3	55.7	315.5	122.3			

Table 2: Drug encapsulation efficacy (w/w) of solid lipid nanoparticles after preparation

No	Drug encapsulation efficacy (%)	Mean
1	97.47	
2	97.08	97.52
3	98.01	

cles (Heydenreich et al. 2003). The SLN were prepared by the solvent diffusion method in an aqueous system. The size of the particles exhibited bimodal distribution, with a volume mean diameter of 76.8 nm and 315.5 nm for the two parts, respectively (as shown in Table 1).

In our previous research on the preparation of SLN by solvent diffusion method, a 1% PVA aqueous solution was used as an aqueous system in order to prevent the submicrometer emulsion droplets of lipid from incorporating or coacervating, caused by the PVA absorbed around the emulsion droplets. In the present research, we only used distilled water as an aqueous system to form nanopaticles prior to the incorporation of the emulsion droplets. This may be attributed to the more hydrophobic stearylamine.

Not using a PVA substance as a dispersive vehicle, will be helpful in the separation of SLN from the suspension solution, and for isolating the solid powder of SLN by lyophilization or spray drying. Furthermore SLN can be produced on large industrial scale.

The drug encapsulation efficiency of SLN is shown in Table 2. Due to the lower solubility of cyclosporin A in water, the possibility of suspending nano-drug particles in the SLN suspension exists, which affects the drug encapsulation efficiency. The drug release data of SLN at time zero confirms that less drug forms the nano-drug particles except for the part of drug adsorbed on the surface of SLN (as shown in Fig. 2).

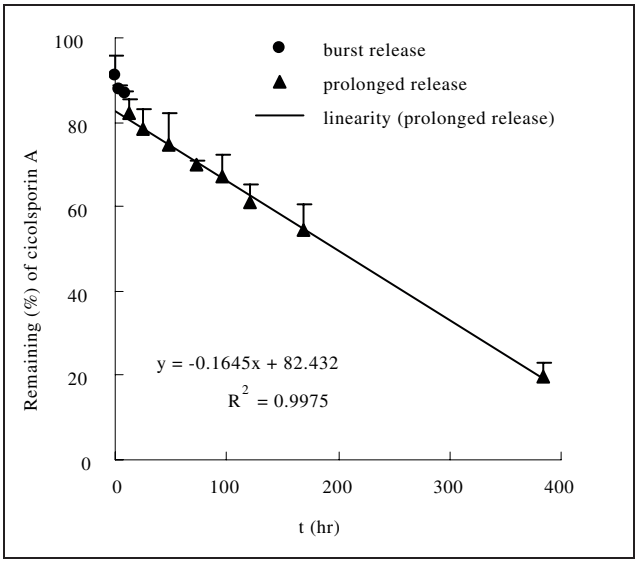


Fig. 2: Release profile of SLN in dissolution medium (composed of 0.1% SDS aqueous solution)

2.2. Drug release properties of solid lipid nanoparticles

The drug release profiles from the SLN prepared by the solvent diffusion method in an aqueous system shows a linear relationship for zero order plotting following the burst of drug (Fig. 2). The burst release was observed at the beginning of the study and released nearly 18% of the drug from the SLN. Afterwards, prolonged release was observed, resulting in a 4% release of drug from the SLN every day, as calculated from the trendline equation (1).

Remaining cyclosporin A = (theoretical weight of drug loaded in system-analysed weight of drug in filtrate) $\times$ 100/theoretical weight of drug loaded in system (1)

Due to the poor solubility of cyclosporin A in water, several drug solubilizers such as sodium dodecylsulphate (SDS) etc. were selected. The aqueous solution containing 0.10% SDS was chosen, which fulfiled sink conditions.

Compared to the usual methods of SLN production by high pressure homogenization or microemulsions, using a large amount of surfactant or drug-solubilizing surfactant, the novel solvent diffusion method in water is simple, does not need any special equipment, results in sustained release and the production can be done on a large industrial scale.

In conclusion, a novel solvent diffusion method in water was used to prepare solid lipid nanoparticles (SLN) with high drug encapsulation efficacy. The size distribution of SLN revealed a bimodal profile (average volumn diameter: 209.8 nm) and exhibited a biphasic drug release pattern with a less initial burst and prolonged release over 16 days, following the zero order.

3. Experimental

3.1. Materials

Stearylamine (Fluka Chemie) was used as the lipid material of SLN. Cyclosporin A was kindly donated by Hangzhou Huadong Pharmaceutical Co., Ltd, China. Sodium dodecylsulphate (SDS) was of chemical reagent grade. Ethanol, acetone and other chemicals were of analytical reagent grade.

3.2. Preparation of stearylamine SLN by the solvent diffusion method in aqueous system

Stearylamine (50 mg) was dissolved in ethanol (20 ml) and cyclosporin A (5 mg) was dissolved in acetone (2 ml), and then were mixed solutions. The resultant organic solution was dropped into distilled water (75 ml) under mechanical agitation with 400 rpm in a water bath at 60 °C and held for 10 min.. The drug-loaded SLN suspension was quickly produced with an azury color. After cooling to room temperature, the SLN suspension was treatment with ultrasound for 1 min (400 W) and then allowed to stand.

3.3. Measurement of physicochemical properties of solid lipid nanoparticles

The morphological examination of the SLN was performed by transmission electron microscopy (TEM) (JEM-1200EX, Japan). The samples were stained with 2% (w/v) phosphotungstic acid and placed on copper grids with films for viewing by TEM.

The mean diameter and zeta potential of SLN in suspension were determined with a Zetasizer (3000HS, Malvern Instruments, UK) after being diluted 20 times with distilled water.

Samples were placed in Ultrafree tubes with a cutoff of 10,000 Da (Ultrafree, MC Millipore, Bedford, USA) and centrifuged for 10 min at 14,000 g. Finally, the amounts of cyclosporin A in the ultrafiltrates were determined by means of a HPLC method (pump: Agilent G1310A Isopump 1100 Series; detector: Agilent G1314A VWD (Variable Wavelength Detector set at 210 nm) 1100 Series; column, Agilent ZORBA $\times$ SB-C18, 250 $\times$ 4.6 mm; mobile phase, methanol/acetonitrile/water (27 : 60 : 13 v/v/v)). The drug recovery in the SLN was calculated from eq. (2).

Drug recovery = (weight of drug added in system-analysed weight of drug in ultrafiltrates) $\times$ 100/weight of drug added in system (2)

3.4. Analysis of drug release properties of solid lipid nanoparticles

Nine milliliters standby SLN suspension were dispersed in a 50 ml glass test-tube and after addition of 30 ml dissolution medium (composed of 0.1% SDS aqueous solution), the mixture was shaken horizontally (Incubator Shaker HZ-8812S, Hualida Laboratory Equipment Company, China) at 37 °C and 60 strokes per min. One milliliter of the dispersion was with-

drawn from the system at each time interval and filtered with a 220 nm filter followed by centrifugation (20,000 rpm for 30 min). The filtrate was determined with the HPLC method as described above.

References

Gasco MR (1993) Method for producing solid lipid microspheres having a narrow size distribution, US Patent 5 250 236.

Heydenreich, AV, Westmeier R, Pedersen N, Poulsen HS, Kristensen HG, (2003) Preparation and purification of cationic solid lipid nanospheres-effects on particle size, physical stability and cell toxicity. Int J Pharm 254: 83–87.

Hu FQ, Yuan H, Zhang HH, Fang M (2002) Preparation of solid lipid nanoparticles with clobetasol propionate by a novel solvent diffusion method in aqueous system and physicochemical characterization. Int J Pharm 239: 121–128.

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

Zur Mühlen A, Mehnert W (1998b) Drug release and release mechanism of prednisolone loaded solid lipid nanoparticles. Pharmazie 53: 552–555.

Müller RH, Lucks JS (1996) Arzneistoffträger aus festen Lipidteilchen, Feste Lipidnanosphären (SLN), European Patent No. 0605497.