ELSEVIER

International Journal of Pharmaceutics 239 (2002) 121–128

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Preparation of solid lipid nanoparticles with clobetasol propionate by a novel solvent diffusion method in aqueous system and physicochemical characterization

F.Q. Hu *, H. Yuan, H.H. Zhang, M. Fang

School of Pharmaceutical Science, Zhejiang University, 353,Yanan Road, Hangzhou 310031, PR China

Received 14 April 2001; received in revised form 11 February 2002; accepted 27 February 2002

Abstract

Solid lipid nanoparticles (SLN) are a colloidal carrier system for controlled drug delivery. Monostearin SLN were prepared by a novel solvent diffusion method in an acidic aqueous system in order to improve the recovery of the method. The lipophilic model drug clobetasol propionate was incorporated to study the recovery of nanoparticles, entrapment efficacy, zeta potential (charge) and drug delivery characterization. The drug and monostearin were dissolved in acetone and ethanol at 50 °C in water bath, the resultant organic solution was poured into an acidic aqueous (pH 1.10) containing 1% polyvinyl alcohol (PVA) under mechanical agitate at room temperature. The drug loaded SLN was quickly produced with an aggregation state and easily separated by centrifugation. The recovery of nanoparticles was markedly increased compared to using a usual aqueous (pH 5.73) containing the same concentration of PVA. After burst drug release at the first 3 h, a distinctly prolonged release over a monitored period of 4 days was observed and nearly 6% drug was released in each day. Further, a novel preparation method and the optimized separation parameters in the present research for SLN were established. These results also demonstrate the principle suitability of SLN as a prolonged release formulation for lipophilic drugs.

Keywords: Solvent diffusion method in aqueous system; Solid lipid nanoparticles; Clobetasol propionate; Monostearin; Entrapment efficacy; Prolonged release

1. Introduction

Solid lipid nanoparticles (SLN) are a colloidal carrier system for controlled drug delivery and followed by the development of emulsion, liposomes, microparticles and nanoparticles based on synthetic polymers since the beginning of the nineties (Müller et al., 2000). Compared to traditional carriers the SLN combine advantages of polymeric nanoparticles and o/w fat emulsions for drug delivery administration, such as a good tolerability compared with polyester nanoparticles (Müller et al., 1996a; Maaßen et al., 1993), a high bioavailability by oral administration (Yang et al., 1999a), a targeting effect on brain (Yang et al., 1999b). Due to the production by high pressure homogenization (Müller and Lucks, 1996b) or

* Corresponding author.
E-mail address: pharmnet@cps.zju.edu.cn (F.Q. Hu).

0378-5173/02/$ - see front matter
PII: S0378-5173(02)00081-9

microemulsions (Gasco, 1993) they can be produced on large industrial scale. In recent years, the number of publications in the research working with SLN has distinctly increased.

The investigations about drug incorporation and release are an important tool in the design and evaluation of a potential drug carrier system. However, there are distinctly less data available about drug release. A major problem during the work with SLN was the burst release observed with these systems. Up to now, a prolonged drug release in vitro was only obtained when studying the incorporation of prednisolone (Mühlen et al., 1998a).

With a high temperature and also with a high surfactant concentration at a given temperature were believed the main reason to produce the burst release (Mühlen and Mehnert, 1998b). The hot homogenization technique with formulating surfactant is the mainly method on SLN preparation now. In most cases, an alternative approach via microemulsion on the production of SLN is also using a high concentration of surfactant and co-surfactant (Gasco, 1993). The lack of a prolonged release would severely limit the applicability of the system for drug delivery. Consequently, it is necessary using a novel method to explore the prolonged drug release.

The aim of this investigation was therefore, to assess if a prolonged release is basically possible, with a novel solvent diffusion method in aqueous system. This method was the first reported about the preparation of nanoparticles with synthetic polymers (Kawashima et al., 1998). Clobetasol propionate was used as a lipophilic model drug. The optimum preparation conditions (i.e. separation from acidic aqueous phase) were established and the release behavior of the drug loaded particles was also observed.

2. Materials and methods

2.1. Materials

Monostearin (Shanghai Chemical Reagent Co., Ltd, China) was used as lipid material of SLN. Clobetasol propionate was kindly donated by Hangzhou Huadong Pharmaceutical Co., Ltd, China. 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 analytical reagent grade.

2.2. Preparation of monostearin SLN by solvent diffusion method in aqueous system

The concept of the preparation method is based on the 'emulsion solvent diffusion method in water' (Kawashima et al., 1998), in which the polymer, such as DL-lactide/glycolide copolymer (PLGA), are generally dissolved in the organic phase. However, lipid could not dissolved in the organic phase completely at room temperature. On the other hand, the separation of SLN from the forming SLN suspension, such as ultracentrifugation, ultrafiltration, are difficult due to the light density of lipid or the effect of high molecular PVA. In the present study, the lipid was dissolved in the organic phase in water bath at 50 °C and used an acidic aqueous phase in order to adjust the zeta potential to form coacervation of SLN, and then easily separation by centrifugation. The weighed monostearin (396 mg) and the weighed drug (clobetasol propionate, 4 mg) were dissolved completely in a mixture of acetone (12 ml) and ethanol (12 ml) in water bath at 50 °C. The resultant organic solution was poured into 240 ml of an acidic aqueous phase containing 1% PVA (w/v) under mechanical agitate (DC-40, Hangzhou Electrical Engineering Instruments, China) with 400 rpm at room temperature for 5 min. The pH value of the acidic aqueous phase was adjusted to 1.10 by addition of 0.1 M hydrochloric acid. The SLN suspension was quickly produced. The entire dispersed system was then centrifuged (4000 rpm for 10 min, 80-2, Shanghai Surgery Instruments, China) and re-suspended in distilled water. The resultant dispersion was dried by lyophilization.

2.3. Lyophilization of SLN dispersion

The SLN dispersion (1.0% of lipid content, w/v) were fast frozen under −75 °C in a deep-freeze for 5 h in ultra low refrigeratory, (Sanyo Ultra-

Low Temperature Freezer MDF-192, Japan) and then the samples were moved to the freeze-drier (LGJ0.5-II, Academy of Martial Medicine Science Experiment Instruments, China). The drying time was controlled in 72 h and then to get the SLN powder. The recovery of SLN was defined as the weight ratio of the freeze dried SLN to the initial loading of monostearin and drug and calculated from Eq. (1).

$$\text{Recovery} = \text{analysed weight of SLN} \times 100/\text{theoretical weight of SLN} \quad (1)$$

2.4. Measurement of physicochemical properties of SLN

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 Zetasizer (3000HS, Malvern Instruments, UK) after diluted 20 times with the original dispersion medium of preparation, respectively. For example, the measurement of the zeta potential data of SLN on the condition of the aqueous system with pH 1.10 containing 1% PVA, was operated after the prepared original SLN suspension diluted with the aqueous system with pH 1.10 containing 1% PVA. After the dilution, the pH value of the system is unchanged. The 'zeta potential of drug' were determined with the original drug crystal suspension, diluted with the aqueous system at pH 5.73 containing 1% PVA or the aqueous system at pH 1.10 containing 1% PVA, at the same solid content as SLN determined sample.

The freeze dried SLN were dissolved in ethanol under water bath at 65 °C for 30 min and then cooled to room temperature to preferentially precipitate the lipid. The drug content in the supernatant after centrifugation (4000 rpm for 15 min) was measured spectrophotometrically at 240 nm by means of a HPLC method (pump, waters 515; detector, waters 486; column, Hypersil C18, 150×3.9 mm^2; mobile phase, methanol/water (74:26 v/v)). The drug recovery and content in the SLN were calculated from Eq. (2) and Eq. (3), respectively.

$$\text{Drug recovery} = \text{analysed weight of drug in SLN} \times 100/\text{theoretical weight of drug loaded in system} \quad (2)$$

$$\text{Drug content} = \text{analysed weight of drug in SLN} \times 100/\text{analysed weight of SLN} \quad (3)$$

2.5. Analysis of drug release properties of SLN

The drug release profits from SLN were investigated in vitro. Thirty milligrams of powdered SLN were dispersed in 50 ml appropriate glass test-tube and added in 30 ml dissolution medium (composed of 40% PEG 400 aqueous solution with 0.5% Tween 80 at pH 7.03), sonic treatment (Sonic Purger CQ250, Academy of Shanghai Shipping Electric Instrument) in water bath at room temperature with 10 min, and then shaken horizontally (Incubator Shaker HZ-88125, Hualida Laboratory Equipment Company, China) at 37 °C and 60 strokes per min. One milliliter of the dispersion was withdrawn from the system at each time interval and filtrated with 220 nm filter. The filtrate was determined using the HPLC method as described above.

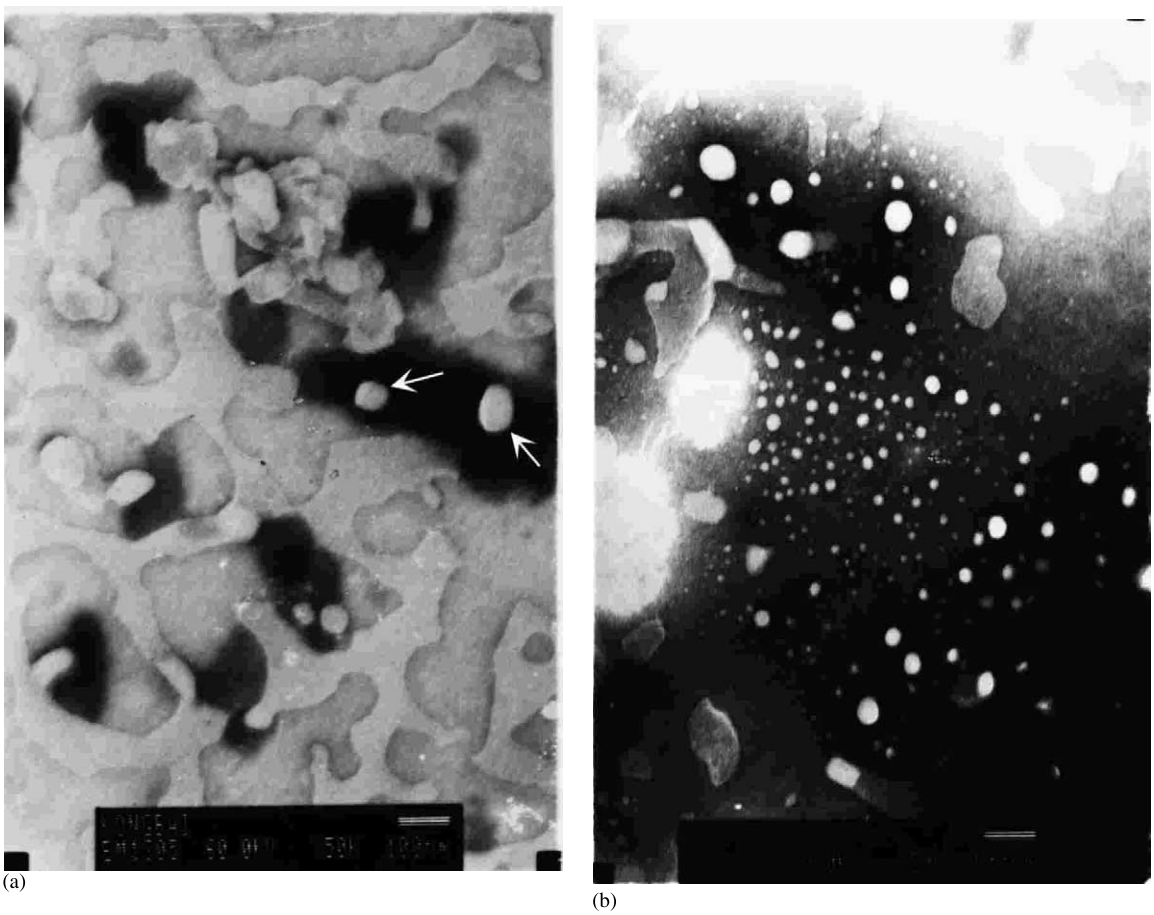
3. Results and discussions

3.1. Solvent diffusion method in an aqueous system

The TEM of SLN with clobetasol propionate prepared by the solvent diffusion method in an aqueous system is shown in Fig. 1. The particles showed a quasi-sphere or cylinder shape and exhibited bimodal particle size distribution, with a volume mean diameter of 143.3 and 432.4 nm, respectively, under the acidic condition (as shown in Table 1).

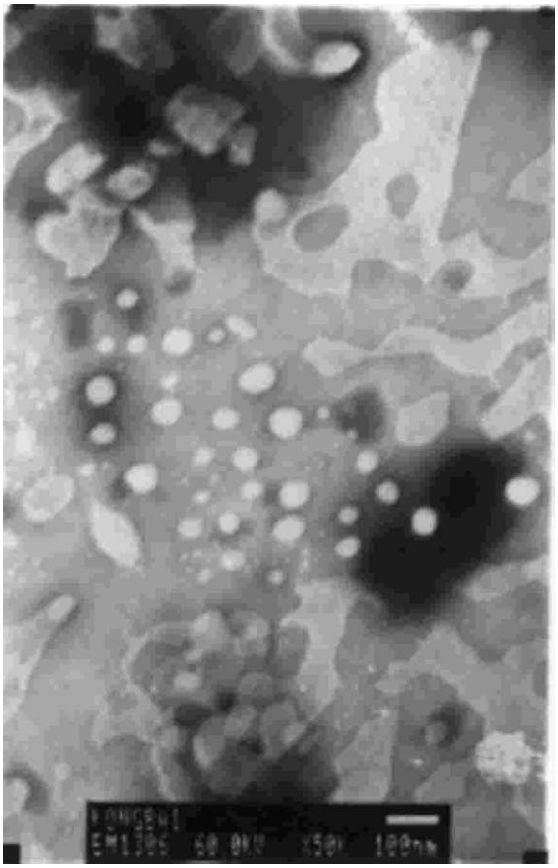
In the solvent diffusion method in an aqueous system, the diffusion rate of the water miscible organic solvent to aqueous phase was very rapid. The turbulence of the interface of the emulsion droplets occurred by the Marangoni effect (Fessi et al., 1989). At this time, the PVA molecular in aqueous phase are absorbed around the emulsion droplets, resulting in spontaneous droplet formation in the submicron range. With the reduced solubility of lipid and drug at the interface of the resultant emulsion droplets, the lipid immediately precipitate, including the drug that is present in this lipid phase.

The irregular shape of produced SLN may mainly decide by the nature property of lipid including soluble property, crystal lattice and film forming capability. As DSC measurement with the powdered SLN (data not shown), the powdered nano-drug produced by the same preparation method without using lipid, we found that the endothermic peck has been changed compare to the original drug crystal. There are two endothermic pecks existed, one is the original endothermic peck and another is a new endothermic peck, which is below the original endothermic peck. We guess that the crystal lattice of drug has been changed after preparation process. The recrystallization process may affect the shape and size distribution of SLN.

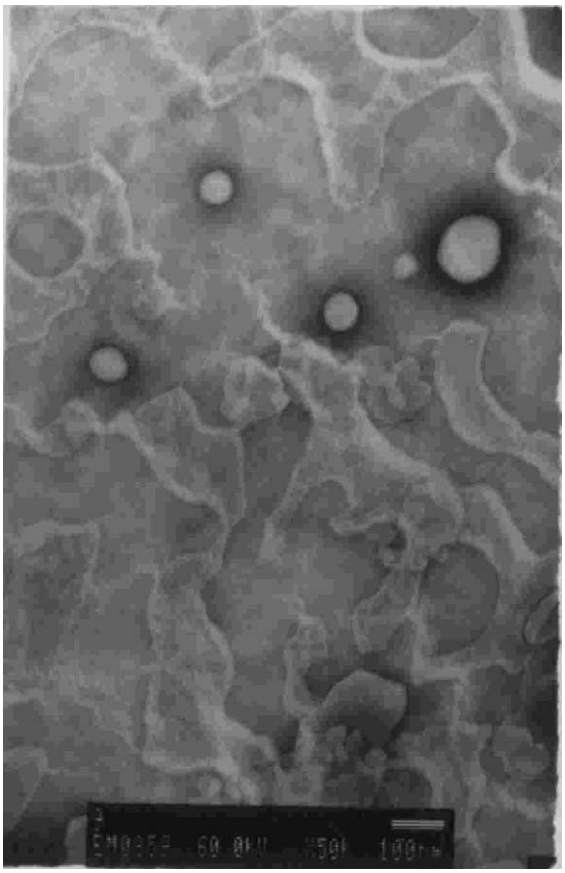


(a) (b)

Fig. 1. TEM of SLN with clobetasol propionate by the solvent diffusion method in an aqueous system. The bar on the photograph means 100 nm. (a) Drug-free SLN prepared in neutral aqueous phase. The arrow point to is nanoparticles. (b) Drug loaded SLN prepared in neutral aqueous phase. (c) Drug-free SLN prepared in acidic aqueous phase. (d) Drug loaded SLN prepared in acidic aqueous phase.



(c)



(d)

Fig. 1. (*Continued*)

Table 1
The size of SLN by the solvent diffusion method in an aqueous system, determined with Zetasizer (3000HS, Malvern Instruments, UK)

Dosage forms	Number average (nm)			Volume average (nm)				Mean volume polydispersity
	Area	Mean	Width	Area	Mean	Width	average (nm)	
Drug-free[a]	75.8	117.3	8.5	13.8	117.3	8.5	289.4	0.25
	24.2	316.1	48.0	86.2	316.8	48.8		
Drug loading[a]	97.2	43.5	22.9	11.8	46.5	25.7	312.2	0.22
	2.8	252.9	271.9	88.2	347.9	200.1		
Drug-free[b]	100.0	89.0	44.5	100.0	138.2	115.7	138.2	0.27
Drug loading[b]	92.7	116.2	69.2	20.0	143.3	89.7		
	7.3	418.4	158.8	80.0	432.4	231.5	381.1	0.22

[a] Preparation of the usual condition at pH 5.73 as comparison.
[b] Preparation of the acidic condition as pH 1.10.

In our preliminary study, we used surfactant, such as Pluronic F68, sodium cholate in the organic phase or/and aqueous phase, and found the unaltered or increased size of SLN (data not show). However, during the production of lipid particles with high pressure homogenization, surfactant is also incorporated into the lipid phase and affect the drug delivery, such as burst release possibility (Mühlen and Mehnert, 1998b). Consequently, the surfactant free formulation was adopted.

With the solvent diffusion method in an aqueous system, the extent of small SLN suspension with light density lipid was gained. Usually, the separation of nanoparticles from solvent system is using ultracentrifugation or ultrafiltration. Unfortunately, when the SLN suspension was ultracentrifuged (HITACHI 80P-T, HITACHI Instrument Ltd) at 45 000 rpm with 1 h, the SLN could not separated completely, the supernatant has more SLN inside and the recovery was very lower. This phenomena may be produced the light density of lipid or the small size of SLN. The similar result also presented the separation with ultrafiltration, when the SLN suspension was ultrafiltered (Labscale, Millipore Instruments Ltd, MW of the film is 100 000, PXB100C50, USA), the recovery was more than one hundred after freeze-dried. It means more PVA molecular also remain in the residual ultrafiltrate.

The measurement of the zeta potential allows predictions about the stability of colloidal dispersion (Komatsu et al., 1995). In general, particle aggregation is less likely to occur for charged particles with high zeta potential due to electric repulsion (Levy et al., 1994; Takamura et al., 1984). Usually, lipid particle has negative charge on the surface (Schwarz and Mehnert, 1999), using nearly neutral charge condition in the preparation system to produce the aggregation of the SLN may separate SLN from its suspension. There are more methods can be used including positive aggregative agent (e.g. aluminum chloride) and adjust the pH of system, etc. Under this acidic condition of system, there is more positive hydrogen ion in the solution and may change zeta potential of lipid particles from negative to nearly zero. The zeta potential of drug, lipid and SLN were shown in Table 2. As expectation, under the lower pH condition, the zeta potential of system is more nearly zero and produced aggregation of the SLN and then the separation of SLN from SLN suspension is easily operated by centrifugation.

Coacervation and finally precipitation of lipid only take place at the condition of the acidic dispersed aqueous medium with pH 1.10, at this time, the zeta potential value of drug load SLN is approached zero. Under the condition of a usual dispersed aqueous medium with pH 5.73, coacervation and finally precipitation of lipid did not take place.

3.2. Recoveries of SLN and encapsulation efficiency of drugs

The effect of the separation method on SLN recovery, drug recovery and drug content of SLN are shown in Table 3. The recoveries of SLN were dependent on the separation condition and were in the rank order of the acidic aqueous phase, followed by the neutral aqueous phase as expectation. These findings indicated that precipitation of SLN was almost completed in the acidic aqueous phase.

Table 2
The zeta potential of drug, lipid and solid lipid nanoparticles (SLN) in the different dispersed aqueous phage (mv)

Dispersed aqueous phage	Drug	Drug-free SLN		Drug load SLN		
	Zeta potential (mv)	Width (mv)	Zeta potential (mv)	Width (mv)	Zeta potential (mv)	Width (mv)
Usual aqueous phase (pH 5.73)	0.5	1.6	−14.8	1.6	−14.3	1.6
Acidic aqueous phase (pH 1.10)	4.7	4.1	3.8	4.4	4.3	4.1

Table 3
Effect of separation conditions on solid lipid nanoparticles (SLN) recovery, drug recovery and drug content (w/w)

Separation conditions	SLN recovery	Drug recovery	Drug content
Aqueous phase	(%)	(%)	(%)
Usual aqueous phase (pH 5.73)	6.50	6.31	0.96
	8.22	7.98	0.96
Acidic aqueous phase (pH 1.10)	94.55	92.53	0.97
	93.33	92.53	0.98

The drug recovery and drug content of SLN were also increased with the enhancement of SLN recovery at the acidic aqueous phase.

3.3. Drug release properties of SLN

The drug release profiles from the SLN prepared by the solvent diffusion method in aqueous system proved linear relationships for Higuchi plotting after the burst of drug (Fig. 2). The burst release was observed at the initial 3 h and released nearly 45% of the drug from the SLN. After that, a prolonged release was obtained and released 5.9% of drug from the SLN every day, calculating from the trend line equation in Fig. 2. As DSC measurement with the powdered SLN (data not shown), we found that the endothermic peck presented at the same temperature point as the original drug crystal. It means that the original drug crystal lattice is forming after preparation. These findings indicated that the internal structure of the SLN was a polymeric lipid matrix. The drug dispersed in the lipid matrix with a microcrystal state as the original drug crystal lattice. As the huge surface of nanoparticles and some drug adsorbed the surface of nanoparticles or precipitated in the superficial lipid matrix, the dissolution profile of the SLN exhibited a burst of drug during the initial stage. During the later stage, drug release was continuous and slow, indicating that the drug release rate was determined by the diffusion of drug from the rigid matrix structure. With the definite percentages of burst followed by prolonged release, the burst can be exploited to deliver an initial dose when desired.

After sonic treatment, the size data of the re-dispersed SLN suspension was determined. The value of number average is 443.5 nm (width with 221.4 nm) and the value of volume average is 476.9 nm (width with 234.4 nm). It means that not so mush aggregated particle is existed in the released medium and the prolonged release may be decided by the structure of SLN.

The drug loading capacity may affect the delivery property of SLN. When the drug loading capacity was increased 10%, a similar result of burst followed by prolonged release also was obtained. The difference is the extent of burst release and nearly 70% dosage was observed with the SLN of high loading capacity produced by the solvent diffusion method in aqueous system (data not shown).

A major problem of the SLN production with the high pressure homogenization or the microemulsions is the burst release and only less research mentioned the sustained release of SLN under using no surfactant or no drug-solubilizing surfactant with the cold homogenization technique (Mühlen and Mehnert, 1998b). It may limit the drug solubility in the system and decrease the

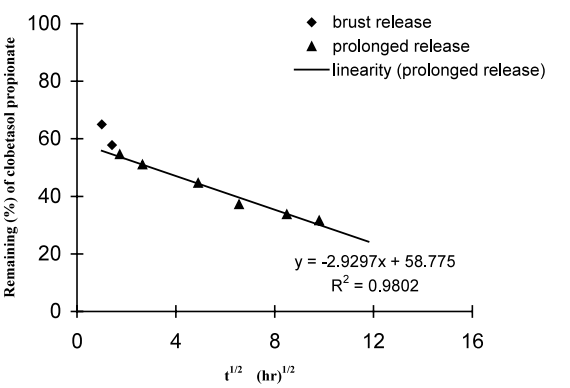


Fig. 2. Release profiles of SLN in dissolution medium (composed of 40% PEG 400 aqueous solution with 0.5% Tween 80).

loading capacity of drug. With a novel solvent diffusion method in aqueous system, the SLN can be obtained under mild conditions according to one step, being very easy, do not require any special equipment and also can obtain the sustained release.

4. Conclusion

A novel solvent diffusion method in aqueous system was demonstrated to prepare the SLN with the highly SLN recovery and drug recovery after the selection of the optimal separated condition. The size distribution of SLN revealed a bimodal profile (average diameter: nm) and exhibited a biphasic drug release pattern with an initial burst and prolonged release over 4 days, following the Higuchi rule. The dissolution properties were characterized by a microcrystal matrix structure.

Acknowledgements

A part of the present study was supported from the Pharmaceutical Engineering of Gifu Pharmaceutical University, Japan.

References

Fessi, H., Puisieux, F., Devissaguet, J.P., Ammoury, N., Bentia, S., 1989. Nanocapsule formation by interfacial polymer deposition following solvent displacement. Int. J. Pharm. 55, R1–R4.

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

Kawashima, Y., Yamamoto, H., Takeuchi, H., Hino, T., Niwa, T., 1998. Properties of a peptide containing DL-lactide/glycolide copolymer nanospheres prepared by novel emulsion solvent diffusion methods. Eur. J. Pharm. Biopharm. 45, 41–48.

Komatsu, H., Kitajima, A., Okada, S., 1995. Pharmaceutical characterization of commercially available intravenous fat emulsions: estimation of average particle size, size distribution and surface potential using photon correlation spectroscopy. Chem. Pharm. Bull. 43, 1412–1415.

Levy, M.Y., Schutze, W., Fuhrer, C., Benita, S., 1994. Characterization of diazepam submicron emulsion interface: role of oleic acid. J. Microencapsul. 11, 79–92.

Maaßen, S., Schwarz, C., Mehnert, W., Lucks, J.S., Yunis-Specht, F., Müller, B.W., Müller, R.H., 1993. Comparison of cytotoxicity between polyester nanoparticles and solid lipid nanoparticles (SLN). Proc. Int. Symp. Control. Release Bioact. Mater. 20, 490–491.

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

Mühlen, A.Z., Mehnert, W., 1998b. Drug release and release mechanism of prednisolone loaded solid lipid nanoparticles. Pharmazie 53, 552.

Müller, R.H., Maaßen, S., Weyhers, H., Specht, F., Lucks, J.S., 1996a. Cytotoxicity of magnetite loaded polylactide, polylactide/glycolide particles and solid lipid nanoparticles (SLN). Int. J. Pharm. 138, 85–94.

Müller, R.H., Lucks, J.S., 1996b. Arzneistoffträger aus festen Lipidteilchen, Feste Lipidnanosphären (SLN), European Patent No. 0605497.

Müller, R.H., Mäder, K., Gohla, S., 2000. Solid lipid nanoparticles (SLN) for controlled drug delivery-a review of the state of the art. Eur. J. Pharm. Biopharm. 50, 161–177.

Schwarz, C., Mehnert, W., 1999. Solid lipid nanoparticles (SLN) for controlled drug delivery. II. Drug incorporation and physicochemical characterization. J. Microencapsul. 16, 205–213.

Takamura, A., Ishii, F., Noro, S., Tanifuji, M., Nakajima, S., 1984. Study of intravenous hyperalimentation: effect of selected amino acids on the stability of intravenous fat emulsions. J. Pharm. Sci. 73, 91–94.

Yang, S.C., Zhu, J.B., Lu, Y., Liang, B.W., Yang, C.Z., 1999a. Body distribution of camptothecin solid lipid nanoparticles after oral administration. Pharm. Res. 16, 751–757.

Yang, S.C., Lu, L.F., Cai, Y., Zhu, J.B., Liang, B.W., Yang, C.Z., 1999b. Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain. J. Control. Release 59, 299–307.