Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 350 (2008) 130–137
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Disodium norcantharidate loaded poly(ε-caprolactone) microspheres I. Preparation and evaluation

Shaobing Wang, Shengrong Guo *, Liang Cheng

School of Pharmacy, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, China

Received 27 April 2007; received in revised form 17 May 2007; accepted 22 August 2007
Available online 25 August 2007

Abstract

Poly(ε-caprolactone) (PCL) microspheres encapsulating disodium norcantharidate (DSNC), a drug in salt form and with high water solubility, were prepared by s/o/w solvent evaporation technique and characterized in terms of size, morphology, encapsulation efficiency and drug release. The viscosity of s/o dispersion was crucial to the successful encapsulation of DSNC. Scanning electron microscopy (SEM) studies showed that the drug-loaded microspheres had coarse surface and porous internal structure. The analysis of X-ray diffraction (XRD) indicated that there was no interaction between DSNC and PCL, but the degree of crystallinity of PCL decreased with the introduction of the drug. The drug release profiles indicated an initial burst release followed by a slow release, and a further investigation into the release mechanism implied that the release of DSNC from PCL microspheres was caused by a combination of diffusion and osmotic pressure.

Keywords: Poly(ε-caprolactone); Microspheres; Disodium norcantharidate; Water solubility; Solvent evaporation

1. Introduction

Disodium norcantharidate (DSNC, Fig. 1), a demethylated analogue of cantharidin isolated from the dried body of blister beetle, is a potent anti-cancer drug against primary hepatic carcinoma, breast cancer and abdominal cancer (Wang, 1989). Despite the increased antihepatoma activity, its toxicity to the gastrointestinal and urinary tracts is not eliminated completely (Tagwireyi et al., 2000). Additionally, DSNC has a short biological half-life of 2–4 h and is eliminated rapidly after oral or intravenous administration. Microencapsulation with biodegradable polymer can provide a feasibility to overcome these side-effects and demerits by controlling drug release (Freiberg and Zhu, 2004), however, few investigations on the microencapsulation of DSNC were reported.

Solvent evaporation technique is one of the most popular ways to accomplish microencapsulation (Bodmeier and McGinity, 1987, 1988; Alex and Bodmeier, 1990; O'Donnell and McGinity, 1997; Viswanathan et al., 1999; Lee et al., 2000; Carrasquillo et al., 2001). As usual, the desired polymer is dissolved in a suitable organic solvent, and the drug can be dissolved, dispersed or emulsified into the polymer solution, which is then emulsified in an aqueous or oil phase containing emulsifier. As the solvent evaporation occurs, the microspheres harden and can be harvested by suitable filtration and drying. Selection of microencapsulation methods is primarily determined by the solubility of the drug and the polymer (Bodmeier and McGinity, 1988; Herrmann and Bodmeier, 1998). The w/o/w and s/o/w solvent evaporation techniques are commonly used to encapsulate water-soluble drugs (Takada et al., 1997; Pérez et al., 2000; Lai and Tsiang, 2005). Compared to w/o/w-technique, s/o/w-technique possesses two major advantages: the first, s/o/w-technique need not prepare w/o primary emulsion, whose stability is a prerequisite for the successful stabilization of a multiple emulsion and the high loading of drug within the solid microparticles (Nihant et al., 1994); the second, the drug in solid state requires a dissolution step prior to the diffusion into continuous phase, thus allowing higher encapsulation efficiency (Lamprecht et al., 2000).

Poly(ε-caprolactone) (PCL) is one of the biocompatible and biodegradable polyester polymers and does not generate an acid environment unlike the polylactide or polyglycolide polymers,

* Corresponding author. Tel.: +86 21 3420 4793; fax: +86 21 3420 4793.
E-mail address: srguo@sjtu.edu.cn (S. Guo).

0378-5173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2007.08.030

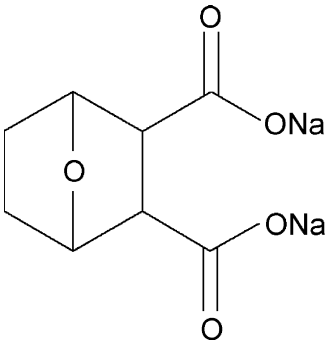

Fig. 1. Chemical structure of disodium norcantharidate.

besides, it possesses high hydrophobicity, non-toxicity and high permeability to low molecular weight drugs (Pitt, 1990). Therefore, many investigations have focused on its application for controlled delivery of various drugs (Kim and Lee, 2001; Lin and Huang, 2001; Dhanaraju et al., 2003; Sinha et al., 2004; Lamprecht et al., 2000; Pérez et al., 2000).

The objectives of this study are first to encapsulate DSNC into PCL microspheres by s/o/w solvent evaporation technique and secondly to characterize the microspheres in terms of particle size, morphology, encapsulation efficiency and drug release.

2. Materials and methods

2.1. Materials

Disodium norcantharidate was purchased from Ange Pharmaceutical Company (Nanjing, China). Poly(ε-caprolactone) (MW 50,000) was supplied by Daicel Polymer Ltd. (Minatoku, Tokyo, Japan). Polyvinylalcohol (PVA, 88% hydrolyzed) was provided by Weicheng Chemical Industry Co. Ltd. (Shanghai, China) as emulsifier. Tween 80 and dichloromethane were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

2.2. Preparation of microspheres

DSNC-loaded PCL microspheres were prepared by a modified s/o/w solvent evaporation technique (Lamprecht et al., 2000). The drug was micronized with a planetary ball mill (Pulverisette 6, Fritsch, Germany). A different amount of micronized drug (50–250 mg) was dispersed in 3 ml of dichloromethane containing PCL (150–750 mg). This s/o dispersion was added dropwise into 40 ml of 1% (w/v) PVA aqueous solution. The resulting emulsion was stirred with a double-bladed propeller continuously for 40 min under ambient pressure, and then stirred for another 20 min under reduced pressure (20 Pa). Finally, the microspheres were collected by filtration, washed with deionized water and dried in a vacuum desiccator at room temperature.

The blank PCL microspheres were prepared by simple o/w solvent evaporation method, and the variables were kept constant during the preparation.

2.3. Particle size analysis

Particle size of microspheres was measured by a particle size analyzer (CIS 100, Ankersmid, Netherlands). For the analysis, the sample was prepared by suspending 50 mg of microspheres in 5 ml of 0.2 μm filtered distilled water containing 2% (w/v) Tween 80 and then sonicating in a water bath for 3 min to prevent aggregation between microspheres. The particle size was expressed as the volume mean diameter in micrometer.

2.4. Morphology of microspheres

The surface and internal morphologies of microspheres were observed with field emission scanning electron microscope (SEM) (JSM-7401F, JEOL, Japan). Dried microspheres were mounted onto stubs using double-sided adhesive tape with conductive effect and analyzed with SEM. To reveal the internal structure, the stuck microspheres were cross-sectioned with razor blade prior to the observation.

2.5. X-ray diffraction

X-ray diffraction patterns were obtained using an X-ray diffractometer (D/max 2200, Rigaku, Japan). The samples were placed in a steel holder and scanned over a 2θ range of 0–40° at the rate of 6° min^{-1}. The degree of crystallinity (X_C %) was evaluated from the following equation (Gonzalez et al., 1999):

$$X_C\% = \frac{I_T - I_A}{I_T} \times 100\% \quad (1)$$

where I_T and I_A are the intensities of the whole sample and the amorphous phase (area under the curve), respectively.

2.6. HPLC method analysis

The drug content of DSNC-loaded PCL microspheres was determined by high performance liquid chromatography (HPLC), equipped with a variable wavelength detector (SPD-10AD*VP*, Shimadzu, Japan). To extract DSNC from the microspheres, 20 mg of microspheres were dissolved in 1.2 ml of acetonitrile, and then 8.8 ml of purified water was added to precipitate the polymer matrix. The resulting solution was centrifuged for 10 min at 10,000 rpm and the supernatant was collected for HPLC analysis. HPLC analysis was carried out under the follow condition: Diamonsil™ C_{18} column (250 mm × 4.6 mm, 5 μm, Dikma Technologies, Beijing, China), wavelength fixed at 208 nm and mobile phase composed of acetonitrile and water (12:88, pH 3.1, adjusted with phosphoric acid). The linearity of the response was verified over the concentration range of 20–640 μg/ml ($r^2 = 0.999$). According to the determination of DSNC in microspheres, drug loading and entrapment efficiency were calculated as follows:

$$\text{Drug loading} = \frac{\text{Mass of drug in microspheres}}{\text{Mass of microspheres}} \times 100\% \quad (2)$$

$$\text{Encapsulation efficiency} = \frac{\text{Actual drug loading}}{\text{Theoretical drug loading}} \times 100\% \quad (3)$$

2.7. In vitro release behavior

Thirty milligram of microspheres were placed in 3 ml of 0.05 M phosphate buffer (PB) adjusted to pH 7.4 and incubated in a horizontal-shaker at 37 °C. At predetermined intervals, microspheres were centrifuged for 2 min at 500 rpm, then 0.1 ml of supernatant was extracted and 0.1 ml of fresh buffer was added. The extracted supernatant was diluted with 0.9 ml of mobile phase for HPLC analysis.

3. Results and discussion

3.1. Preparation of microspheres

In this study, the s/o/w solvent evaporation technique was selected to encapsulate DSNC, a highly water-soluble and low molecular weight drug. First, PCL polymer was dissolved in 3 ml of dichloromethane, and then the micronized drug was dispersed in the polymer solution. This s/o dispersion was added dropwise into 40 ml of 0.1% PVA aqueous solution, and the resulting mixture was stirred with a double-bladed propeller at 20 °C. Since the residual dichloromethane easily led to the agglomeration of the microspheres, reduced pressure was adopted at the final stage of stirring to ensure complete evaporation of dichloromethane. After the removal of dichloromethane, the microspheres were collected by filtration, washed with deionized water (three times and 10 ml per time) and dried in a vacuum desiccator at room temperature.

3.2. Encapsulation efficiency and particle size

Among several parameters of the formulation, the polymer concentration played an important role in the encapsulation of DSNC. As shown in Table 1 (B1–B5), at a constant feed ratio of PCL to DSNC and the fixed stirring rate, the encapsulation efficiency of DSNC increased with the increasing concentration of the polymer. The increase in entrapment efficiency was mainly attributed to the increased viscosity of s/o dispersion, which prevented the leakage of drug into the aqueous phase during the hardening stage. Fig. 2 shows that the viscosity of s/o dispersion increased with the increasing concentration of the polymer, and the more viscous the s/o dispersion was, the higher entrapment efficiency was achieved. This result is in agreement with other studies (Takada et al., 1997; Yang et al., 2000). The increased polymer concentration can accelerate the solidification of the phase-separated polymer domain, which answers for the increase in particle size as well as the decrease in drug loss (Kim et al., 2006). In addition, the increasing amount of drug addition led to an increase in encapsulation efficiency (Table 1, B4, B8 and B9). The probable reason for the increase in encapsulation efficiency is that the polymer was not overloaded with the amount of the drug (Benoit et al., 1999). Finally, the effect of stirring rate was taken into account. Higher stirring rate led to smaller mean diameter and lower encapsulation efficiency (Table 1, B6–B8), which was due to the fact that higher stirring rate resulted into smaller emulsion droplets, facilitating the leakage of drug into the water phase (Sansdrap and Moës, 1993; Yang et al., 2000).

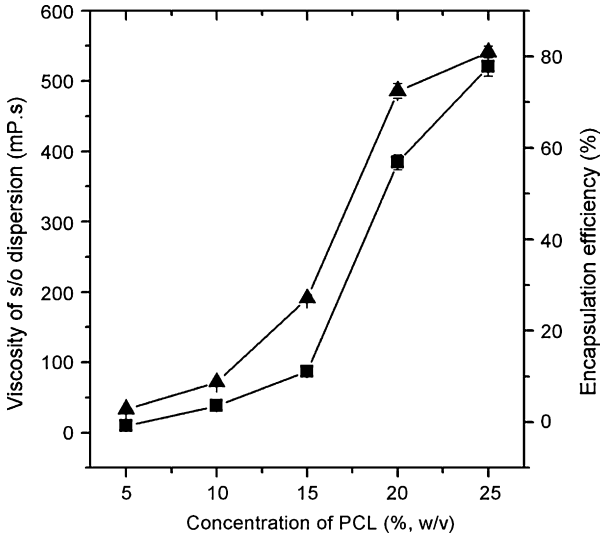

Fig. 2. Effect of polymer concentration on the viscosity of s/o dispersion and corresponding encapsulation efficiency of DSNC-loaded PCL microspheres: (▲) viscosity of s/o dispersion; (■) encapsulation efficiency.

Table 1
Particle size, drug loading and entrapment efficiency of DSNC-loaded PCL microspheres obtained under various preparation conditions ($n = 3$)

Batch	C_{PCL} (%)	W_{DSNC} (mg)	Stirring (rpm)	Particle size (μm)	D.L. (% ± S.D.)	E.E. (% ± S.D.)
B1	5	50	1000	39.2 ± 1.6	0.70 ± 0.03	2.80 ± 0.12
B2	10	100	1000	67.7 ± 2.1	2.17 ± 0.11	8.68 ± 0.44
B3	15	150	1000	122.3 ± 3.1	6.76 ± 0.18	27.04 ± 0.72
B4	20	200	1000	178.7 ± 4.8	18.11 ± 0.40	72.44 ± 1.60
B5	25	250	1000	229.4 ± 4.2	20.23 ± 0.32	80.92 ± 1.28
B6	20	100	800	257.5 ± 5.7	7.87 ± 0.19	55.09 ± 1.33
B7	20	100	1200	143.4 ± 3.4	7.12 ± 0.21	49.84 ± 1.47
B8	20	100	1000	172.9 ± 3.9	7.63 ± 0.24	53.41 ± 1.68
B9	20	300	1000	181.6 ± 4.4	25.25 ± 0.67	75.75 ± 2.01

C_{PCL}, concentration of PCL in organic phase; W_{DSNC}, weight of the drug dispersed in polymer solution; D.L., drug loading of the microspheres; E.E., encapsulation efficiency of DSNC. The volumes of organic phase and water phase were 3 and 40 ml, respectively.

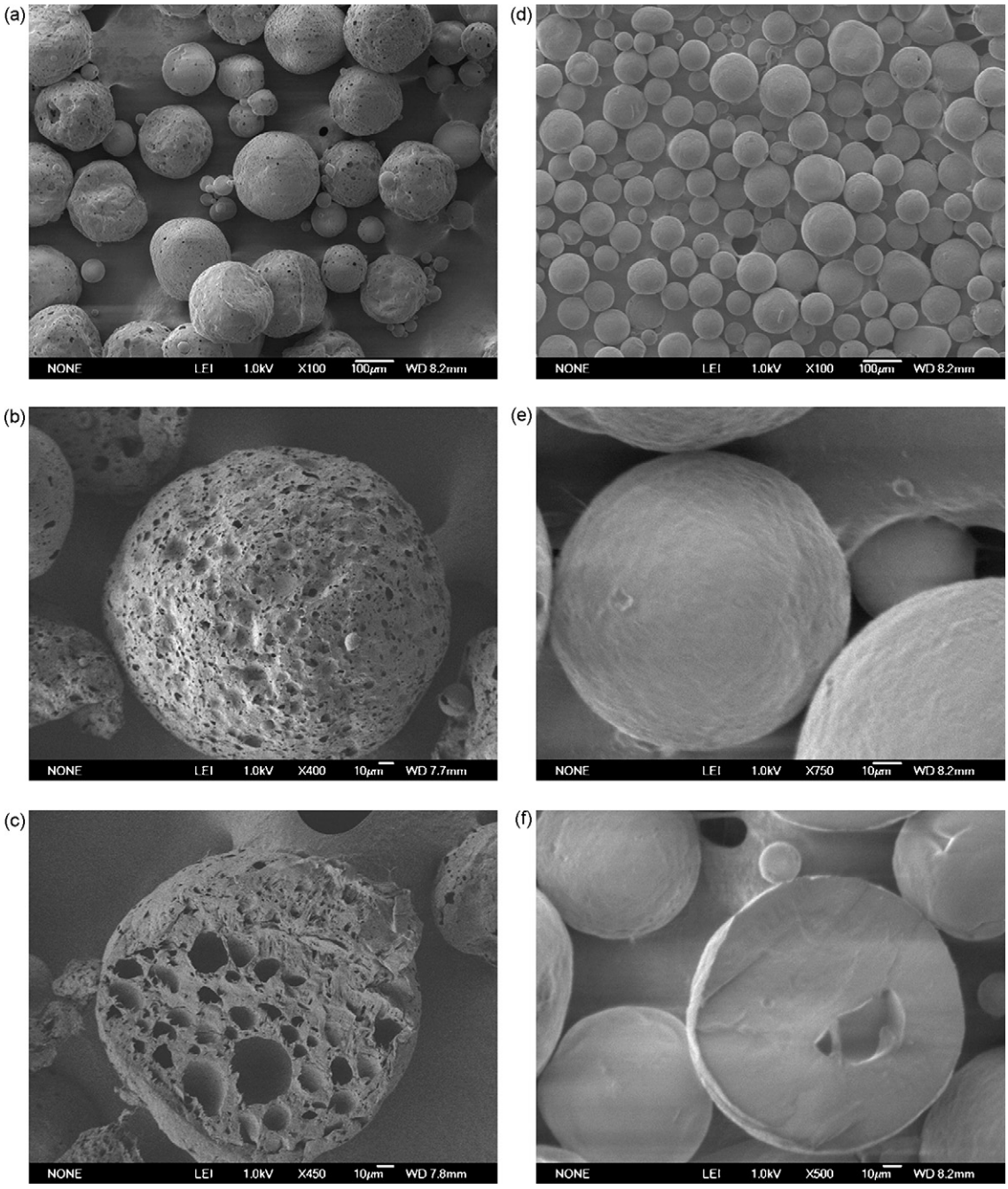

Fig. 3. Scanning electron microscopic photographs of DSNC-loaded (a, b and c) and blank (d, e and f) PCL microspheres prepared by the s/o/w solvent evaporation technique: (a and d) external morphology; (b and e) surface; (c and f) internal morphology.

3.3. Morphology of microspheres

The surface morphology and internal structure of microspheres were observed by SEM. The drug-loaded microspheres appeared spherical with diameters in the range of 39–258 μm (Fig. 3a), and were easily found to be coarse and porous at a high magnification (Fig. 3b). The coarseness and porosity was commonly considered as a result of solvent removal during the terminal stage of the formulation (Dash, 1997). However, the blank PCL microspheres prepared under the same conditions revealed the smooth surface without pores (Fig. 3d–f). Thus, the coarseness and porosity should not be caused by the preparation procedure itself, including the operation of reducing pressure. Much to our surprise, the cross-section of DSNC loaded microspheres showed highly porous internal structure (Fig. 3c), which was quite different from other microspheres also prepared by s/o/w-technique (Takada et al., 1997; Lamprecht et al., 2000), especially, in the investigation conducted by Lamprecht and his team, PCL was also chosen to encapsulate drug. The marked differences in morphology were probably due to the differences in physicochemical characteristics of the model drugs, because the drug in salt form not only can generate osmotic pressure,

but also easily leads to a phase separation phenomenon during the polymer coacervation (Weidenauer et al., 2003). In order to confirm this explanation, an attempt to encapsulate varied drugs or compounds was made under the same conditions. When NaCl was encapsulated, the microspheres showed coarse surface and porous structure, conversely, the microspheres revealed smooth surface and non-porous structure when encapsulating 5-fluorouracil or other model drugs in non-salt form, including norcantharidin, the acid anhydride form of DSNC (photographs not shown here). These results supported the explanation that the coarseness and porosity was a result of the salt form of the model drug.

3.4. X-ray diffraction analysis

X-ray diffractometry is widely used for the characterization and evaluation of microspheres (Gonzalez et al., 1999; Dash et al., 2002). Fig. 4 shows the XRD spectra of pure drug, PCL and the drug-loaded microspheres. As for the XRD of PCL, it displayed two characteristic peaks in the range of 20–25°, confirming the semi-crystalline structure of PCL polymer (Pérez et al., 2000). The characteristic peak of DSNC was observed in the diffraction pattern of the drug-loaded microspheres, which indicated that DSNC retained its original crystallinity during the preparation of the microspheres. Since DSNC is hardly dissolved in dichloromethane, crystalline fragments of the drug were incorporated in the final microspheres in the same state as initially dispersed in the polymer solution.

PCL maintained its semi-crystalline characteristics throughout the preparation, however, the degree of crystallinity of the polymer varied with the amount of the drug. As shown in Fig. 5, the drug-free microspheres had a crystallinity value of 45.3%, but the value decreased with the introduction of DSNC, and the more amount of the drug was added, the lower crystallinity value was obtained. The result was probably due to the fact that the presence of DSNC was unfavorable for the recrystallization of PCL polymer. Besides, there was no interaction between DSNC and PCL, because the diffraction pattern of the microspheres was only the summation of diffraction patterns of the individual constituents.

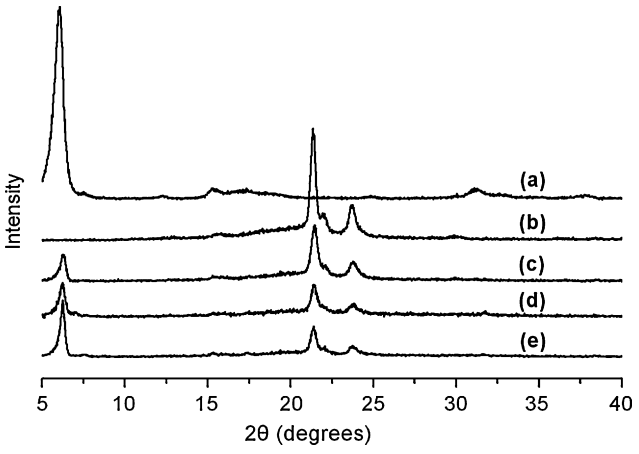

Fig. 4. X-ray diffraction patterns of: (a) DSNC; (b) PCL; (c–e) microspheres with drug loading of 7.63, 18.11 and 25.25%, respectively.

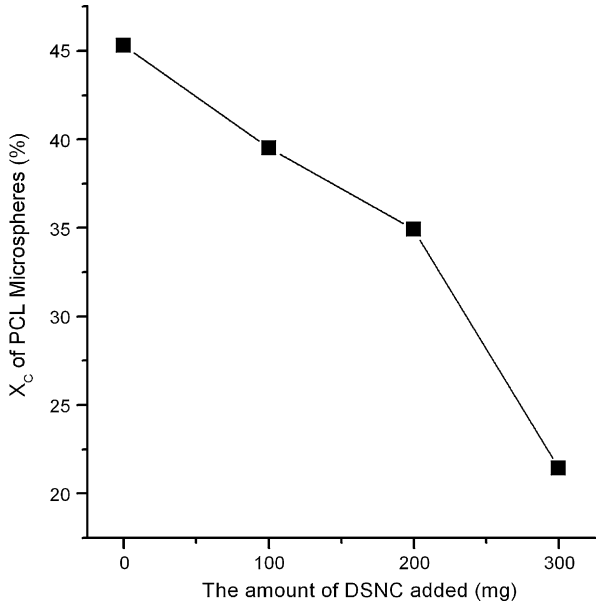

Fig. 5. Influence of the amount of DSNC added on the degree of crystallinity (X_C) of PCL microspheres (the amount of PCL was fixed at 600 mg).

3.5. In vitro release behavior

In vitro release profiles of DSNC from the microspheres of different drug loading in phosphate buffer (0.05 M, pH 7.4) were shown in Fig. 6. Regardless of drug loading, the release profiles exhibited a rapid release phase followed a slow release phase. 65–83% of the entrapped drug was released within 8 h. The rapid release was probably contributed to two aspects: one is the high water-solubility of DSNC (347 mg/ml), in favor of its rapid migration to the dissolution medium; the other is the coarseness

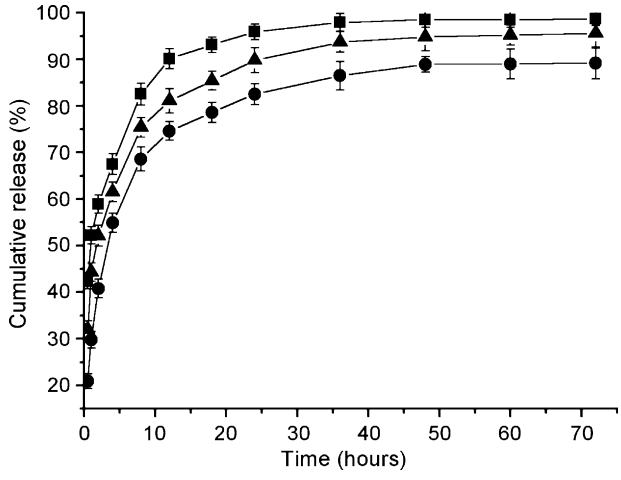

Fig. 6. In vitro release profiles of NCDS from the microspheres of different drug loading in phosphate buffer (0.05 M, pH 7.4) at 37 °C (●, 7.63%; ▲, 18.11%; ■, 25.25%; $n = 3$).

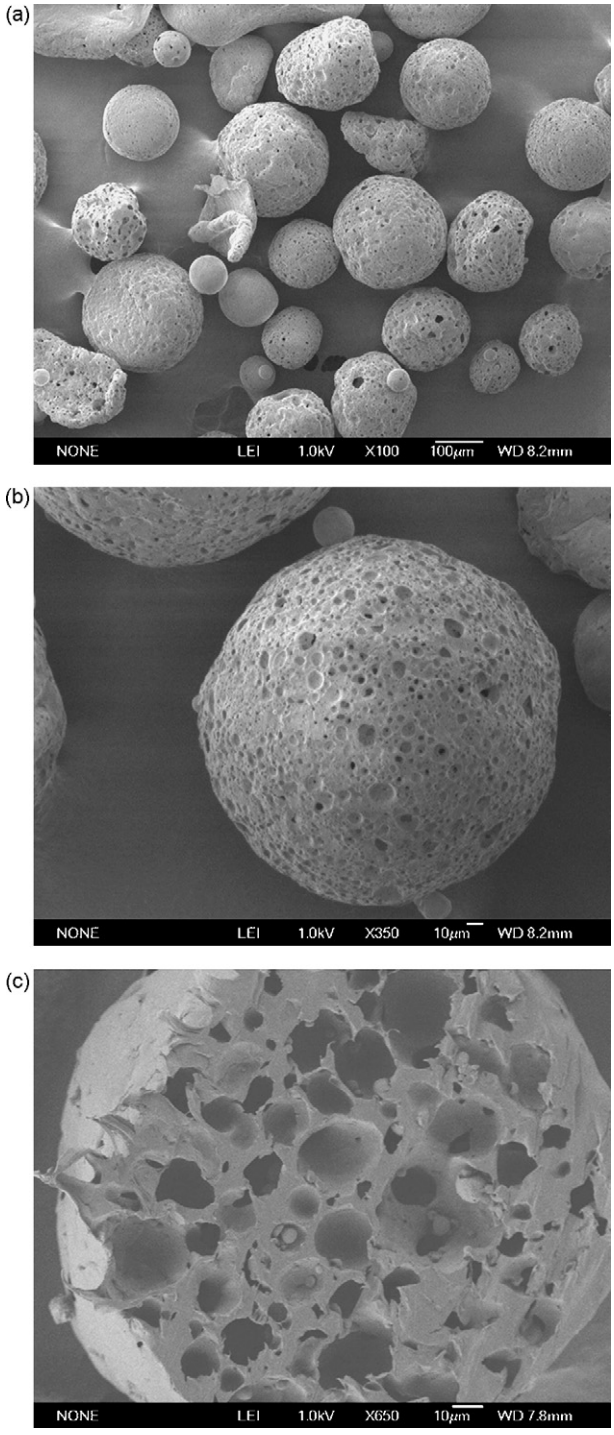

Fig. 7. Morphologies of DSNC-loaded PCL microspheres after 72 h release for: (a) external morphology; (b) surface; (c) internal morphology.

and porosity of the microspheres, which facilitates the diffusion of the water-soluble drug. Moreover, the morphologies of microspheres after release (72 h) were almost alike to those before release (Fig. 7). The insignificant differences in morphology between before and after release can be attributed to the rapid release of DSNC as well as the slow erosion or degradation of PCL polymer.

Table 2
Coefficients and exponents of the drug release from PCL microspheres with various drug loading

Drug loading (%)	Coefficient constant (k)	Diffusional exponent (n)	Correlation coefficient (r^2)
7.63	0.2926	0.4311	0.9935
18.11	0.4151	0.3062	0.9668
25.25	0.5041	0.2200	0.9848

3.6. Kinetics of drug release

Since PCL possesses semi-crystallinity, slow degradation and high permeability to low molecular weight drug (Pitt, 1990), drug release from PCL microparticles was commonly considered as a result of drug diffusion, not of the degradation of the polymer (Ha et al., 1997; Pérez et al., 2000). In order to examine the release mechanism of DSNC from PCL microspheres, the release data (≤70%) were fitted to the following power law equation (Ritger and Peppas, 1987):

$$\frac{M_t}{M_\infty} = kt^n \tag{4}$$

where M_t/M_∞ is the fraction of drug released at time t, k a coefficient constant, and n is the diffusional exponent indicative of release mechanism. With regard to non-swellable microspheres, the kinetics of drug release indicates Fickian diffusion, non-Fickian diffusion and zero-order transport when n is 0.43, 0.43∼1 and 1, respectively. The results were summarized in Table 2, and the n value decreases with the increasing loading level. So far as the microspheres of low drug loading (7.63%) was concerned, the corresponding release data fit Fickian diffusion, however, the release kinetics for the microspheres of higher drug loading was appropriate to neither Fickian nor non-Fickian diffusion. The above result implied that the release mechanism of DSNC from PCL microspheres was not caused by pure diffusion.

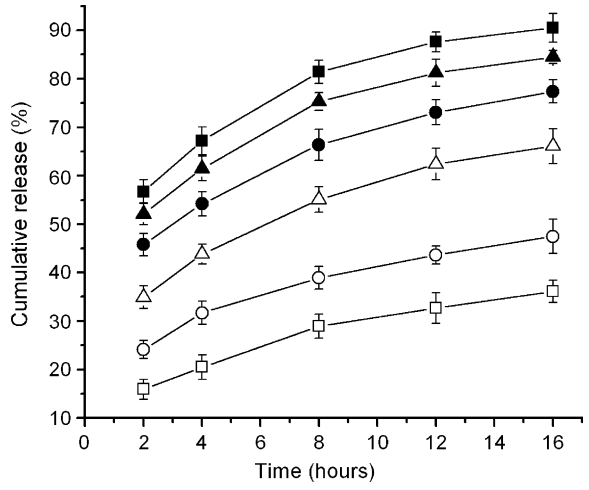

Fig. 8. Release profiles of DSNC from PCL microspheres in phosphate buffers (pH 7.4) of different concentrations (■, 0.01 M; ▲, 0.05 M; ●, 0.2 M) and in 0.05 M phosphate buffer containing different amounts of NaCl (△, 4%; ◯, 8%; □, 12%), $n = 3$.

In order to investigate the release mechanism of DSNC, the effect of osmotic pressure was taken into consideration, because drug salt distributed in the porous polymeric matrix can generate high osmotic pressure, thus influencing the release profiles (Weidenauer et al., 2003). The effect of osmotic pressure on the drug release was testified by studying the release profiles in the solutions of different phosphate concentrations and different ionic strengths. As shown in Fig. 8, the release of DSNC markedly slowed with the increasing concentration of phosphate or NaCl. In other words, the drug release from PCL microspheres decreased with the increasing ionic strength of the buffer, which is consistent with the investigations conducted by Lemmouchi and Schacht (1997). The results implied that internal osmotic pressure generated by DSNC played a significant role in the rapid release phase.

As the drug release proceeded, the drug content decreased and the contribution of osmotic pressure gradually diminished. So the slow release phase can be considered to represent the drug release by diffusion through the polymer matrix.

4. Conclusion

This study demonstrates that it is feasible to encapsulate drug salt in the polymeric microspheres employing the s/o/w solvent evaporation technique. Interestingly, the salt form of the drug was responsible for the porosity of the microspheres as well as the inner osmotic pressure. The drug release profiles can be divided into a rapid release phase and a slow release phase. During the rapid release phase, the release of DSNC is caused by a combination of diffusion and osmotic pressure, whereas during the slow release phase, the drug release was mainly governed by diffusion.

An investigation into modifying both the microspheres morphology and the drug release has been carried out, and the results will be discussed in a forthcoming paper.

References

Alex, R., Bodmeier, R., 1990. Encapsulation of water-soluble drugs by a modified solvent evaporation method. I. Effect of process and formulation variables on drug entrapment. J. Microencapsul. 7, 347–355.

Benoit, M.-A., Baras, B., Gillard, J., 1999. Preparation and characterization of protein-loaded poly(ε-caprolactone) microparticles for oral vaccine delivery. Int. J. Pharm. 184, 73–84.

Bodmeier, R., McGinity, J.W., 1987. The preparation and evaluation of drug-containing poly(dl-lactide) microspheres formed by the solvent evaporation method. Pharm. Res. 4, 465–471.

Bodmeier, R., McGinity, J.W., 1988. Solvent selection in the preparation of poly(DL-lactide) microspheres prepared by the solvent evaporation method. Int. J. Pharm. 43, 179–186.

Carrasquillo, K.G., Stanley, A.M., Aponte-Carro, J.C., Jésus, P.D., Costantino, H.R., Bosques, C.J., Griebenow, K., 2001. Non-aqueous encapsulation of excipient-stabilized spray-freeze dried BSA into poly(lactide-*co*-glycolide) microspheres results in release of native protein. J. Control. Release 76, 199–208.

Dash, A.K., 1997. Determination of the physical state of drug in microcapsule and microsphere formulations. J. Microencapsul. 14, 101–112.

Dash, A.K., Khin-Khin, A., Suryanaryanan, R., 2002. X-ray powder diffractometric method for quantitation of crystalline drug in microparticulate systems. I. Microspheres. J. Pharm. Sci. 91, 983–990.

Dhanaraju, M.D., Vema, K., Jayakumar, R., Vamsadhara, C., 2003. Preparation and characterization of injectable microspheres of contraceptive hormones. Int. J. Pharm. 268, 23–29.

Freiberg, S., Zhu, X.X., 2004. Polymer microspheres for controlled drug release. Int. J. Pharm. 282, 1–18.

Gonzalez, M.F., Ruseckaite, R.A., Cuadrado, T.R., 1999. Structural changes of polylactic-acid (PLA) microspheres under hydrolytic degradation. J. Appl. Polym. Sci. 71, 1223–1230.

Ha, J.-H., Kim, S.-H., Han, S.-Y., Sung, Y.-K., Lee, Y.-M., Kang, I.-K., Cho, C.-S., 1997. Albumin release from bioerodible hydrogels based on semi-interpenetrating polymer networks composed of poly(ε-caprolactone) and poly(ethylene glycol) macromer. J. Control. Release 49, 253–262.

Herrmann, J., Bodmeier, R., 1998. Biodegradable, somatostatin acetate containing microspheres prepared by various aqueous and non-aqueous solvent evaporation methods. Eur. J. Pharm. Biopharm. 45, 75–82.

Kim, H.K., Chung, H.J., Park, T.G., 2006. Biodegradable polymeric microspheres with "open/closed" pores for sustained release of human growth hormone. J. Control. Release 112, 167–174.

Kim, S.Y., Lee, Y.M., 2001. Taxol-loaded block copolymer nanospheres composed of methoxy poly(ethylene glycol) and poly(epsilon-caprolactone) as novel anticancer drug carriers. Biomaterials 22, 1697–1704.

Lai, M.K., Tsiang, R.C.-C., 2005. Microencapsulation of acetaminophen into poly(L-lactide) by three different emulsion solvent-evaporation methods. J. Microencapsul. 22, 261–274.

Lamprecht, A., Rodero-Torres, H., Schäfer, U., Lehr, C.M., 2000. Biodegradable microparticles as a two-drug controlled release formulation: a potential treatment of inflammatory bowel disease. J. Control. Release 69, 445–454.

Lee, J.-H., Park, T.G., Choi, H.-K., 2000. Effect of formulation and processing variables on the characteristics of microspheres for water-soluble drugs prepared by w/o/o double emulsion solvent diffusion method. Int. J. Pharm. 196, 75–83.

Lemmouchi, Y., Schacht, E., 1997. Preparation and in vitro evaluation of biodegradable poly(ε-caprolactone-co-D,L lactide) (X-Y) devices containing trypanocidal drugs. J. Control. Release 45, 227–233.

Lin, W.-J., Huang, L.-I., 2001. Fabrication of porous poly(ε-caprolactone) microparticles for protein release. J. Microencapsul. 18, 577–584.

Nihant, N., Schugens, C., Grandfils, C., Jérôme, R., Teyssié, P., 1994. Polylactide microparticles prepared by double emulsion/evaporation technique. I. Effect of primary emulsion stability. Pharm. Res. 11, 1479–1484.

O'Donnell, P.B., McGinity, J.W., 1997. Preparation of microspheres by the solvent evaporation technique. Adv. Drug Deliv. Rev. 28, 25–42.

Pérez, M.H., Zinutti, C., Lamprecht, A., Ubrich, N., Astier, A., Hoffman, M., Bodmeier, R., Maincent, P., 2000. The preparation and evaluation of poly(ε-caprolactone) microparticles containing both a lipophilic and a hydrophilic drug. J. Control. Release 65, 429–438.

Pitt, C.G., 1990. Poly(ε-caprolactone) and its copolymers. In: Chasin, M., Langer, R. (Eds.), Biodegradable Polymers as Drug Delivery Systems. Marcel Decker Inc., New York, NY, pp. 71–120.

Ritger, P.L., Peppas, N.A., 1987. A simple equation for description of solute release. I. Fickian and non-fickian release from non-swellable devices in the form of slabs, spheres, cylinders or discs. J. Control. Release 5, 23–36.

Sansdrap, P., Moës, A.J., 1993. Influence of manufacturing parameters on the size characteristics and the release profiles of nifedipine from poly(DL-lactide-co-glycolide) microspheres. Int. J. Pharm. 98, 157–164.

Sinha, V.R., Bansal, K., Kaushik, R., Kumria, R., Trehan, A., 2004. Poly-ε-caprolactone microspheres and nanospheres: an overview. Int. J. Pharm. 278, 1–23.

Tagwireyi, D., Ball, D.E., Loga, P.J., Moyo, S., 2000. Cantharidin poisoning due to "Blister beetle" ingestion. Toxicon 38, 1865–1869.

Takada, S., Kurokawa, T., Miyazaki, K., Iwasa, S., Ogawa, Y., 1997. Utilization of an amorphous form of a water-soluble GPIIb/IIIa antagonist for controlled release from biodegradable microspheres. Pharm. Res. 14, 1146–1150.

Viswanathan, N.B., Tomas, P.A., Pandit, J.K., Kulkarni, M.G., Mashelkar, R.A., 1999. Preparation of non-porous microspheres with high entrapment efficiency of proteins by a (water-in-oil)-in-oil emulsion technique. J. Control. Release 58, 9–20.

Wang, G.S., 1989. Medical uses of mylabris in ancient China and recent studies. J. Ethnopharmacol. 26, 147–162.

Weidenauer, U., Bodmer, D., Kissel, T., 2003. Microencapsulation of hydrophilic drug substances using biodegradable polyesters. Part I: Evaluation of different techniques for the encapsulation of Pamidronate di-sodium salt. J. Microencapsul. 20, 509–524.

Yang, Y.-Y., Chung, T.-S., Bai, X.-L., Chan, W.-K., 2000. Effect of preparation conditions on morphology and release profiles of biodegradable polymeric microspheres containing protein fabricated by double-emulsion method. Chem. Eng. Sci. 55, 2223–2236.