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European Journal of Pharmaceutics and Biopharmaceutics 69 (2008) 1176–1181
European Journal of Pharmaceutics and Biopharmaceutics
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Note

Formation mechanism and release behavior of poly(ε-caprolactone) microspheres containing disodium norcantharidate

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Received 24 October 2007; accepted in revised form 26 February 2008
Available online 29 February 2008

Abstract

Disodium norcantharidate (DSNC) loaded poly(ε-caprolactone) (PCL) microspheres were prepared by s/o/w solvent evaporation technique, and the formation mechanism and release behavior of the microspheres were investigated. The particle formation of the microspheres was influenced by the osmotic effect of DSNC. During the microsphere preparation, water diffused into the emulsion droplets and dissolved the particles of DSNC. Thereafter, DSNC generated osmotic effect and drove the water to flow in the emulsion droplets more quickly, thus forming an inner water phase. As the water influx proceeded, the state of the emulsion was transferred from s/o/w to w/o/w, thus resulting in the porosity of the microspheres. The release tests were carried out in the release media of different osmotic pressures achieved by adding different amounts of dextrose. The results indicated that the initial release of DSNC from the microspheres was controlled by a combination of osmotic effect and diffusion, but the release after the initial was mainly controlled by diffusion. This study demonstrated that the osmotic effect of DSNC not only was responsible for the particle formation but also contributed to the release from the microspheres.

Keywords: Particle formation; Porosity; Microspheres; Release behavior; Disodium norcantharidate

1. Introduction

Polymeric microspheres for controlled drug delivery possess lots of advantages. They can be administrated orally, intravenously or via implantation, and can be tailored for desired release profiles and in some cases can even provide organ-targeted release [1].

Solvent evaporation technique is the most popular way to accomplish encapsulation [2]. As usual, the desired polymer is dissolved in an organic solvent, and drug can be dissolved, dispersed or emulsified into the polymer solution, which is then emulsified in a continuous phase containing emulsifier. As solvent evaporation proceeds, the microspheres solidify and can be harvested by filtration and drying. Although many literatures focus on the influence of various parameters, only a few investigations detailed the encapsulation mechanism [3,4]. In particular, no literature reported the particle formation mechanism pertaining to s/o/w-technique. On the other hand, the release of a water-soluble drug from polymeric microspheres was commonly attributed to the diffusion of drug rather than the degradation of polymer [5].

In general, w/o/w and s/o/w solvent evaporation techniques respectively achieve porous and non-porous microspheres because of the presence and absence of the inner water phase [6]. Besides, the addition of NaCl in the continuous phase was frequently applied in w/o/w-technique to modify microsphere structure and encapsulation efficiency by modulating the osmotic gradient between the outer and inner water phase [7,8]. However, it was seldom applied in s/o/w-technique because the technique lacks

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doi:10.1016/j.ejpb.2008.02.020

the inner water phase. In our previous study, DSNC-loaded PCL microspheres prepared by s/o/w-technique were highly porous, and the microsphere morphology, encapsulation efficiency and release behavior were able to be modified by the addition of NaCl in the continuous phase during the preparation [9], which indicated that the characteristics of DSNC-loaded microspheres were different from most polymeric microspheres containing other water-soluble drugs.

This study investigated the formation mechanism and release behavior of DSNC-loaded PCL microspheres prepared by s/o/w solvent evaporation technique.

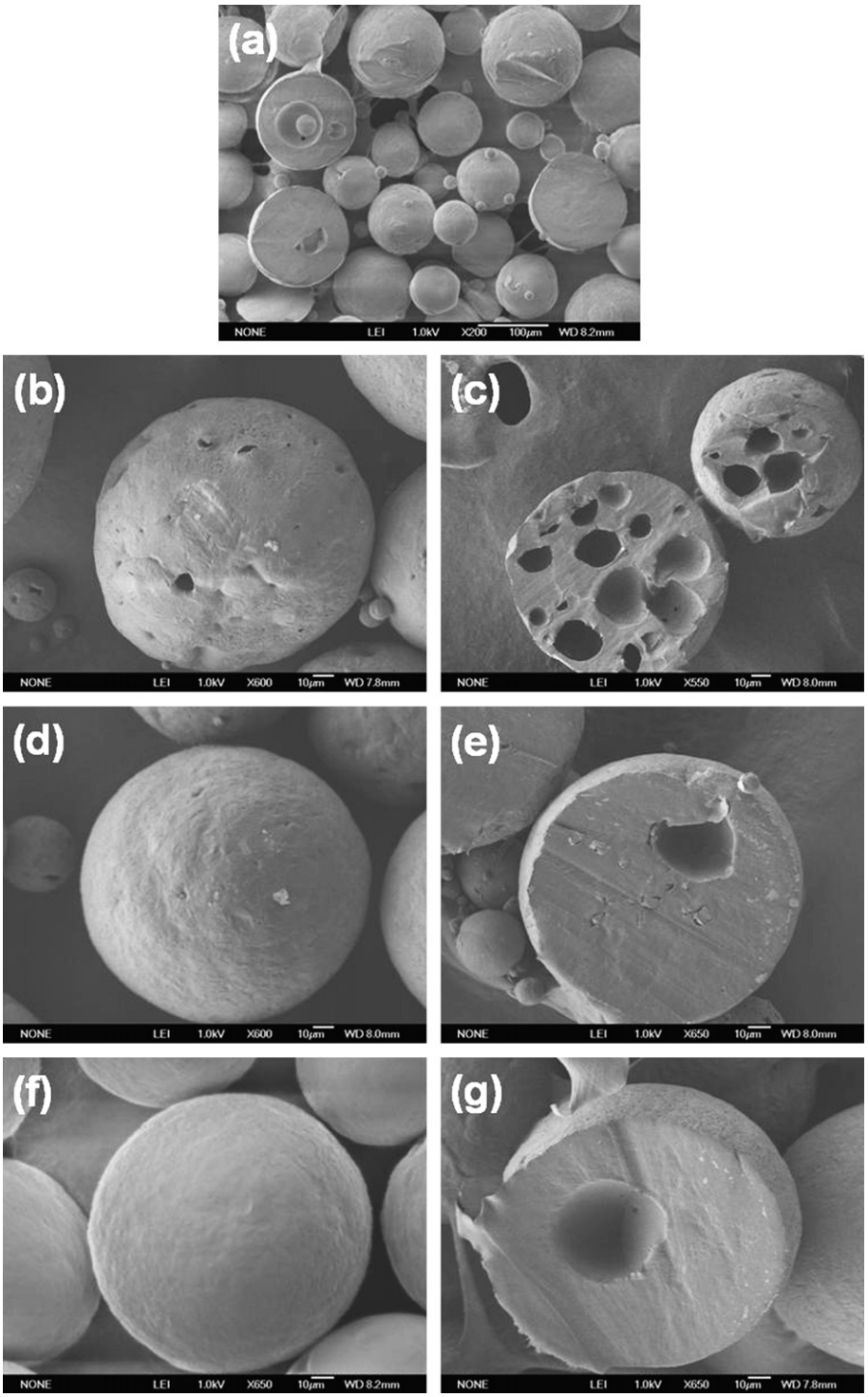

Fig. 1. SEM photographs of drug-free microspheres (a) and the microspheres containing NaCl (b and c), BSA (d and e) and 5-Fu (f and g).

2. Materials and methods

2.1. Materials

Poly(ε-caprolactone) (MW 50,000) was purchased from Daicel Polymer Ltd. (Tokyo, Japan). DSNC was bought from Ange Pharmaceutical Company (Nanjing, China). 5-Fluorouracil (5-Fu) was purchased from Nantong Jinghua Pharmaceutical Co., Ltd. (Jiangsu, China). Bovine serum albumin (BSA) and succinic acid (SA) were obtained from Sigma–Aldrich, China. Polyvinylalcohol (PVA, 88% hydrolyzed) was supplied by Weicheng Chemical Industry Ltd. (Shanghai, China).

2.2. Microsphere preparation

DSNC-loaded PCL microspheres were prepared by the s/o/w solvent evaporation method [9]. Briefly, 3 ml of dichloromethane mixture containing 200 mg of DSNC and 600 mg of PCL was vigorously homogenized with a homogenizer (GF-1, Qilinbeier, Jiangsu, China) to form s/o dispersion. The dispersion was added dropwise into 40 ml of 1% (w/v) PVA aqueous solution and emulsified by mechanical stirring (S-212, Shenshun, Shanghai, China) at 20 °C and 1000 rpm for 60 min, 40 min under ambient pressure and followed by 20 min under reduced pressure (20 kPa). The microspheres were collected by filtration, washed with deionized water and dried in a vacuum desiccator at room temperature.

As a comparison, PCL microspheres were prepared under the same condition to encapsulate NaCl, 5-Fu or BSA.

2.3. Microsphere morphology

To observe the microsphere morphology, dried microspheres were mounted onto stubs using double-sided carbon tape and analyzed with scanning electron microscopy (SEM, JSM-7401F, JEOL, Japan). To reveal the internal morphology, the microspheres were cross-sectioned with razor blade prior to the observation.

2.4. Water influx and osmotic effect

The water influx (*WI*, %) into the microspheres during the preparation was estimated according to the literature [9]. Briefly, the solidified microspheres were filtered under a reduced pressure to remove the water on the microsphere surface, and then the microsphere weights before (W_b) and after (W_a) drying were recorded, respectively. The value of *WI* was calculated according to the following equation:

$$WI = \frac{W_b - W_a}{W_a} \times 100 \quad (1)$$

To demonstrate that the water influx during the preparation of DSNC-loaded microspheres was caused by the osmotic effect of DSNC, 2 g of SA as an influx tracer was dissolved in the continuous phase, and subsequently the amount of SA incorporated in the dried microspheres was determined by high performance liquid chromatography (HPLC).

2.5. Determination of SA and DSNC

Both SA and DSNC were determined by HPLC (SPD-10AD*VP*, Shimadzu, Japan) under the same conditions: a reversed phase column (Diamonsil™ C_{18}, 250 × 4.6 mm, 5 μm, Dikma, China), a mobile phase composed of acetonitrile and water (12:88, pH 3.1 adjusted with phosphoric acid), an ultraviolet detector at 208 nm and at a flow rate of 1.0 ml/min. The retention time of SA and DSNC was 4.0 and 6.7 min, respectively. To extract SA or DSNC from the microspheres, 20 mg of microspheres was 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.

2.6. In vitro release behavior

Dried DSNC-loaded microspheres (30 mg) were accurately weighted in triplicate and placed in vials containing 3 ml of dissolution medium. The vials were maintained at

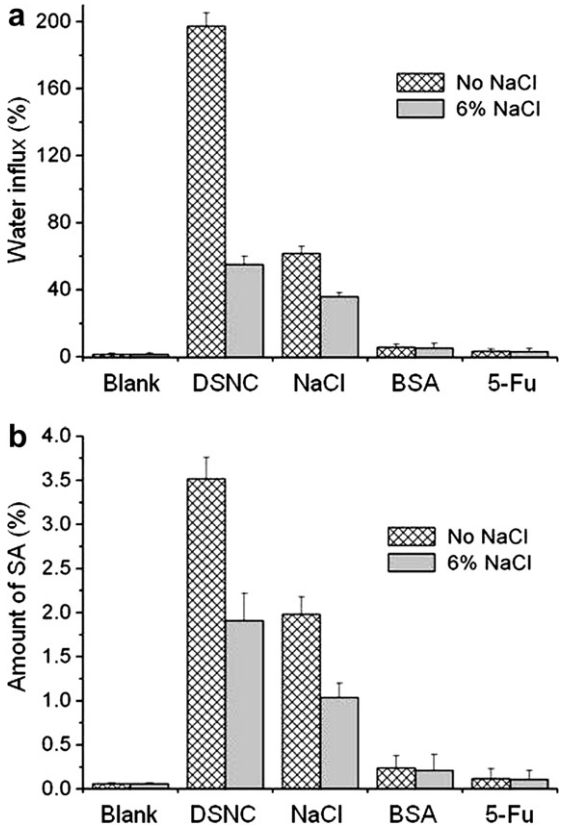

Fig. 2. Effect of a model drug on the water influx (a) and the amount of SA (b) incorporated into the microspheres prepared with and without the addition of NaCl in the continuous phase ($n = 3$).

37 °C in a horizontal-shaker and shaken at a rate of 100 rpm (SHA-C, Jinbo-tech, China). At predetermined intervals, 0.1 ml of the supernatant was collected after centrifugation (TGL-16C, Anting Instruments, Shanghai, China) and replaced with 0.1 ml of fresh medium. The collected supernatant was diluted with 0.9 ml of mobile phase for HPLC analysis.

3. Results and discussion

To investigate the cause of the porosity of DSNC-loaded microspheres, PCL microspheres were prepared under the same conditions to encapsulate some other compounds. When encapsulating NaCl, the microspheres were also of porous surface and internal structure (Fig. 1b and c). Conversely, the microspheres revealed non-porous surface and dense structure when encapsulating BSA or 5-Fu (Fig. 1d–g). It must be pointed out that some of the microspheres containing BSA or 5-Fu had one large pore. The large pore was probably caused by the stirring itself or by the embedment of a small microsphere in a large one, since some of the drug-free microspheres had a similar large pore and others had no pore (Fig. 1a). Nevertheless, the pores in microspheres containing BSA or 5-Fu were obviously different from those in microspheres containing DSNC or NaCl. These results indicated that the porosity of DSNC-loaded microspheres was a result of the characteristics of the drug salt.

The encapsulation efficiencies of DSNC, BSA and 5-Fu were 56.3%, 43.2% and 47.4%, respectively. Although the encapsulation efficiencies of BSA and 5-Fu were quite low, the resultant microspheres possessed smooth surface and dense internal structure, which implied that the porosity of DSNC-loaded microspheres was not caused by the leakage of a drug particle into the continuous phase. The porosity of DSNC-loaded microspheres can be attributed to the water influx into the microspheres during the preparation. Fig. 2 shows the effect of model drug on the water influx and the amount of SA incorporated into the microspheres. On one hand, both the water influx and the amount of SA into DSNC-loaded microspheres were higher than those into both drug-free microspheres and the microspheres containing BSA or 5-Fu. On the other hand, the addition of NaCl (6%, w/v) obviously resulted in a decrease in the water influx and the amount of SA into DSNC-loaded microspheres, but exerted an insignificant effect on the water influx and the amount of SA into both drug-free microspheres and the microspheres containing BSA or 5-Fu. These results indicated that DSNC generated an obvious osmotic effect, which resulted in the water influx into the microspheres during the preparation. Therefore, the porosity of DSNC-loaded microspheres can be attributed to the osmotic effect of DSNC in the preparation.

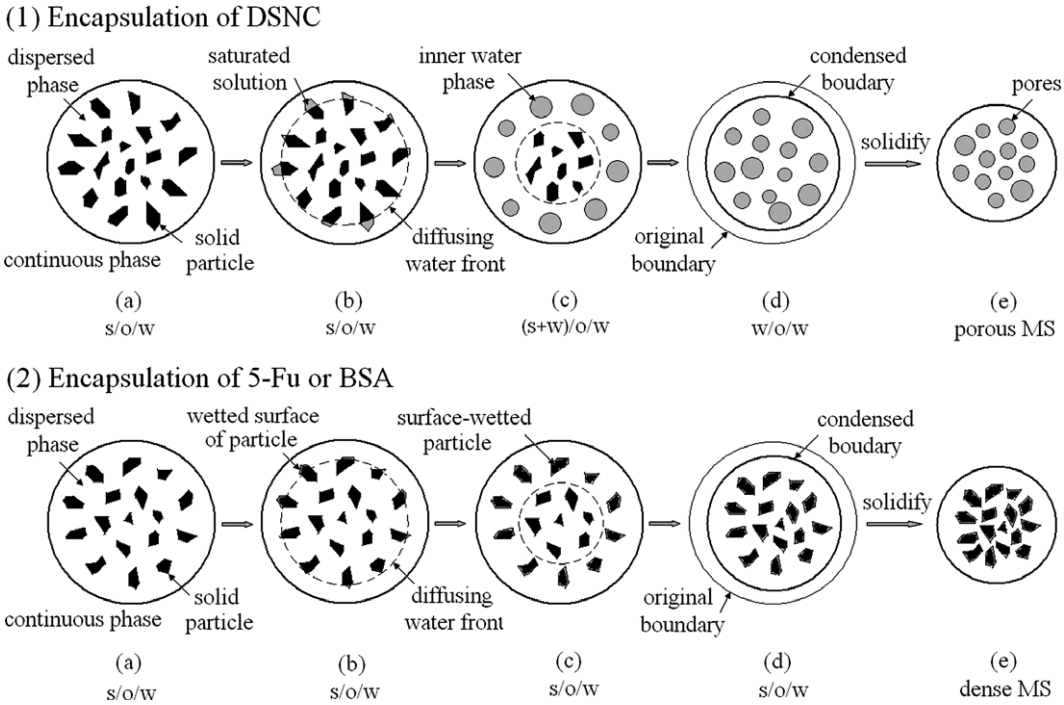

Fig. 3. Proposed mechanism of the particle formation of PCL microspheres containing a different drug by s/o/w solvent evaporation technique. (1) Encapsulation of DSNC: (a) s/o/w emulsion droplets were initially formed; (b) diffusing water reached the particles of DSNC and dissolved a portion of the particles; (c) water was drawn in more quickly under the osmotic effect and an inner water phase was formed; (d) the state of the emulsion was transferred from s/o/w to w/o/w; (e) porous microspheres were formed after the solidification. (2) Encapsulation of 5-Fu or BSA: (a) s/o/w emulsion droplets were initially formed; (b) diffusing water reached the drug particles and the particles were only wetted or slightly dissolved; (c) no driving force to drive water to flow in quickly, and no inner water phase was formed; (d) the emulsion remained in the s/o/w state; (e) dense microspheres were formed after the solidification.

Based on the above investigations, a mechanism of the microsphere formation was proposed as shown in Fig. 3. Since water has about 0.2% (v/v) solubility in dichloromethane, the water in the continuous phase can diffuse into the emulsion droplets during the microsphere preparation. Once the water reached a particle of DSNC, it was able to dissolve a portion of the particle and form a drug solution. Since DSNC has a high water solubility (346.7 mg/ml, 20 °C) and is a disodium salt, it can generate a high osmotic pressure which drove the water to flow in the emulsion droplets more quickly. As the water influx processed, the particle was dissolved entirely, and an inner aqueous phase was formed prior to the microsphere solidification. Due to the formation of the inner water phase, the state of the emulsion was transferred from s/o/w to w/o/w during the preparation, which consequently resulted in the porosity of the microspheres (Fig. 3(1)). Conversely, after the water diffused into the emulsion droplets and reached the particles of 5-Fu and BSA, the particles were just wetted or slightly dissolved because of their relatively low water solubility. Besides, 5-Fu and BSA are non-ionic compounds. Both of them hardly generated or only generated a slight osmotic pressure in the emulsion droplets in the preparation, which indicated there was no driving force to drive the water to flow into the emulsion droplets continuously. Namely, no inner aqueous phase was formed and the emulsion remained in s/o/w state in the preparation. Thus, the dense microspheres were obtained after solidification and drying (Fig. 3(2)).

To investigate the contribution of the osmotic effect to the release of DSNC, the release tests were carried out in release media of different osmotic pressures achieved by modulating the concentration of dextrose (0, 0.8 and 1.6 mol/l), and the results are shown in Fig. 4a. The similarity factor (f_2) was employed to compare the drug release profiles [10]:

$$f_2 = 50 \times \log_{10}\left\{\left[1 + \frac{1}{N}\sum_{i=1}^{N}(T_i - R_i)^2\right]^{-1/2} \times 100\right\} \qquad (2)$$

where N is the number of time points determined, and T_i and R_i represent the average release percentage of test profile and reference profile at i time point, respectively. Commonly, f_2 value above 50 indicates a statistical similarity between the two release profiles, respectively. The release profile in purified water was chosen as the reference profile, and the f_2 values of release profiles in 0.8 and 1.6 mol/l dextrose solutions were 47.2% and 37.1%, respectively. These results indicated that the drug release profiles were significantly dependent on the concentration of dextrose, namely dependent on the osmotic pressure of the dissolution medium. Thus, osmotic effect generated by DSNC was demonstrated to play a role in the drug release. On the other hand, only the release rate in the first hour was obviously dependent on the osmotic pressure of the release media (Fig. 4b). This result indicated that the osmotic effect generated by DSNC only played an important role in the initial release of DSNC. Therefore,

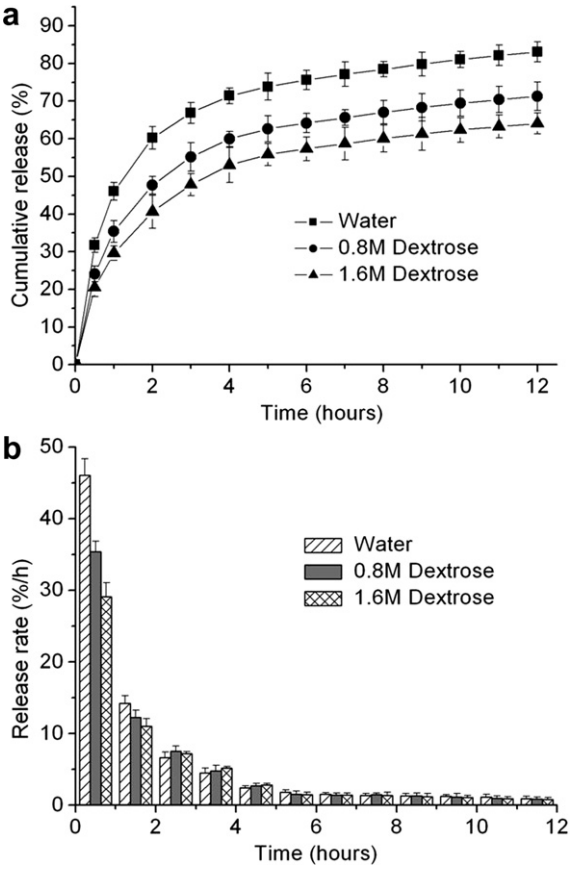

Fig. 4. Cumulative release and release rate of DSNC from the microspheres in dissolution medium containing a different concentration of dextrose at 37 °C ($n = 3$).

the release mechanism of DSNC from PCL microspheres can be considered as a combination of osmotic effect and diffusion. So far as the initial release was concerned, the drug release was attributed to osmotic effect of DSNC and diffusion through the pores. After the initial release, the drug content decreased and the osmotic effect gradually diminished, thus the release was mainly attributed to the diffusion through the polymer matrix.

In conclusion, the particle formation and drug release behavior had a close correlation with the physicochemical characteristics of the model drug. The osmotic effect generated by DSNC not only was responsible for the porosity of the microspheres but also contributed to the initial release of the drug.

Acknowledgement

The authors would like to thank Professor Shun-ai Che (Shanghai Jiao Tong University, China), who provided SEM for the observation of microsphere morphology.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejpb.2008.02.020.

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