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International Journal of Pharmaceutics 353 (2008) 15–20
INTERNATIONAL JOURNAL OF PHARMACEUTICS
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Disodium norcantharidate-loaded poly(ε-caprolactone) microspheres II. Modification of morphology and release behavior

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Received 28 May 2007; received in revised form 31 October 2007; accepted 5 November 2007
Available online 20 February 2008

Abstract

Disodium norcantharidate (DSNC)-loaded poly(ε-caprolactone) (PCL) microspheres were prepared by s/o/w solvent evaporation technique, and their morphology and drug release behavior were modified by adding NaCl (3, 6 and 9%) in the continuous phase during the preparation. The addition of NaCl decreased the water influx into the emulsion droplets and the porosity of the resultant microspheres. Higher NaCl concentration resulted in smaller particle size, lower density and higher drug loading of the microspheres. Despite higher drug loading and smaller particle size, the microspheres prepared with NaCl in the continuous phase exhibited slower drug release. The modification of the release profiles was correlated with the changes in the surface and internal morphology of the microspheres. Therefore, by adding NaCl in the continuous phase during the preparation, both morphology and release behavior of the microspheres can be modified to a certain extent.

Keywords: Microspheres; Morphology; Release behavior; Solvent evaporation technique

1. Introduction

Over the past two decades, much attention has been paid to biodegradable polymeric microspheres for drug delivery. Biodegradable polymeric microspheres can control the drug release rate, thus prolonging the biological activity and decreasing the administration frequency as well as the drug side effects (Pekarek et al., 1994; Jeong et al., 1997; Freiberg and Zhu, 2004).

Drug release from biodegradable polymers is governed by various properties of the polymer, drug and carrier system (Washington, 1990; Kissel et al., 1991), and the mechanism of drug release from biodegradable polymers can be summarized as diffusion, degradation or a combination of diffusion and degradation (Fung and Saltzman, 1997). For the diffusion-controlled release, the desirable release behavior can be achieved by modulating the surface and internal morphology of microspheres (Ghaderi et al., 1996; Jeyanthi et al., 1996; Yang et al., 2000; Miyazaki et al., 2006). NaCl addition is frequently employed in w/o/w-technique to modify microsphere morphology and drug release behavior by means of modulating osmotic pressure (Herrmann and Bodmeier, 1995; Pistel and Kissel, 2000; Jiang et al., 2002). However, few literatures were found to report its application in s/o/w-technique to modify the morphology and drug release behavior of microspheres.

Disodium norcantharidate (Fig. 1) is a potent anti-cancer drug against primary hepatic carcinoma, breast cancer and abdominal cancer, but easily produces irritant effects on the urinary organs (Wang, 1989). In our previous study (Wang et al., 2007), DSNC-loaded PCL microspheres were prepared by s/o/w-technique. The microspheres revealed highly porous surface and internal structure due to the salt form of DSNC, and consequently the drug was rapidly released from the microspheres. Therefore, the objective of the present study is to modify the morphologic characteristics and drug release behavior of the microspheres by adding NaCl in the continuous phase during the preparation.

2. Materials and methods

2.1. Materials

Poly(ε-caprolactone) (MW 50,000) was obtained from Daicel Polymer Ltd. (Minato-ku, Tokyo, Japan). Polyvinylalcohol (PVA, 88% hydrolyzed) was supplied by Weicheng Chemical

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doi:10.1016/j.ijpharm.2007.11.007

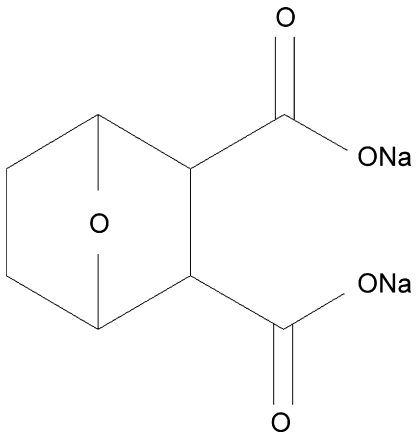

Fig. 1. Chemical structure of disodium norcantharidate.

Industry Ltd. (Shanghai, China) as an emulsifier. Disodium norcantharidate was bought from Ange Pharmaceutical Company Ltd. (Nanjing, China). 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 s/o/w solvent evaporation technique. Briefly, 100 mg of micronized drug was first dispersed in 3 ml of dichloromethane containing 600 mg of PCL. The s/o dispersion was added dropwise into 40 ml of 1% (w/v) PVA aqueous solution containing a different concentration of NaCl (0, 3, 6 and 9%, w/v). The resulting emulsion was stirred (1000 rpm) with a double-bladed propeller continuously for 40 min at 20 °C and under ambient pressure. To ensure complete evaporation of dichloromethane, the emulsion was stirred for another 20 min under reduced pressure (20 kPa). The microspheres were collected by filtration under a decompressed condition, washed with deionized water and dried in a vacuum desiccator at room temperature.

2.3. Morphology of microspheres

In order to observe the surface morphology and internal structure of the microspheres, dried microspheres were mounted onto stubs using a double-sided adhesive tape with conductivity and analyzed with scanning electron microscope (SEM, JSM-7401F, JEOL, Japan). To reveal the internal morphology, the stuck microspheres were cross-sectioned with razor blade prior to the observation.

2.4. Water influx and porosity

After the solidification in the preparation, the microspheres were filtered under a decompressed condition to remove the water on the microsphere surface. To evaluate the water influx into the dispersed phase during the preparation, the weight of the microspheres was measured before (W_b) and after (W_a) drying, respectively. The water influx (WI, %) was calculated according to the following equation:

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

The porosity of the resultant microspheres was measured by mercury porosimetry (Autopore IV 9500, Micromeritics, USA). An intrusion and extrusion measurement was performed between 3.5 kPa and 228 MPa. The porosity of the microspheres was calculated according to the following equation:

$$\text{porosity} = \frac{\text{skeletal density} - \text{bulk density}}{\text{skeletal density}} \times 100 \quad (2)$$

2.5. Particle size analysis

About 50 mg of dried microspheres were dispersed in 5 ml of 0.2 μm filtered distilled water containing 2% (w/v) Tween 80 and then sonicated in a water bath for 3 min to prevent aggregation between the particles. Size measurement was performed by a particle size analyzer (CIS 100, Ankersmid, The Netherlands). The particle size was expressed as the mean volume diameter in micrometer.

2.6. Tapped density

Tapped density was achieved by tapping a measuring cylinder containing the microspheres (Pistel and Kissel, 2000). A known weight of microspheres were passed through a 0.3-mm screen to break up agglomerates and then transferred to a 2 ml glass graduated cylinder (readable to 0.02 ml). Then the cylinder containing the sample was raised and allowed to drop under its own weight, and the drop was fixed at 14 ± 2 mm. Tapped volume was measured after initial 500 taps and additional 750 taps, respectively. If the difference between the two volumes is less than 2%, the tapped volume after total 1250 taps was regarded as the final tapped volume. Otherwise, additional 1250 taps were repeated until the difference between succeeding measurements is less than 2%. Tapped density of the microspheres was calculated and expressed as g/ml using the following equation:

$$\text{tapped density} = \frac{\text{weight of microspheres}}{\text{final volume after 1250 taps}} \quad (3)$$

2.7. Encapsulation efficiency

The actual drug loading of the microspheres was determined by high performance liquid chromatography (HPLC, SPD-10AD*VP*, Shimadzu, Japan) using a reversed phase column (Diamonsil™ C_{18}, 250 mm × 4.6 mm, 5 μm, Dikma, China) and a mobile phase composed of acetonitrile and water (12:88, pH 3.1, adjusted with phosphoric acid). The flow rate was 1.0 ml/min and the determination was monitored at 208 nm by an ultraviolet detector. The linearity of the response was verified over the concentration range of 20–640 μg/ml ($r^2 = 0.999$). 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. The encapsulation efficiency was expressed as the ratio of the actual loading to the theoretical loading.

2.8. In vitro release behavior

In vitro release tests were carried out in triplicate at 37 °C. Dried microspheres (30 mg) were placed in 3 ml of 0.05 M phosphate buffer (pH 7.4), incubated in a horizontal-shaker and shaken at a rate of 100 rpm. At predetermined intervals, 0.1 ml of the supernatant was extracted after centrifugation (500 rpm for 2 min) and replaced with 0.1 ml of the fresh buffer. The extracted supernatant was diluted with 0.9 ml of mobile phase for HPLC analysis.

3. Results and discussion

3.1. Morphological characteristics

Fig. 2 shows SEM photographs of DSNC-loaded PCL microspheres prepared with a different concentration of NaCl in the continuous phase. When prepared without the addition of NaCl, the microspheres showed the highly coarse surface and porous internal structure (A1–A3). On the contrary, the microspheres

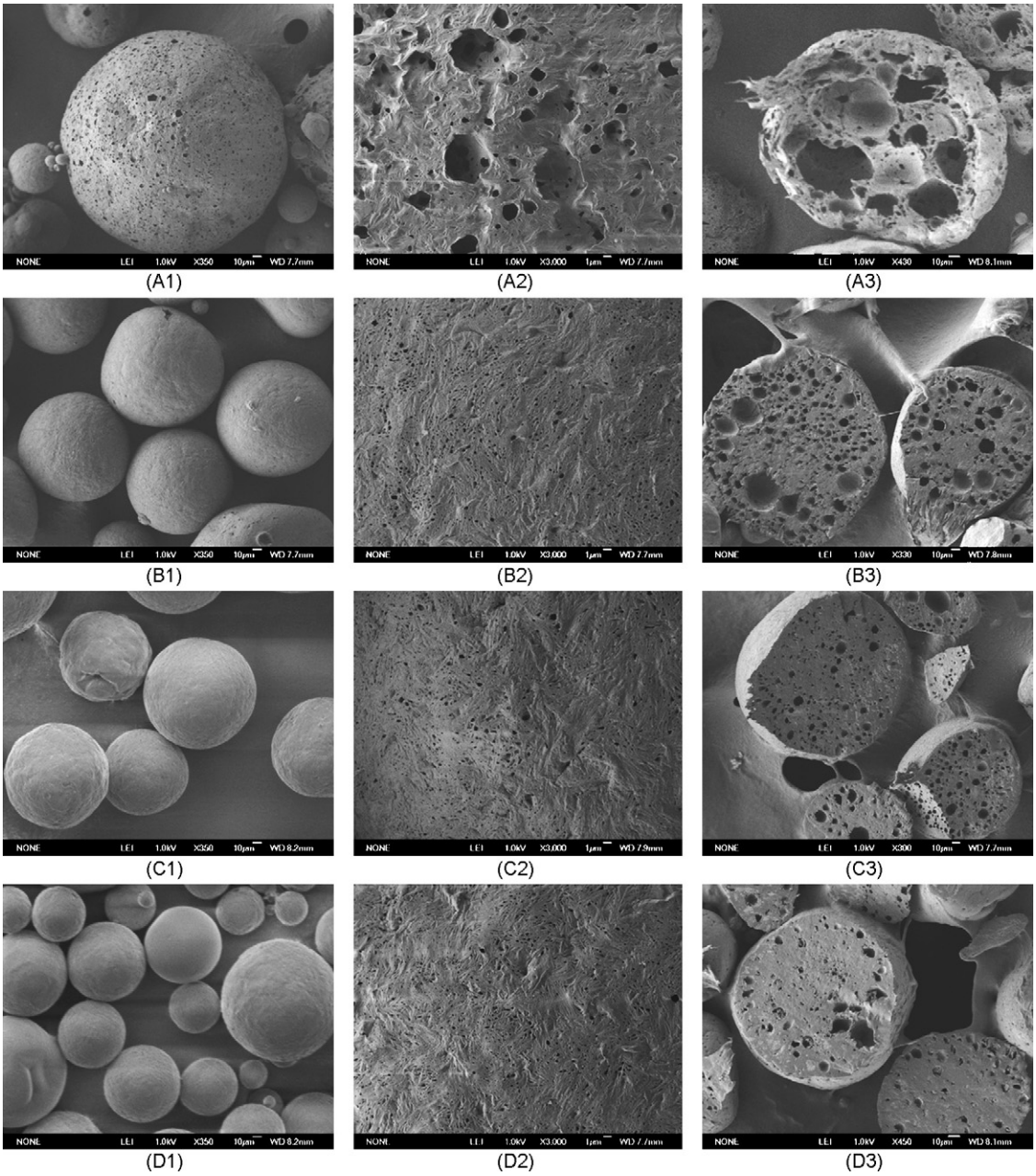

Fig. 2. External (A1–D1), surface (A2–D2) and internal (A3–D3) morphology of DSNC-loaded PCL microspheres prepared with a different concentration of NaCl in the continuous phase: A1–A3, 0%; B1–B3, 3%; C1–C3, 6%; D1–D3, 9%.

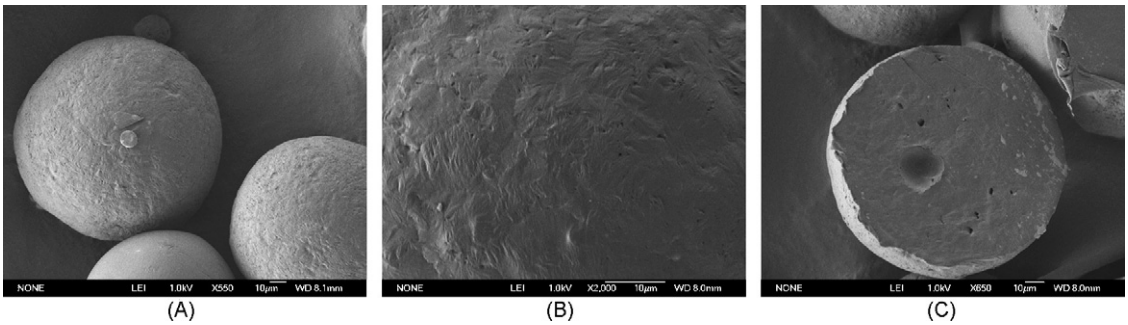

Fig. 3. External (A), surface (B) and internal (C) morphology of norcantharidin-loaded PCL microspheres prepared with s/o/w solvent evaporation technique.

prepared with NaCl addition possessed rather smooth surface, but tiny pores were easily observed at a high magnification (B2–D2). With regard to the internal structure, the addition of NaCl produced less porous microspheres but was unable to eliminate the porosity of the microspheres completely (B3–D3).

As a comparison, PCL microspheres were prepared to encapsulate norcantharidin, the acid anhydride form of DSNC. An excessive amount of norcantharidin was added in the polymer solution to form a dispersed solution, and the microspheres were prepared under the same process conditions. As a result, the encapsulation efficiency of norcantharidin was quite low (below 2%), but the microspheres had the smooth surface and non-porous structure (Fig. 3). These results indicated that the porosity of DSNC-loaded microspheres was caused by the water influx into the dispersed phase rather than the leakage of drug particles into the continuous phase. The water influx into the dispersed phase and the porosity of the resultant microspheres were measured and summarized in Table 1. The results indicated that both the water influx and the porosity decreased with the increase in the concentration of NaCl in the continuous phase.

The water influx can be attributed to the osmotic effect caused by the presence of DSNC. In general, drug with a water solubility of 50–300 mg/ml is considered as an ideal candidate for osmotic delivery (McClelland et al., 1991), and osmotic pressure of a solution is dependent on the moles of particles (molecules or ions) of solute present in the solution (Amsden, 2003). Since DSNC has a high water solubility (measured as 346.6 mg/ml at 20 °C) and is a disodium salt, it easily generates osmotic pressure in an aqueous solution. On the other hand, water has about 0.2% (v/v) solubility in CH_2Cl_2, and it can diffuse into the emulsion droplets during the microsphere preparation. Though the amount of water diffusing into the emulsion droplets was quite slight, it was sufficient to dissolve the drug particles and form a solution due to the extremely high water solubility of DSNC. The drug solution formed in the emulsion droplets was able to generate a high osmotic pressure which drove the water to flow in subsequently. Based on such an explanation, the addition of NaCl in the continuous phase decreased the osmotic gradient between the continuous phase and the drug solution formed in the emulsion droplets, thus decreasing the water influx into the dispersed phase and the porosity of the resultant microspheres.

Table 1
Water influx into the dispersed phase and porosity of the resultant microspheres[a]

Batches	WI (%)	BD (g/ml)	SD (g/ml)	MR_V (μm)	Porosity (%)
MS0	197.5	0.1528	0.5516	30.1	72.3
MS3	92.8	0.3178	0.6580	19.7	51.7
MS6	53.1	0.4095	0.6614	13.5	38.1
MS9	34.3	0.4503	0.6451	9.4	30.2

[a] WI, water influx into the dispersed phase; BD, bulk density; SD, apparent skeletal density; MR_V, median pore radius (volume).

3.2. Particle size and tapped density

Particle size of microspheres is commonly determined by the size of emulsion droplets formed in the preparation (Sansdrap and Moës, 1993), and the presence of electrolytes in the continuous phase exerts no influence on the droplet size and its distribution of oil–water (o/w) emulsions (Hunt and Dalgleish, 1996; Srinivasan et al., 2000).

Fig. 4 shows the mean diameter and tapped density of DSNC-loaded and DSNC-free microspheres prepared with a different concentration of NaCl in the continuous phase. The addition of NaCl hardly influenced the particle size and tapped density of the drug-free microspheres and, however, resulted in a decrease in particle size and an increase in tapped density of the drug-loaded microspheres. Since the osmotic effect of DSNC can bring about the water influx into the dispersed phase and produce many pores inside the microspheres, the drug-loaded microspheres had larger particle size and lower density than the drug-free microspheres. On the other hand, for the drug-loaded microspheres, a higher concentration of NaCl resulted in a smaller particle size and a higher tapped density, which can be attributed to the fact that the addition of NaCl decreased the water influx into the dispersed phase and the porosity of the resultant microspheres. Therefore, the increase in tapped density and decrease in particle size of the drug-loaded microspheres supported the fact that the microsphere morphology can be modified by adding NaCl in the continuous phase.

3.3. Encapsulation efficiency

Fig. 5 shows the solubility of DSNC in different NaCl solutions at 20 °C and the encapsulation efficiency of the microspheres prepared with a different concentration of NaCl in the continuous phase. The encapsulation efficiency increased with the increase in NaCl concentration in the continuous phase. The increase in encapsulation efficiency was probably because the

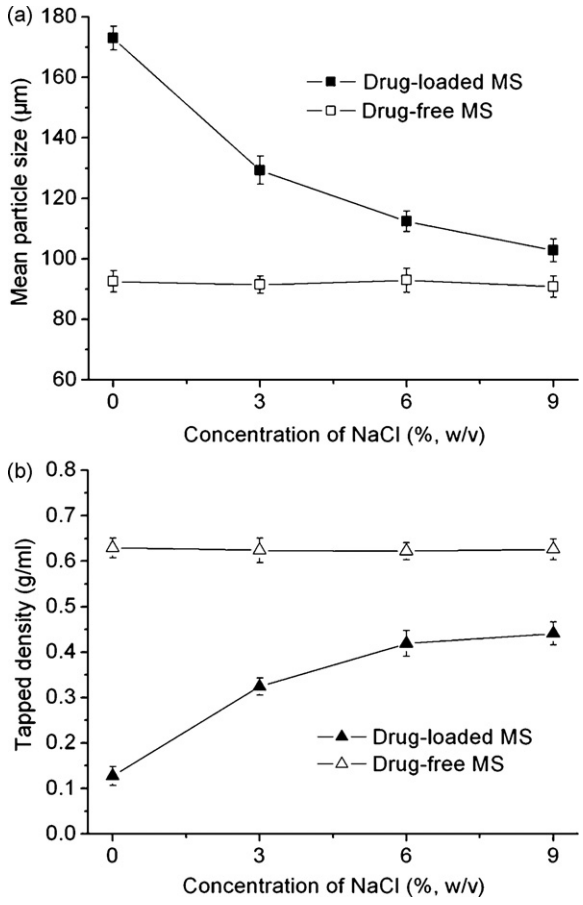

Fig. 4. Particle size (a) and tapped density (b) of the drug-loaded (■ and ▲) and drug-free (□ and △) microspheres prepared with a different concentration of NaCl in the continuous phase ($n = 3$).

addition of NaCl decreased the water influx into the emulsion droplets thus decreasing the subsequent drug diffusion into the continuous phase. Takada et al. (1997) and Weidenauer et al. (2003) also demonstrated that NaCl addition employed in s/o/w-technique resulted in an increase in encapsulation efficiency. On the other hand, the solubility of DSNC decreased from 346.6

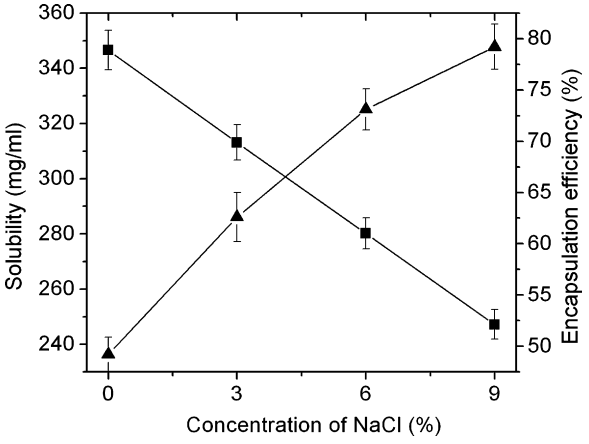

Fig. 5. Solubility (■) of DSNC in different NaCl solutions at 20 °C and encapsulation efficiency (▲) of the microspheres with a theoretical drug loading of 14.3% prepared with a different concentration of NaCl in the continuous phase ($n = 3$).

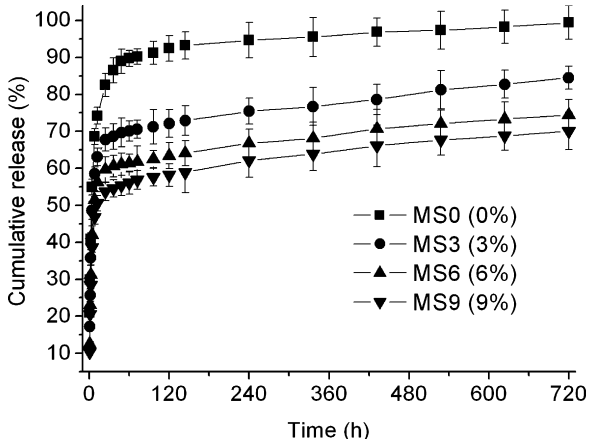

Fig. 6. In vitro release of DSNC in 0.05 M phosphate buffer at 37 °C from PCL microspheres prepared with a different concentration of NaCl in the continuous phase ($n = 3$).

to 247.2 mg/ml when the concentration of NaCl increased from 0 to 9%. However, the influence of NaCl on the solubility of DSNC was considered as a minor contribution to the increase in the encapsulation efficiency, because the amount of the drug (100 mg) was far from saturating the continuous phase (40 ml) during the microspheres preparation.

3.4. In vitro release behavior

Fig. 6 shows the drug release profiles of the microspheres prepared with a different concentration of NaCl in the continuous phase. The release profiles were composed of a rapid release phase and a slow release phase. In the rapid release phase, the drug release was controlled by the diffusion through the pores of the microspheres; whereas in the slow release phase, the drug release was controlled by the diffusion through the polymer matrix. Interestingly, higher concentration of NaCl resulted in slower drug release, despite leading to higher drug loading and smaller particle size of the microspheres.

Difference factor (f_1) and similarity factor (f_2) are commonly employed to compare the drug release profiles (Moore and Flanner, 1996; Costa, 1999):

$$f_1 = \frac{\sum_{i=1}^{N} |T_i - R_i|}{\sum_{i=1}^{N} (T_i + R_i)/2} \times 100 \tag{4}$$

$$f_2 = 50 \log_{10} \left\{ \left[1 + \frac{1}{N} \sum_{i=1}^{N} (T_i - R_i)^2 \right]^{-1/2} \times 100 \right\} \tag{5}$$

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. The values of f_1 and f_2 range from 0 to 100, and a higher f_1 value or a lower f_2 value indicates a better difference between two release profiles. Commonly, f_1 value above 15 (15–100) or f_2 value below 50 (0–50) indicates the statistical difference between the two release profiles, otherwise representing the similarity of the two release profiles. The values of f_1 and f_2 were calculated and summarized in Table 2. On one hand, the release profiles of

Table 2
Values of the difference factor (f_1) and similarity factor (f_2) between the release profiles of the microspheres prepared with a different concentration of NaCl in the continuous phase

Pairs of profiles	Difference factor (f_1)	Similarity factor (f_2)
MS0–MS3	20.5	40.1
MS0–MS6	33.0	31.3
MS0–MS9	41.1	27.5
MS3–MS6	12.7	54.8
MS3–MS9	21.2	44.9
MS6–MS9	8.3	66.0

MS3, 6 and 9 were statistically different from the release profile of MS0, which indicated that the addition of NaCl in the continuous phase was favorable for the slow drug release. On the other hand, MS3–MS9 behaved the statistical difference, however, MS3–MS6 and MS6–MS9 showed the insignificant difference, which implies that whether the addition of NaCl leads to a significant decrease in the drug release or not depends on the concentration of NaCl in the continuous phase in the microsphere preparation.

The modification of release behavior of the microspheres can be correlated with the changes in the surface and internal morphology of the microspheres. The addition of NaCl was favorable for not only the smooth surface but also the decreases in porosity and median pore radius of the microspheres (Table 1), which decreased the drug diffusion into the release medium.

4. Conclusion

This study demonstrated that the addition of NaCl in the continuous phase can be employed in s/o/w-technique to modify the morphology and release behavior of the microspheres, despite being frequently applied to w/o/w-technique. Since DSNC was able to generate osmotic effect and form an inner aqueous solution in the emulsion droplets during the preparation, the addition of NaCl in the continuous phase decreased the osmotic gradient between the inner aqueous solution and the continuous phase, thus resulting in smooth surface and a decrease in the porosity of the microspheres. Furthermore, by means of modifying the surface and internal morphology of the microspheres, the drug release behavior of the microspheres was modified to a certain extent.

In vivo investigations based on the above-described formulation are being conducted.

Acknowledgement

We would like to thank Dr. Lijuan Sun (East China University of Science and Technology, China) for her earnest assistance in the measurement of porous characteristics by mercury porosimetry.

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