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International Journal of Pharmaceutics 324 (2006) 144–151
INTERNATIONAL JOURNAL OF PHARMACEUTICS
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Effect of temperature-increase rate on drug release characteristics of dextran microspheres prepared by emulsion solvent evaporation process

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Received 3 February 2006; received in revised form 18 May 2006; accepted 6 June 2006
Available online 9 June 2006

Abstract

Microspheres containing theophylline (TH) were prepared from a hydrophobic dextran derivative by an emulsion solvent evaporation process using an acetone/liquid paraffin system. The effects of solvent evaporation rate on particle properties and drug release characteristic of the microspheres were evaluated. The solvent evaporation rate was controlled by the rate of increase in temperature of the water bath, ranging 7.5–30 °C/h. Drug release from the microspheres was examined using JPXIV 2nd fluid (pH 6.8) containing 0.1% Tween 80, and was found to be greatly affected by the solvent evaporation rate. The percentage of drug released until 8 h varied; from 28% to 84% for 30 and 7.5 °C, respectively. Differential scanning calorimetry and powder X-ray diffraction studies revealed that TH partially interacted with the polymer and drug crystallinity was maintained intact in the microspheres. According to scanning electron microscopy observations, all microspheres showed a well-formed spherical particle with a solid interior. The appearances of the microspheres were, however, extremely different. Microspheres prepared at 30 °C/h had a very smooth surface, while those prepared at 7.5–15 °C/h had a rough surface with large craters. These findings demonstrated that the surface morphology and drug release characteristic were controlled by the rate of increase of temperature.

Keywords: Microencapsulation; Solvent evaporation; Temperature-increase rate; Controlled release; Hydrophobic dextran derivate; Theophylline

1. Introduction

The microencapsulation technique based on the emulsification solvent evaporation process has been widely reported in recent years for the preparation of polymeric microspheres. One of the basic problems with the solvent evaporation method is that solvent removal can be a time-consuming process requiring up to several hours for complete evaporation (Sprockel and Prapaitrakul, 1990). Many researchers have thus studied methods to reduce the solvent evaporation time. One technique for enhancing solvent evaporation is known as the reduced pressure process (Izumikawa et al., 1991; Chung et al., 2001). In this process the solvent is rapidly removed, although the solvent evaporation rate is rarely controlled. Another method to save time is the production of microspheres under higher temperature based on the phenomenon that solvent evaporation depends largely on the temperature (Bodmeier and McGinity, 1987; Yang et al., 2000, 2001). Although an elevated temperature shortened the necessary preparation time, many drawbacks were also encountered: the recovered total weight decreased; the size distribution shifted toward the larger size; the drug loading efficiency decreased; the morphology became coarser. Thus, uncontrollable acceleration of the solvent evaporation rate led to deterioration in the quality of the microspheres for controlled drug release.

In order to precisely control the emulsion solvent evaporation rate and to modify the drug release property of microspheres, a constant temperature-increase method was proposed in the present study. A modification, heating up the system at a constant rate of temperature change, was made to a conventional solvent evaporation process, while all other formulation and preparation variables were kept the same because several variables can influence the properties of microspheres (Freiberg and Zhu, 2004). The aim of this study was to evaluate the effects of constant temperature-increase of the water bath on solvent evaporation rate and drug release characteristics of the microspheres.

In this study, model microspheres were prepared from theophylline (TH) and hydrophobic dextran derivate. TH was used

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doi:10.1016/j.ijpharm.2006.06.003

as a representative drug since sustained-release formulations are desirable because of the short elimination half-life in humans. Hydrophobically modified dextran is a water-insoluble polymer used for contact lenses in the industrial field. The solvent used for the dispersed phase solution was acetone, while the continuous phase medium was liquid paraffin. The microspheres were characterized by their size, drug loading efficiency and drug release kinetics. In order to reveal the drug release mechanism of the microspheres, the morphology of the microspheres was examined by scanning electron microscopy (SEM). In addition, drug crystallinity in the microspheres and interaction between drug and polymer were evaluated by powder X-ray diffraction analysis (XRD) and differential scanning calorimetry (DSC), respectively.

2. Materials and methods

2.1. Materials

TH as an anhydrous form was purchased from Sigma Chemical Co. (St. Luis, MO) and was used after sieving through a 200-mesh sieve (less than 75 μm). Hydrophobic dextran derivate, PDME, was kindly donated by Meito Sangyo Co. Ltd. (Nagoya, Japan) and was used without further purification. PDME is prepared from dextran (Mw 40,000) by substitution of 0.58 mol acetyl, 0.81 mol propionyl, 1.42 mol butyryl and 0.16 mol methacryloyl per anhydroglucose unit of dextran, as shown in Fig. 1. Liquid paraffin, defined to Japanese Pharmacopoeia (JP), was obtained from Iwaki Seiyaku Co. Ltd. (Tokyo, Japan). Sucrose-ester (DKF-10) was generously supplied by Dai-ichi Kogyo Seiyaku Co. Ltd. (Kyoto, Japan). Polyoxyethylene (20) sorbitan monooleate (Tween 80) was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). All other chemicals were of reagent grade and were used as received.

2.2. Preparation of microspheres

PDME (1.5 g) was dissolved into 15 mL of acetone. Then, 0.5 g of theophylline was suspended in the PDME solution by stirring with a magnetic stirrer. The resultant mixture was poured into 150 mL of liquid paraffin containing 0.75 g of DKF-10, as an emulsifier, in a vessel settled in water bath under agitation (300 rpm, 1 propeller) at 20 °C. Following emulsification for 30 min at this temperature, the system was heated up to 50 °C at the predetermined temperature-increase rate (7.5, 10, 15 or 30 °C/h). In order to remove the organic solution completely, additional heating for 30 min at 50 °C was conducted. After allowing the microspheres to settle and cool to 20 °C, the clear supernatant liquid paraffin was decanted into a waste bottle. The microspheres were washed three times by 50 mL of *n*-hexane, and dried under reduced pressure at room temperature overnight.

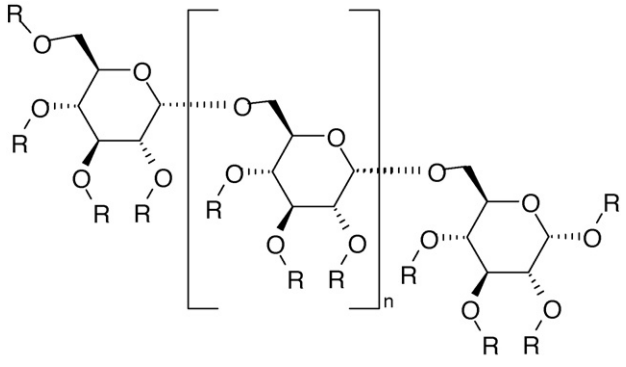

Fig. 1. Schematic structure of PDME. R = –CO–CH_3, –CO–CH_2–CH_3, –CO–$(CH_2)_2$–CH_3, –CO–$C(CH_2)$–CH_3 or H.

2.3. Determination of solvent evaporation rate

In order to examine the solvent evaporation rate, the preparation procedures mentioned above were conducted without drug and polymer. At a specific time during the procedures, all of the dispersed phase and continuous phase were removed into a vessel and weighed, and were then dried at 105 °C for 6 h in an oven and weighed again. The amount of residual solvent was calculated from the weight loss after drying. The percentage of solvent evaporated until that time was determined from the following equation:

$$\text{percent evaporated} = \frac{\text{initial amount} - \text{residual amount}}{\text{initial amount}} \times 100 \quad (1)$$

2.4. Recovery and particle size

Product recovery was determined from the weight ratio of dried microspheres to the loaded drug and polymer. Drug content was analyzed as follows. Microspheres containing approximately 10 mg of TH were accurately weighed and were completely dissolved in methylene chloride, and then drug concentration was determined spectrophotometrically at 274 nm. Encapsulation efficiency was calculated as in the following equation:

$$\text{encapsulation efficiency (\%)} = \frac{\text{drug content (\%)}}{\text{theoretical loading (\%)}} \times 100 \quad (2)$$

The particle size and distribution of microspheres were determined by sieving through a set of standard sieves with openings of 75, 106, 150, 212, 250, 300, 355, 425, 500, 600 and 710 μm. Each sample was placed on the uppermost sieve and shaken enough to separate. The individual sieves were weighed to determine the amount of microspheres retained in each. From the weight distribution, the geometric mean diameter was calculated for each sample. Microspheres with diameters in the range 75–250 μm were used for further studies.

2.5. Release test

In vitro release studies were carried out at 37 ± 0.5 °C in 900 mL of JPXIV 2nd fluid (pH 6.8, 0.05 M H_2KPO_4 and 0.0236 M NaOH) with 0.1% Tween 80 using a standard JPXIV dissolution apparatus with paddle stirrers (100 rpm). Since the microspheres had a tendency to float in water, Tween 80 was

added to dissolution medium in order to improve the wettability of microspheres. Five milliliters of samples were withdrawn from the dissolution vessels at 0.25, 0.5, 1, 2, 4, 6, and 8 h and immediately replaced with an equal volume of the same test fluid. Samples were filtered with a membrane filter (pore size 0.45 μm). The filtrate was analyzed spectrophotometrically at 274 nm for TH content. The data represent an average of three experiments.

2.6. SEM

The surface morphology and internal structure of microspheres were observed with a scanning electron microscope (JSM-5600LV, JEOL, Japan). Prior to observation, samples were coated with gold using a vacuum evaporator.

2.7. DSC

The thermal analysis was conducted using a differential scanning calorimeter (DSC 8240D, Rigaku Co., Tokyo, Japan). Approximately 5 mg of samples were hermetically sealed into aluminum pans and scanned over 25–300 °C against aluminum oxide at a heating rate of 10 °C/min.

2.8. XRD

Powder X-ray diffraction patterns were obtained using an X-ray diffractometer (RINT 1400, Rigaku Co., Tokyo, Japan) at 200 mA and 60 kV. The samples were placed in a glass holder and scanned over $3° < 2\theta < 60°$ at a rate of 2°/min.

3. Results

3.1. Solvent evaporation rate

Fig. 2 shows the preset temperature-increase schedules and the plots of actual temperature in the liquid paraffin during the preparation procedure. The temperature of the system was maintained at 20 °C for the initial 30 min to form uniformly dispersed emulsion droplets. The system was then heated up to 50 °C at the preset rate; either 7.5, 10, 15 or 30 °C/h. It can be seen that the temperature of the continuous phase increased at a constant rate after heating up, with a slight delay compared to the preset temperature-increase schedule in all cases.

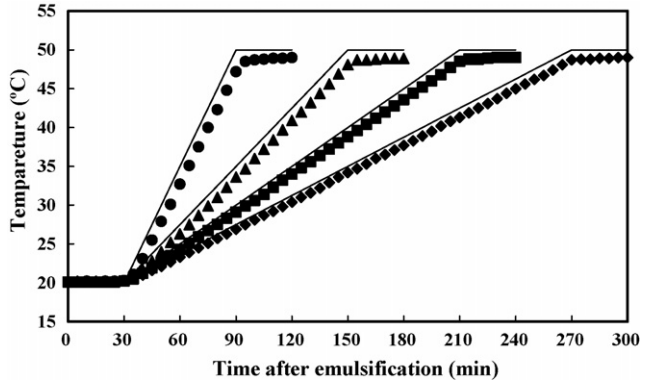

Fig. 2. Change in temperature of the continuous phase during preparation. Solid lines represent the preset temperature-raising schedules. Temperature-increase rate: ●, 30 °C/h; ▲, 15 °C/h; ■, 10 °C/h; ♦, 7.5 °C/h.

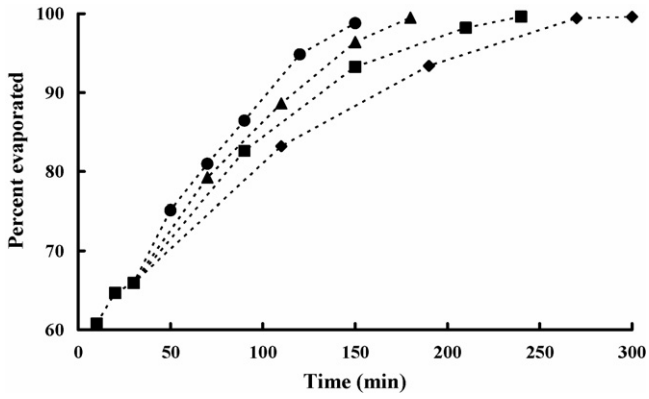

Fig. 3. Evaporation profiles of acetone from preparative systems at various heating rates. Temperature-increase rate: ●, 30 °C/h; ▲, 15 °C/h; ■, 10 °C/h; ♦, 7.5 °C/h.

Fig. 3 shows solvent evaporation profiles from the systems during the preparation procedures. Most of the acetone was rapidly dispersed into the liquid paraffin and was removed from the system within the initial few minutes. This is due to the fact that acetone is soluble in liquid paraffin to a limited extent, depending on the affinity between liquid paraffin and acetone. Acetone was then slowly evaporated until 30 min. After the temperature began to rise at a constant rate, acetone evaporated faster according to the preset temperature-increase rate. The partitioned acetone in the continuous phase evaporated from the surface of the continuous phase and was replaced by further partitioning of acetone from the droplets, until complete evaporation. These results illustrated that the constant rate of change of temperature was very useful in achieving a precisely controlled solvent evaporation rate.

3.2. Recovery and particle size

All of the microspheres prepared at various temperature-increase rates, 7.5–30 °C/h, were spherical, free-flowing, and non-aggregated. Any additional increase in the rate of change of temperature over 30 °C/h led to aggregation of the emulsion droplets. Particle characteristics are summarized in Table 1. MS30, MS15, MS10 and MS7.5 represent the microspheres prepared at temperature-increase rates of 30, 15, 10 and 7.5 °C/h, respectively. Product recovery varied 60.1–67.5, and drug content slightly increased as the evaporation rate increased. Thereby, TH was entrapped in the microspheres prepared from PDME with a high encapsulation efficiency of above 110%. It is likely that acetone is exuded from the solidifying microspheres along with the dissolved polymer during the dispersion and hardening process of the primary emulsion in the dispersion medium. This appeared to be responsible for the rather low product recovery and excess drug loading. The mean diameter increased from 97 to 187 μm as the evaporation rate decreased from 30 to 7.5 °C/h. This was due to the increase in viscosity of the dispersed phase

Table 1
Recovery and particle size of microspheres

Preparation	Product recovery (%)	Drug content (%)	Encapsulation efficiency (%)	Mean diameter (μm)
MS30	63.8	33.1	132.4	97
MS15	67.5	31.5	126.0	134
MS10	60.1	29.2	116.8	170
MS7.5	65.2	28.1	112.4	187

caused by the lower temperature. In general, increase in viscosity of the dispersed and dispersion phases resulted in increased mean droplet size (Kılıçarslan and Baykara, 2003).

3.3. Drug release behavior

TH release profiles from the microspheres prepared at various solvent evaporation rates are shown in Fig. 4. The drug release was significantly influenced by the solvent evaporation rate. MS30 released only 28% during 8 h, while MS7.5 released more than 80% within the same period. Several researchers have reported that a high evaporation rate causes rapid release of drug (Sato et al., 1988; Yang et al., 2000). In contrast, our results illustrated that a higher solvent evaporation rate led to a slower drug release rate. This surprising finding warrants to further examination of the drug release mechanisms.

3.4. Kinetics of drug release

In order to examine the mechanism of drug release from the microspheres, the release data (≦70%) were fitted to the following power law equation (Ritger and Peppas, 1987):

$$\frac{M_t}{M_\infty} = kt^n \quad (3)$$

where M_t/M_∞ is the fraction of drug released at time t, k the coefficient constant which accounts for the structural and geometrical properties of the matrix, and n is the diffusional exponent indicative of the mechanism of drug release. According to Ritger and Peppas (1987), a value of the exponent, $n = 0.5$, $0.5 < n < 1$, $n = 1.0$ indicates Fickian diffusion, non-Fickian diffusion and zero-order transport, respectively. The results are summarized in Table 2. The values of diffusional exponent, n, for the microspheres were between 0.658 and 0.828, indicating non-Fickian kinetics. The drug release mechanisms of these microspheres were the same, while coefficient parameter, k, of MS7.5 was seven-fold larger than that of MS30. In polymeric microspheres, an initial fast release can be caused by a large proportion of drug near the surface (Kristmundsdóttir and Ingvarsdóttir, 1994) and the release rate of the remaining drugs in the polymeric matrix is governed by the channels formed by the dissolving drug (Pongpaibul and Whitworth, 1986). When the higher rate of change of temperature was used, the solvent evaporation and subsequent precipitation of drug and polymer proceeded rapidly, thereby minimizing the amount of drug gathering at the droplet boundary. At the lower temperature-increase rate, the evaporation was slower and the microspheres took longer to form, leading to more drug on or near the interface.

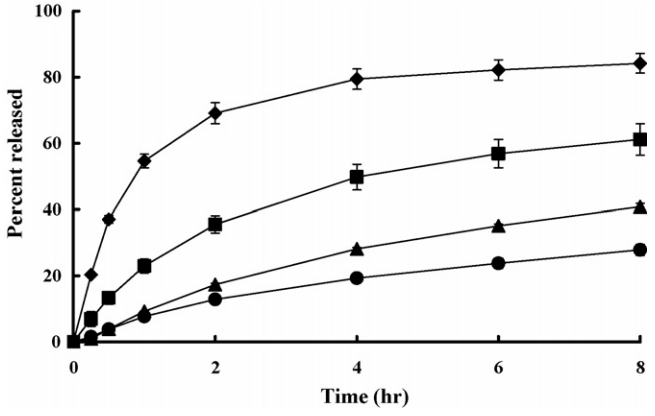

Fig. 4. Release profiles of TH from the microspheres prepared at various evaporation rates. Temperature-increase rate: ●, 30 °C/h; ▲, 15 °C/h; ■, 10 °C/h; ♦, 7.5 °C/h.

3.5. Surface morphology and internal structure

In order to clarify the causes of the difference in drug release, the surface morphology and internal structure of the microspheres were examined by SEM. Fig. 5 displays the appearance and surface of the microspheres. MS30 had an apparently smooth surface. In contrast, MS7.5, MS10 and MS15 showed a rough surface with several cracks and pores. The depth of the cracks seemed to become greater as the evaporation rate decreased while the formulation variables were kept the same. The formation of the large pores led to faster drug release because of the shorter diffusion pathway. Fig. 6 shows the cross-section of the microspheres. SEM revealed that all microspheres had a monolithic structure. Although MS30 appeared to be more rigid than the others, the differences in internal structure were unclear. Furthermore, the microspheres were removed from dissolution medium at 8 and 24 h after the release test started, and were observed by SEM. No significant structural change, however, was found (data not shown).

Table 2
Coefficients and exponents of drug release functions based on Eq. (3) for various microspheres

Preparation	Coefficient constant (k)	Diffusional exponent (n)	Correlation coefficient (r^2)
MS30	7.08	0.695	0.985
MS15	8.30	0.828	0.977
MS10	20.1	0.658	0.978
MS7.5	56.6	0.713	0.985

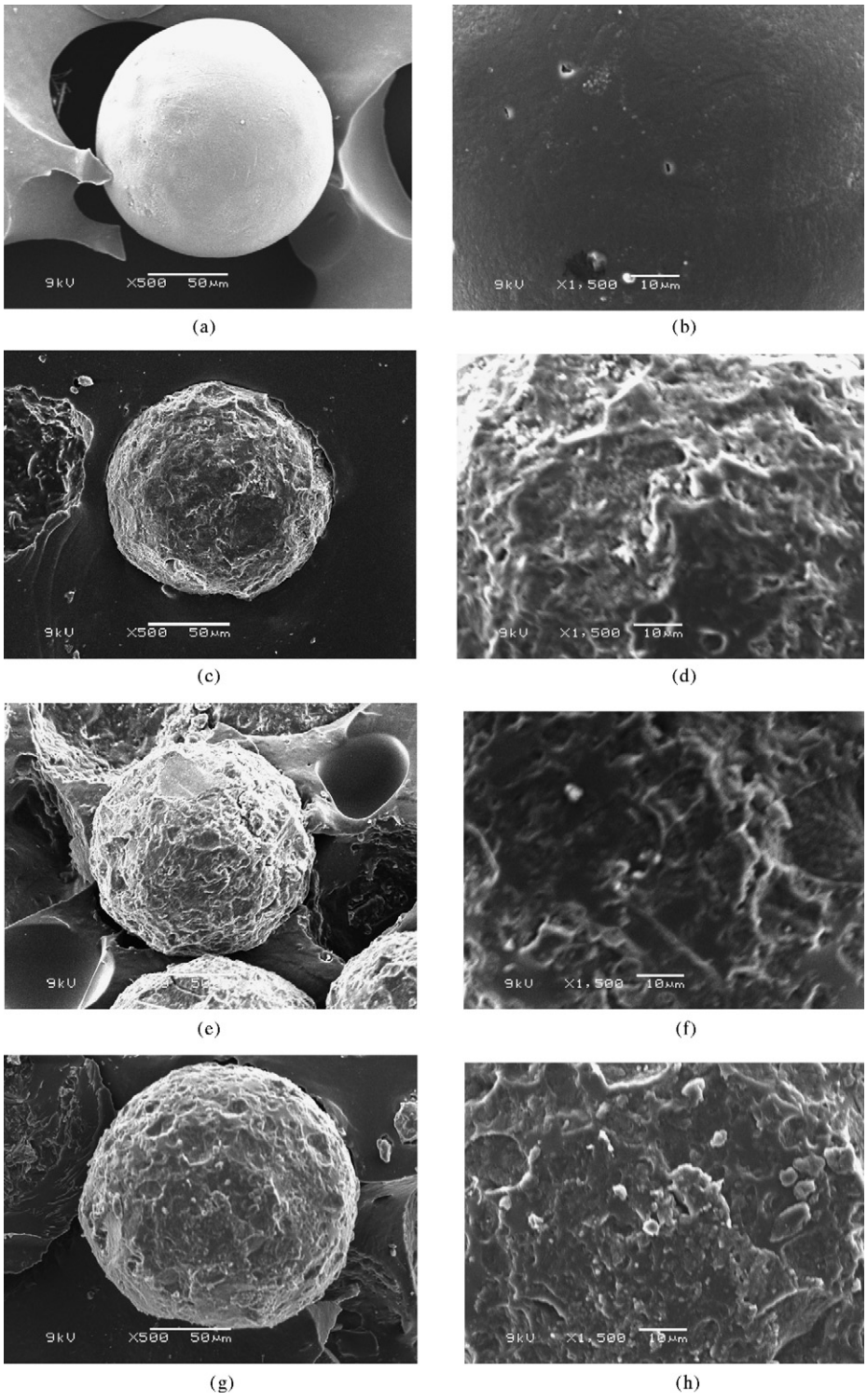

Fig. 5. Scanning electron micrographs of microspheres on magnification of 50× (a, c, e and g) and 3000× (b, d, f and h). Key: (a and b) MS30; (c and d) MS15; (e and f) MS10; (g and h) MS7.5.

3.6. Drug crystallinity

In order to evaluate the effect of the solvent evaporation rate on the thermal properties of the microspheres, intact drug, polymer and drug-loaded microspheres were introduced to DSC analysis. The DSC thermograms are shown in Fig. 7. The polymer did not show any characteristic peaks. TH showed a sharp endothermic peak around 274 °C, which was the same temperature as its reported melting point (Lin and Nash, 1993). Thermal analysis of MS30 and MS15 revealed two thermal events around 274 °C, corresponding to the recrystallization and melting of TH. These results suggested that small amount of the drugs

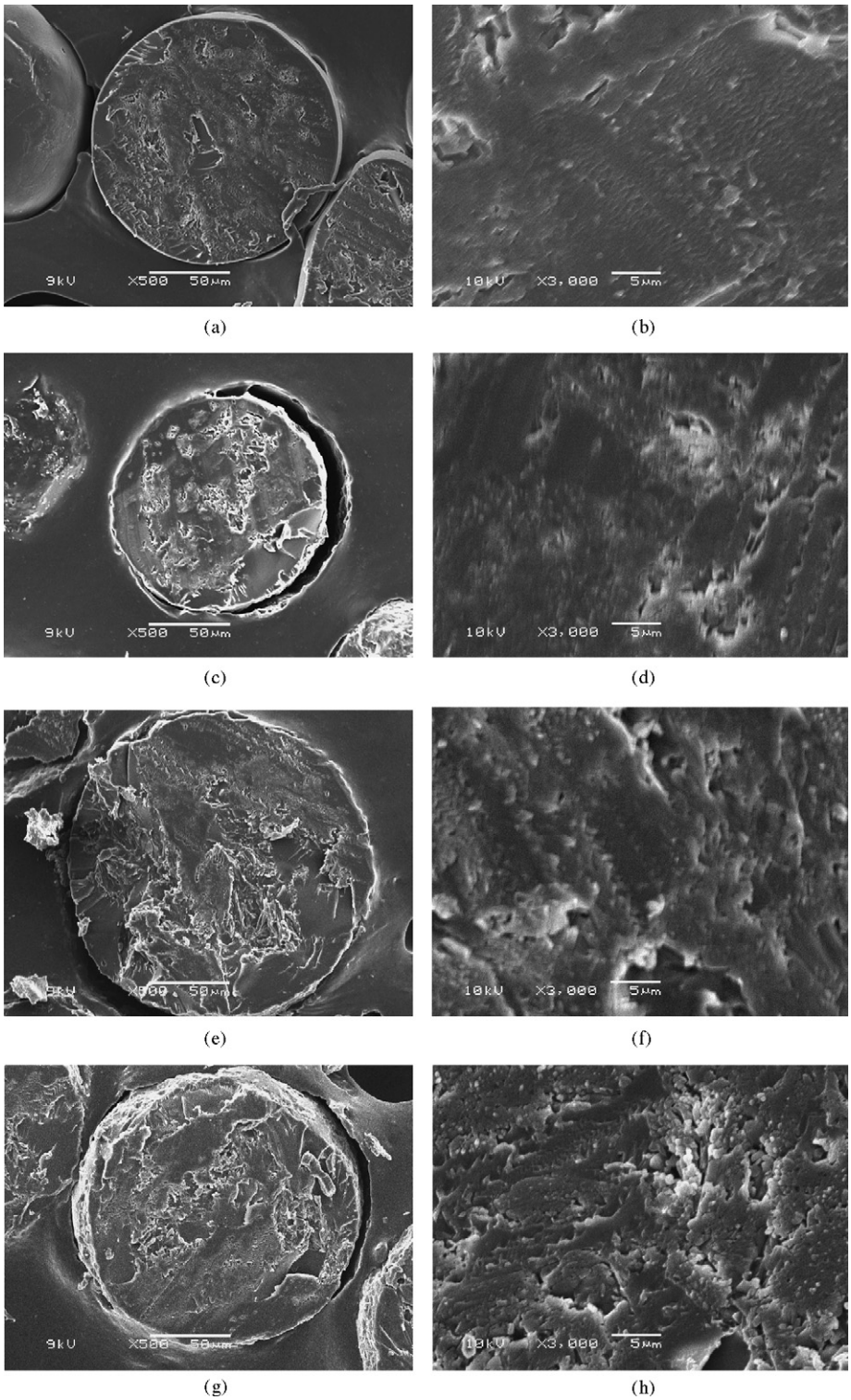

Fig. 6. Scanning electron micrographs of microspheres in cross-sectional view on magnification of 50× (a, c, e and g) and 3000× (b, d, f and h). Key: (a and b) MS30; (c and d) MS15; (e and f) MS10; (g and h) MS7.5.

existed in the microspheres as an amorphous state. For MS10 and MS7.5, the TH melting endotherms are broad, suggesting that the drug is distributed over the microsphere, even on the surface. Dubernet et al. (1991) reported that a diffuse endotherm around the drug melting point was attributed to surface drug crystals in ibuprofen-loaded ethylcellulose microspheres. Moreover, the TH melting point of MS10 and MS7.5 in the thermograms was lowered from 5 to 10 °C, suggesting possible interaction between TH and the polymer.

Fig. 8 shows the XRD spectra of pure drug, polymer and drug-loaded microspheres. The diffraction pattern for TH showed a high intensity peak at $2\theta = 12.4^{\circ}$, identical to stable anhydrous

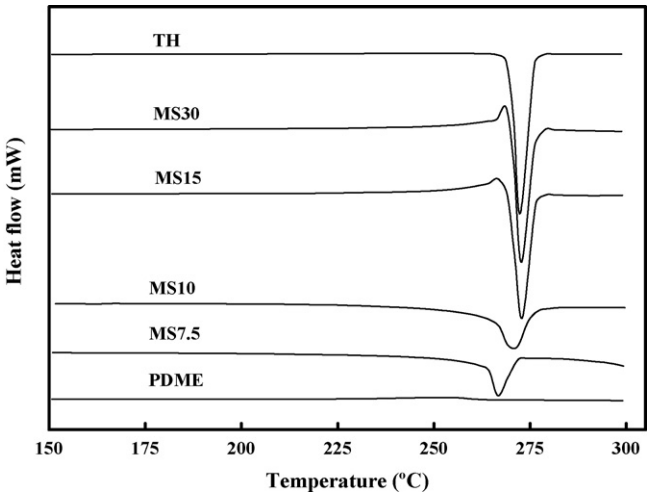

Fig. 7. DSC thermograms of TH, PDME and TH loaded microspheres.

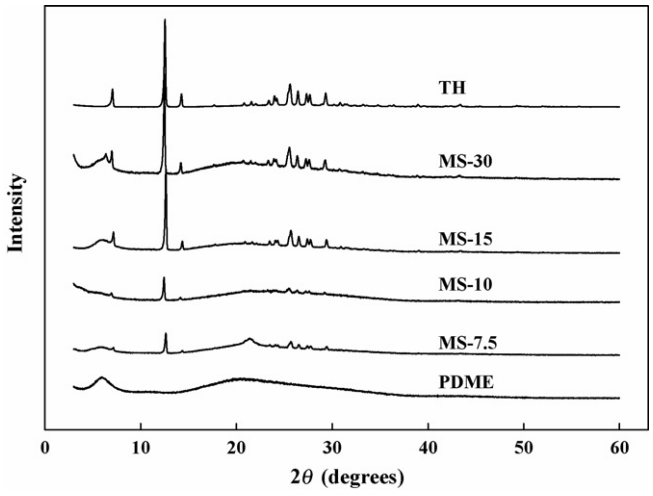

Fig. 8. XRD spectra of TH, PDME and TH loaded microspheres.

theophylline crystal (Phadnis and Suryanarayanan, 1997). The drug-loaded microspheres exhibited the characteristic peaks for TH, suggesting that the crystalline state of the drug was preserved in the microspheres. Therefore, the results supported the notion that the peaks observed in DSC charts for MS10 and MS7.5 could not be due to the melting of by-products or to partial degradation of the drug.

The above results thus indicated that TH retained its original crystallinity during the preparation of the microspheres as expected. Since TH is hardly dissolved in acetone, crystalline fragments of the drug will be incorporated in the final microsphere in the same state as initially suspended in the polymer solution. In addition, the physicochemical property of PDME was also unchanged throughout the preparation procedure.

4. Discussion

The rate of solvent removal from the solidifying microspheres prepared by the solvent evaporation method impacts on the physical properties of the microspheres. In this study, the heating rate of the system was introduced to control the solvent evaporation rate. As the heating rate increased, the evaporation rate of acetone rose in proportion (Fig. 3). The microspheres prepared at the highest solvent evaporation rate were spherical and had an extremely smooth surface (Fig. 5). Some large pores and cracks were, however, noticed on the surface of the microspheres prepared at the lower solvent evaporation rate. By use of the higher heating rate, the solvent removal rate from the droplets to the oily phase was accelerated, and the polymer dissolved in the emulsion was rapidly deposited, forming a rigid outer-shell on the surface of the emulsion. After that hard core was formed more slowly. Thus, the appearance of the microspheres became smoother and the structure became more compact as the solvent evaporation rate increased. At the lower evaporation rate, the polymer solidified at the same time as the hard core was formed, resulting in the creation of coarser microspheres.

Furthermore, it can be seen in Fig. 4 that a significant decrease in drug release rate is achieved by increasing the rate of change of temperature rate from 7.5 to 30 °C/h. The microspheres were then evaluated for their internal structure, but no visible differences were observed among all preparations (Fig. 6). Thus, it appeared that the evaporation rate did not have an appreciate effect on the release mechanism (Table 2). The factors affecting the drug release rate involve not only the structure of the matrix where the drugs are contained but also the chemical properties associated with both the polymer and the drug (Izumikawa et al., 1991). However, DSC and XRD studies revealed that the drug retained its original crystallinity during the preparation of the microspheres (Figs. 7 and 8). Unlike poly (L-lactide) (Izumikawa et al., 1991) or poly (ε-caprolactone) (Lin and Yu, 2002), no change in crystallinity of polymer was found. In general, TH release from polymeric microspheres depends on various factors including the polymer composition, polymer molecular weight, drug loading, particle size, porosity and the microstructure of microspheres (Obeidat and Price, 2003, 2004; Shukla and Price, 1989, 1991). In this study, the surface morphology and microstructure of the microsphere seemed to be critical parameters in the drug release rate because the formulation factors were fixed throughout the study. Overall, these results demonstrated that one of the major advantages of the temperature-increase method was the modification of drug release rate by maintaining control over the physical properties of the microspheres. These trends observed in PDME microspheres were practically the same as those observed in the microspheres prepared from cellulose acetate or cellulose acetate butyrate (data not shown).

5. Conclusion

In the fabrication of microspheres by solvent evaporation process, the temperature-increase method was applied to controlled solvent removal. The solvent evaporation rate was precisely controlled by the rate of change of temperature. The properties of the microspheres prepared by this method were then evaluated, and the results revealed that the surface characteristics of the microspheres and drug release rate were modified by the solvent evaporation rate. At a temperature-increase rate of 30 °C/h, the appearance of the microspheres was spherical with an extremely

smooth surface, and drug release was suppressed. In addition, the necessary time for preparation was shortened. These results demonstrated that the constant temperature-increase method was useful for the fabrication of microparticulate controlled release systems.

Acknowledgment

The authors are grateful to Meito Sangyo Co. Ltd. (Nagoya, Japan) for supplying PDME.

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