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Modulation of drug release from glyceryl palmitostearate–alginate beads via heat treatment

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Abstract

Diclofenac calcium alginate (DCA) beads containing glyceryl palmitostearate (GPS) were prepared by ionotropic gelation method. The effect of GPS amount and heat treatment on characteristics of the DCA beads was investigated. Incorporation of GPS into the DCA beads increased particle size and entrapment efficiency of diclofenac sodium (DS), but decreased water uptake in distilled water, and DS release rate. The heat treatment caused the DCA beads to be irregular shape particles and to possess higher water uptake. A slower release rate of DS in distilled water was found because of interaction of DS and alginate polymer matrix, and a restriction of water sorption into the inside region of the beads, which caused by the shrinkage of the beads after heating. However, the heat treatment did not affect particle shape and water uptake in distilled water of the 3%GPS–DCA beads. Differential scanning calorimetric study showed that GPS in the DCA beads was resolidified to different polymorph after cooling. Furthermore, the micro-Raman spectra indicated the existence of DS in the GPS matrix particles in the beads due to the partition of DS into the melted GPS during heat treatment. This led to a decrease in release rate of DS in pH 6.8 phosphate buffer and a change in DS release pattern in distilled water. Thus, not only the calcium alginate matrix, but also the resolidified GPS matrix in the alginate beads controlled the DS release from the 3%GPS–DCA beads with heat treatment.

Keywords: Calcium-alginate bead; Glyceryl palmitostearate; Heat treatment; Micro-Raman spectroscopy; Diclofenac sodium

1. Introduction

Sodium alginate (SA) is a sodium salt of alginic acid, a naturally occurring non-toxic polysaccharide found in brown algae. Alginate has been widely used as food and pharmaceutical additives, such as a tablet disintegrant and gelling agent. It contains two uronic acids, α-L-guluronic and β-D-mannuronic acids, and is composed of homopolymeric blocks and blocks with an alternating sequence (Draget, 2000). Gelation occurs by cross-linking of the uronic acids with divalent cations, such as Ca^{2+}. This phenomenon has been used to prepare an alginate bead for drug delivery system. The formation of calcium-alginate beads by ionotropic gelation was achieved by dropping the drug-containing SA dispersion into a calcium chloride bath (Østberg et al., 1994; Sugawara et al., 1994). The calcium alginate beads could protect an acid-sensitive drug from gastric juice, and the drug was consequently released from the beads in the intestine (Hwang et al., 1995; Fernández-Hervás et al., 1998). Thus, drug-loaded alginate beads are advantageous for nonsteroidal anti-inflammatory drugs, which caused gastric irritation, and for proteins, which were unstable in stomach. This bead also protects protein against enzymatic degradation in the body, and allows proteins to be released at a controllable rate into a localized area (Gu et al., 2004). Moreover, the alginate beads exhibited a potential for a pulsatile release system of macromolecular drugs (Kikuchi et al., 1997).

Drug released from calcium-alginate beads depends on the swelling of the beads and the diffusion of the drug in the gel matrix (Sugawara et al., 1994). The release characteristics of the entrapped substances could be improved by surface complexation of alginate with chitosan, cationic polysaccharide (Murata et al., 1993a; González-Rodríguez et al., 2002; Anal and Stevens, 2005), and incorporation of some water-soluble polymers into the beads, such as chondroitin sulfate (Murata et

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al., 1996), konjac glucomannan (Wang and He, 2002), gelatin (Almeida and Almeida, 2004), and sodium starch glycolate (Puttipipatkhachorn et al., 2005). Furthermore, the addition of chitin (Murata et al., 2002) and magnesium aluminum silicate (Puttipipatkhachorn et al., 2005), water insoluble substances, could retard drug release of the beads in pH 6.8 dissolution medium. These results were explained by the complexation between the carboxyl groups of alginate, and the amino groups of chitin and the silanol groups of magnesium aluminum silicate, respectively.

The other approach for modifying drug release from the calcium alginate beads was the dispersion of drug into melted wax before encapsulating in calcium alginate beads. The beads obtained from this method gave a sustained-release behavior of drug (Mirghani et al., 2000). In this present study, we intended to prepare diclofenac calcium alginate (DCA) beads incorporating wax particles using ionotropic gelation method and followed by heat treatment. This preparation method might cause a change in physicochemical properties of the DCA beads. Glyceryl palmitostearate (GPS), a mixture of mono-, di-, and triglycerides of palmitic and stearate acid, was chosen for this study because it had a low melting point (53–57 °C) and has been used traditionally as a lubricant and an excipient in sustained release preparations in oral dosage forms (Bodmeier et al., 1990). Therefore, the effect of GPS and heat treatment (at 65 °C) on the particle size, thermal behavior, drug entrapment efficiency, water uptake, and in vitro drug release of the DCA beads was investigated. Moreover, a change in resolidified GPS particles in the alginate beads was also examined using micro-Raman spectroscopy.

2. Materials and methods

2.1. Materials

Diclofenac sodium (DS) was a gift from Biogena Ltd (Limassol, Cyprus). Glyceryl palmitostearate (Precirol® ATO5) and sodium alginate NF17 were purchased from Gattefossé (Gennevilliers, France) and Srichand United Dispensary Co., Ltd. (Bangkok, Thailand), respectively. All other reagents used in this study were of analytical grade and used as received.

2.2. Preparation of DCA beads

SA (1.5% w/v) was dissolved in distilled water with agitation, and then DS (1% w/v) was added and completely dissolved with a homogenizer for 5 min. DS–SA dispersion (80 ml) was dropped through a 1.2 mm inner diameter nozzle into 0.45 M calcium chloride solution (200 ml) with gentle agitation. The gel beads were cured in this solution for 1 h, then filtered, rinsed with distilled water, and dried at room temperature (26–27 °C) for 4 and 5 days. To prepare DCA beads containing GPS, GPS (0.5, 1 or 3% w/v) was incorporated into the DS–SA dispersion using a homogenizer and followed the preparation process as described above. After curing, the 3%GPS–DCA gel beads in calcium chloride solution were heated for 10, 20 or 45 min in a water bath that temperature was controlled at 65 °C. Then, the gel beads were cooled down to 45 °C at a rate of 6 °C/min, filtered, rinsed with distilled water, and dried at room temperature.

2.3. Particle size analysis

Particle size of DCA beads was determined using an optical microscope (Nikon, Japan). Three hundreds beads were randomized and their Feret diameters were measured.

2.4. Drug content and entrapment efficiency determinations

Weighed DCA beads were immersed and dispersed in 100 ml of 0.033 M phosphate buffer at pH 6.8 for 12 h. The solution was then filtered, and the DS content was assayed by UV-spectrophotometer (Shimadzu UV1201, Kyoto, Japan) at wavelength of 260 nm. The DS entrapment efficiency was calculated according to the ratio of actual to theoretical drug contents in the DCA beads (Wang and He, 2002).

2.5. Scanning electron microscopic studies

The surface morphology and internal structure of DCA beads were observed using scanning electron microscopy (SEM). Samples of the dried beads were mounted onto stubs, sputter coated with gold in a vacuum evaporator, and photographed using scanning electron microscope (Jeol Model JSM-5800LV, Tokyo, Japan).

2.6. Differential scanning calorimetry (DSC)

The DSC thermograms of GPS, DCA beads and GPS–DCA beads were recorded using a differential scanning calorimeter (DSC822, Mettler Toledo, Switzerland). Each sample (2.0–2.5 mg) was accurately weighed into a 40-μl aluminum pan without an aluminum cover. The measurements were performed over 30–370 °C at a heating rate of 10 °C/min.

2.7. Fourier transform infrared (FTIR) spectroscopy

FTIR spectra of DS, GPS, DCA beads, and 3%GPS–DCA bead with and without heat treatment were recorded with a FTIR spectrophotometer (Spectrum One, Perkin-Elmer, Norwalk, CT) using KBr disc method. Each sample was gently triturated with KBr powder in a weight ratio of 1:100 and then pressed by a hydrostatic press at a pressure of 10 tonne for 5 min. The disc was placed in the sample holder and scanned from 4000 to 450 cm^{-1} at a resolution of 4 cm^{-1}.

2.8. Micro-Raman spectroscopy

Raman spectra of DS and GPS, which were compressed using a hydrostatic press, and GPS particle in the 3%GPS–DCA beads without and with heat treatment for 45 min were measured using a micro-Raman spectrophotometer (Jobin Yvon T64000, Horiba, France). The 514.5 nm argon ion laser operated at 30 mW of power was focused over less than a 2 μm diameter

Table 1
Characteristics of DCA beads

Component/condition	Mean diameter[a] (mm)	DS content[b] (% w/w)	DS entrapment efficiency[b] (%)	Water uptake in pH 6.8 phosphate buffer[b] (%)		Water uptake in distilled water[b] (%)	
				30 min	60 min	30 min	60 min
1.5% (w/v) DCA beads	1.19 ± 0.16	19.2 ± 0.2	47.9 ± 0.6	69.6 ± 5.7	160.8 ± 15.7	64.7 ± 5.0	63.1 ± 9.4
Heated 20 min	1.24 ± 0.11	15.8 ± 0.4	39.5 ± 0.9	79.3 ± 8.4	131.6 ± 24.7	61.1 ± 2.2	58.9 ± 2.2
Heated 45 min	1.09 ± 0.12	19.9 ± 0.2	49.7 ± 0.5	135.6 ± 21.4	554.2 ± 58.4	72.1 ± 4.7	91.5 ± 8.2
+0.5% (w/v) GPS	1.25 ± 0.15	18.3 ± 0.1	55.0 ± 0.2	99.2 ± 5.7	180.4 ± 21.1	58.4 ± 1.2	67.3 ± 13.5
+1.0% (w/v) GPS	1.23 ± 0.13	18.9 ± 0.2	66.2 ± 0.6	98.4 ± 5.7	216.6 ± 19.3	65.7 ± 9.6	61.9 ± 10.2
+3.0% (w/v) GPS	1.38 ± 0.14	12.9 ± 0.1	70.8 ± 0.4	97.3 ± 13.7	145.1 ± 70.8	54.1 ± 4.8	50.7 ± 3.6
Heated 10 min	1.69 ± 0.17	9.3 ± 0.1	51.0 ± 0.6	81.2 ± 11.9	146.9 ± 4.7	51.5 ± 1.8	64.1 ± 9.6
Heated 20 min	1.45 ± 0.11	11.4 ± 0.4	62.5 ± 2.1	83.4 ± 10.6	206.4 ± 50.0	56.0 ± 1.6	54.0 ± 2.6
Heated 45 min	1.45 ± 0.13	10.7 ± 0.2	58.7 ± 0.9	88.3 ± 6.5	218.1 ± 42.9	52.2 ± 2.8	49.5 ± 5.1

[a] Data are mean ± S.D., $n = 300$.
[b] Data are mean ± S.D., $n = 3$.

circle area by using a Raman microprobe with an 100× eyepiece. The scattered light, dispersed by the spectrophotometer, was detected by a charge-coupled device with a spectra resolution at 0.3 cm^{-1}. The system was calibrated using Si spectra at 521 cm^{-1} before and after measurement.

2.9. Water uptake determination

Weighed DCA beads were placed in a small basket and soaked in 0.033 M phosphate buffer at pH 6.8 or distilled water in medium-filled containers at 26 ± 1 °C. The containers were shaken occasionally. After a predetermined time interval, each basket was withdrawn, blotted to remove excess water and immediately weighed (W_t). The wet beads were then dried in a hot air oven at 37 °C for 16–20 h to obtain constant weight (W_d). The water uptake was calculated from the following equation:

$$\text{Water uptake}\,(\%) = \left(\frac{W_t - W_d}{W_d}\right) \times 100$$

where W_t and W_d are the wet and dry mass of beads, respectively. Water uptake study of the beads in pH 6.8 phosphate buffer was performed for 60 min because the swollen beads was broken and could not be blotted to remove an excess water at the longer time.

2.10. In vitro drug release studies

A USP dissolution apparatus I (Hanson Research, Northridge, CA) was used to characterize the release of DS from the DCA beads. The baskets were rotated with a rate of 50 rev/min at 37 ± 0.5 °C. The dissolution media used were 0.033 M phosphate buffer at pH 6.8 and distilled water. The amount of the DCA beads added to 750 ml dissolution medium was equivalent to DS 25 mg. Samples (5 ml) were collected and replaced with a fresh medium at various time intervals. The amount of drug released was analyzed spectrophotometrically at 260 nm (Shimadzu UV1201, Japan). All dissolution runs were performed in triplicate. According to drug release profiles, the times (in minutes) to achieve 20, 50, or 75% of drug released in dissolution media were calculated for comparing the investigated factors.

2.11. Statistical analysis

One-way analysis of variance (ANOVA) with the least significant difference (LSD) test for multiple comparisons was performed using SPSS program for MS Windows, release 10.0 (SPSS Inc., Chicago, IL) to determine the significant effect of the investigated parameters on DS entrapment efficiency, water uptake, and drug release of the DCA beads. The significance of the difference was determined at 95% confident limit ($\alpha = 0.5$) and considered to be significant at a level of $P < 0.05$.

3. Results and discussion

3.1. Physical properties of the GPS–DCA beads

The particle size of the DCA beads is shown in Table 1. Incorporation of GPS caused an increase in particle size of the DCA beads. The heat treatment for 45 min slightly decreased particle size of the DCA beads, but tended to increase particle size of 3%GPS–DCA beads. The beads produced without heat treatment were spherical as shown by SEM photographs (Fig. 1a and b). The DCA beads heated for 45 min had an irregular shape (Fig. 1c) but no change of particle shape was observed when heated for 20 min. For the 3%GPS–DCA beads, the particle shape did not change after heat treatment (Fig. 1d). The internal structure of the 3%GPS–DCA beads with heat treatment presented that the resolidified GPS particles were distributed and embedded in the calcium alginate matrix (Fig. 1e). The DS entrapment efficiency of the DCA beads increased significantly ($P < 0.05$) with increasing amount of GPS added (Table 1). Heat treatment provided a significant decrease ($P < 0.05$) in the DS entrapment efficiency of the 3%GPS–DCA beads. Heating for 20 min gave a statistically lower DS entrapment efficiency ($P < 0.05$) of the DCA beads. In contrast, the DS entrapment efficiency of the DCA beads heated for 45 min had a significantly higher value ($P < 0.05$) than that without heating.

Incorporation of GPS into the DCA beads tended to increase the particle size and improved the DS entrapment efficiency. The DS entrapment efficiency of the DCA beads increased with increasing amount of GPS added. This indicated that a

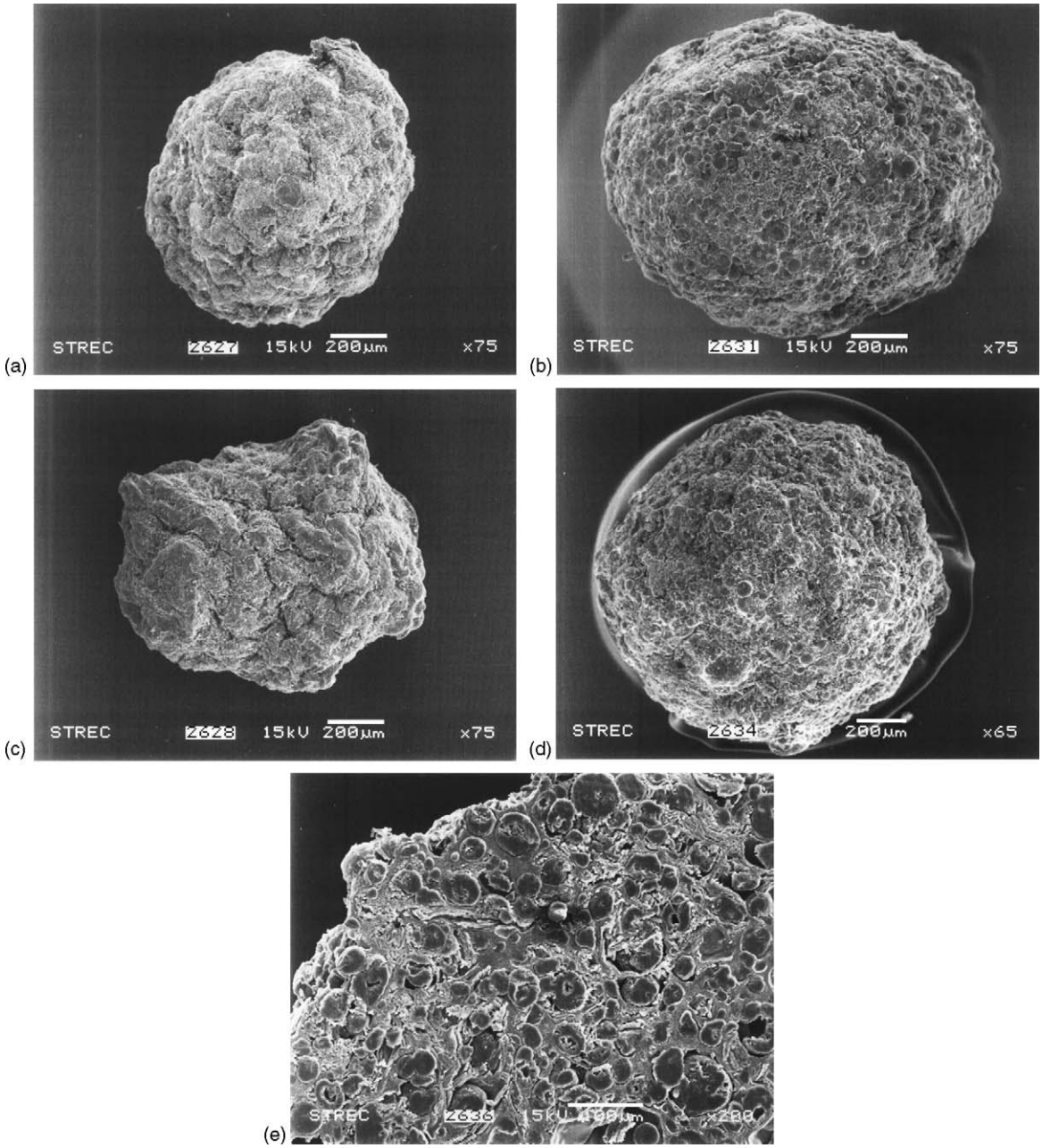

Fig. 1. Microscopic morphology of DCA bead (a), 3%GPS–DCA bead (b), DCA bead (c) and 3%GPS–DCA bead heated for 45 min (d), and internal structure of 3%GPS–DCA bead heated for 45 min (e).

hydrophobic property of GPS caused an increase in barrier for preventing a water leakage from the beads during the preparation period (Dashevsky, 1998), which led to reduce the DS loss from the beads.

Heat treatment at 65 °C seemed to decrease the DS entrapment efficiency of the DCA beads and 3%GPS–DCA beads. It was due to higher liberation of DS from the beads at higher temperature. The decrease of the DS entrapment efficiency was only 10–20% because a saturation of DS in calcium chloride solution reduced concentration gradient of DS in the beads and a lower release rate of DS during heat treatment was thus obtained. However, the DCA beads heated for 45 min gave a higher DS entrapment efficiency than the DCA beads without heating because a longer duration of heating might induce a leaching of some alginates that did not interact with calcium ions. This led to decreasing of total solid content, and increasing of DS content and entrapment efficiency was thus obtained. The leaching of alginate might be caused a higher porosity of an internal structure of the wet DCA beads. Besides, a decrease of gel strength of calcium alginate after heating might be occurred (Leo et al., 1990). Then, a rearrangement and a change in microscopic structure of a matrix structure of the beads during the drying step after heat treatment might be occurred. These led to shrinkage of some regions of the DCA beads. Therefore the irregular shape and smaller particle size of the DCA beads headed for 45 min was observed. On the other hand, the 3%GPS–DCA beads could hold

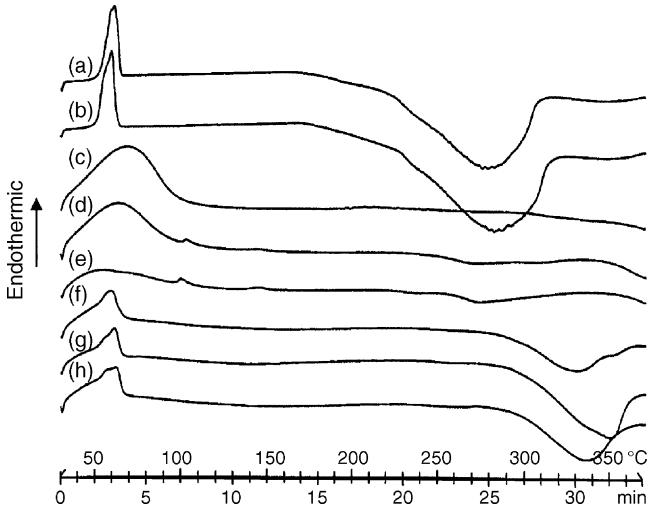

Fig. 2. DSC curves of GPS (a), resolidified GPS (b), calcium alginate bead (c), DCA bead (d), DCA bead heated for 45 min (e), 3%GPS–DCA bead heated for 0 min (f), 10 min (g), and 45 min (h).

the particle shape after heating because melted GPS could retard the leaching of large molecules of alginates from the beads.

3.2. Thermal behavior of the GPS–DCA beads

GPS and resolidified GPS showed a different shape of melting peak around 60–62 °C (Fig. 2a and b) and a broad decomposition peak at 280 °C. The calcium alginate beads and the DCA beads without heat treatment presented a broad endothermic peak at 70 °C (Fig. 2c and d). This peak was due to an evaporation of water residue in the beads. It can be observed that the water residue in the DCA beads with heat treatment for 45 min was less than that without heat treatment (Fig. 2e). This was due to a higher evaporation of water inside the DCA beads during drying process that occurred from a decrease of gel strength and an increase of porosity of the beads after heating. Consequently, the DCA beads with heat treatment shrank and gave an irregular shape when observed using SEM (Fig. 1c). DSC curves of the 3%GPS–DCA beads showed a melting peak of GPS at 60 °C and an exothermic peak of around 320–350 °C (Fig. 3f) that was assigned to the decomposition of the melted GPS. The different shape of the endothermic peaks around 61–62 °C was observed in the 3%GPS–DCA beads with heat treatment for 10 and 45 min (Fig. 2g and h). This indicated that GPS particles in the beads were melted by heat treatment and subsequent resolidified to different polymorphic forms by cooling (Sutananta et al., 1994; Hamdani et al., 2003).

3.3. FTIR and micro-Raman studies

FTIR spectra of the calcium alginate bead and the DCA beads are presented in Fig. 3a and b, respectively. The peaks of the calcium alginate beads around 1637, 1428, and 1031 cm^{-1} represented the stretching of COO^- (asymmetric), COO^- (symmetric), and C–O–C, respectively (Fig. 3a). These peaks presented a lower intensity when compared with the peaks of SA because an ionic bonding between calcium ions and carboxyl

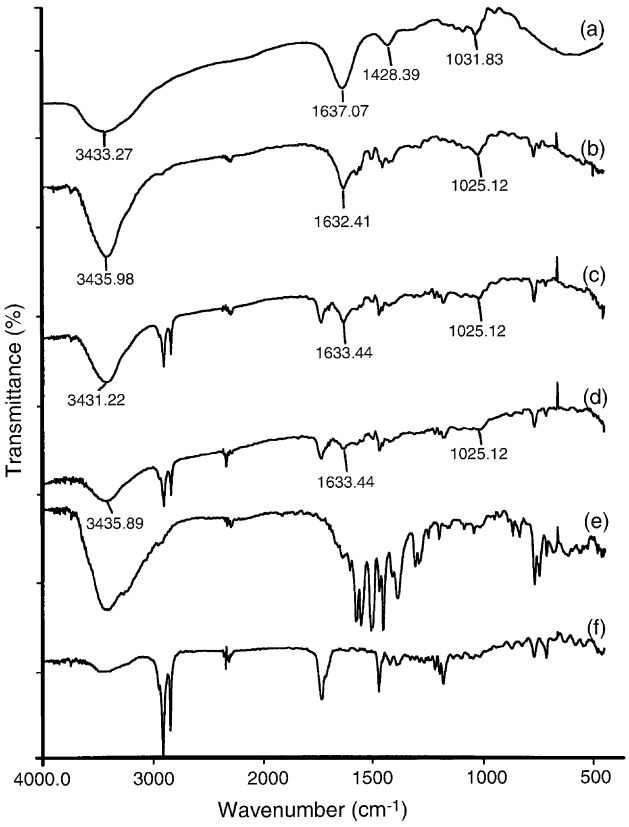

Fig. 3. FTIR spectra of calcium alginate bead (a), DCA bead (b), 3%GPS–DCA bead heated for 0 min (c) and 45 min (d), DS (e), and GPS (f).

groups of SA and a partial covalent bonding between calcium and oxygen atom, which has been previously reported by Sartori et al. (1997). The spectra of the DCA beads showed the peaks around 1575–1386 cm^{-1} of DS, and a remarkable shift to lower wavenumber of COO^- (asymmetric) and C–O–C stretching peaks of calcium alginate. This suggested DS–alginate interaction in the bead matrix. It was possible to describe that amino groups of diclofenac could protonate in SA dispersion and then interacted with carboxyl and ether groups of alginate before cross-linking process. Incorporation of GPS into the DCA beads did not affect the peaks of the DCA beads (Fig. 3c). Moreover, the similar result was observed in the 3%GPS–DCA beads with heat treatment for 45 min (Fig. 3d). This suggested no interaction of GPS and the components in the beads with and without heat treatment.

Micro-Raman spectroscopy was used for investigating a change in the resolidified GPS particles in the 3%GPS–DCA beads. The spectra of DS showed a broad peak at around 1590 cm^{-1} (Fig. 4a), which correlated with the FTIR spectra (Fig. 3e). GPS spectra had two obvious peaks at 2849 and 2882 cm^{-1} (Fig. 4b). The GPS particle in the 3%GPS–DCA beads without heat treatment gave the similar spectra with GPS (Fig. 4c), whereas the resolidified GPS particle in the 3%GPS–DCA beads with heat treatment for 45 min showed a different pattern of spectra (Fig. 4d), which presented a broad peak at the same wavenumber of DS. This suggested that the existence of DS in the resolidified GPS. In the heat treatment

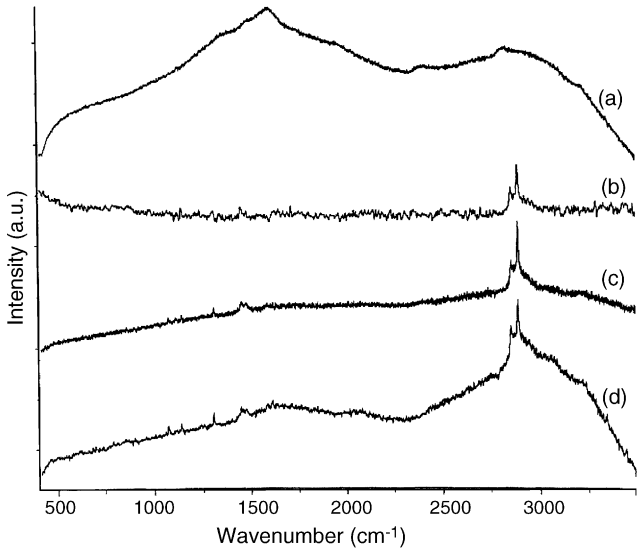

Fig. 4. Micro-Raman spectra of DS (a), GPS (b), GPS particle in 3%GPS–DCA bead without heat treatment (c) and resolidified GPS particle in 3%GPS–DCA bead with heat treatment for 45 min (d).

condition, the ratio of DS, pK_a = 4 (Adeyeye and Li, 1990), to diclofenac acid in 0.45 M calcium chloride solution (pH 5.63) was about 42.7. The DS in the form of free acid could partition into the melted GPS during heating. Moreover, the ionized form of DS could form an ion pair with sodium ions in more hydrophobic environment of the beads with melted GPS. These ion-pairs of DS had a higher hydrophobicity (Fini et al., 1999), which could also partition into the melted GPS. When the DCA beads were cooled, the DS was solidified and dispersed in the GPS matrix at higher extent. This change could affect the release characteristics of the 3%GPS–DCA beads.

3.4. Water uptake of the GPS–DCA beads

The water uptake of DCA beads in various media is listed in Table 1. In pH 6.8 phosphate buffer, the water uptake at 60 min of the DCA beads tended to decrease when incorporating GPS. Heat treatment for 45 min of the DCA beads caused a significantly higher water uptake ($P < 0.05$) when compared with the beads without or with heat treatment for 20 min. Moreover, the water uptake at 60 min of the 3%GPS–DCA beads slightly increased with increasing duration of heating. The water uptake of the calcium alginate beads in pH 6.8 phosphate buffer occurred because calcium ions cross-linked with alginate were exchanged with sodium ions in the medium (Østberg et al., 1994). The partial formation of SA induced water uptake into the beads. Moreover, calcium alginate gels could be solubilized by the addition of phosphate ion, which acted as calcium ions complexing agent at a pH above 5.5 (Remuñán-López and Bodmeier, 1997). Therefore the water uptake of the DCA beads in phosphate buffer occurred mainly from the formation of SA. This led to a significantly higher water uptake ($P < 0.05$) when compared with using distilled water (Table 1). The effect of the addition of GPS into the DCA beads and heat treatment of the 3%GPS–DCA beads on the water uptake in this medium was unclear. However, the highest amount of GPS in the DCA beads decreased the water uptake at 60 min because GPS increased hydrophobic property in the swollen beads and led to retard the water uptake of the DCA beads. The heat treatment gave an obvious increase the water uptake of the DCA beads. This may be due to a higher porosity of the beads after heating and a change in microscopic structure of calcium alginate matrix, which led to a faster uptake of water and a large amount of SA changed in the beads. In the case of the 3%GPS–DCA beads with heating, heat treatment could change a property of interface between resolidified GPS and calcium alginate matrix. This resulted in a more hydrophobic property of the interface channels. However, the change of microscopic structure of calcium alginate matrix after heating for 45 min and the rapid occurrence of SA in this medium had obviously affected the water uptake. This might lead to a higher water uptake of the 3%GPS–DCA beads with heating for 45 min.

In distilled water, a statistically lower water uptake at 30 and 60 min of the 3%GPS–DCA beads ($P < 0.05$) was observed when compared with the DCA beads. The DCA beads with heat treatment for 45 min had the highest water uptake, whereas the

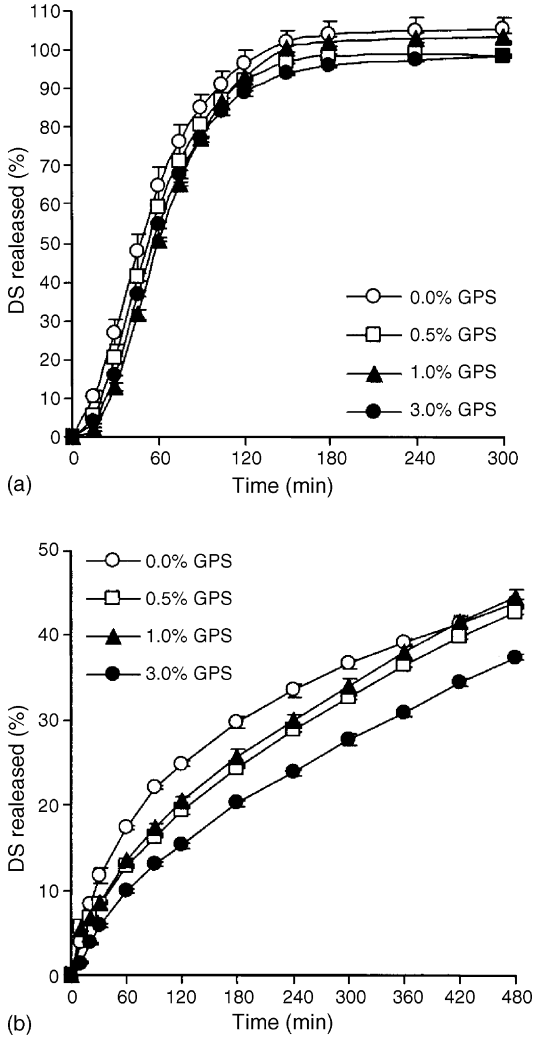

Fig. 5. Effect of GPS on the release of DS from the DCA beads in pH 6.8 phosphate buffer (a), and distilled water (b). Each point is the mean ± S.D., $n = 3$.

Table 2
DS release rate of DCA beads in various dissolution media

Component/condition	pH 6.8 phosphate buffer		Distilled water, T_{20} (min)
	T_{50} (min)	T_{75} (min)	
1.5% (w/v) DCA beads	47.31 ± 3.86	73.39 ± 6.27	76.43 ± 1.46
Heated 20 min	45.09 ± 0.73	66.16 ± 2.19	75.62 ± 4.15
Heated 45 min	49.29 ± 0.45	69.57 ± 1.25	316.74 ± 8.67
+0.5% (w/v) GPS	52.22 ± 0.74	81.24 ± 1.32	128.36 ± 3.15
+1.0% (w/v) GPS	59.21 ± 0.42	87.22 ± 0.16	116.06 ± 5.39
+3.0% (w/v) GPS	56.11 ± 0.56	86.87 ± 1.40	177.40 ± 4.51
Heated 10 min	66.10 ± 1.01	115.65 ± 2.25	125.29 ± 3.94
Heated 20 min	69.98 ± 1.59	115.46 ± 2.47	282.10 ± 1.05
Heated 45 min	73.06 ± 0.67	137.11 ± 1.10	239.93 ± 5.64

Data are mean ± S.D., $n = 3$. T_{20}, T_{50}, and T_{75} = the times to achieve 20, 50, and 75% of DS released from the DCA beads.

heat treatment did not change obviously the water uptake of the 3%GPS–DCA beads. The DCA beads were more stable in distilled water. This led to a restrict of water uptake into the beads. Incorporation of GPS decreased the water uptake of the DCA beads because of a more hydrophobic property of the DCA beads after adding GPS. Heat treatment for 45 min provided an increase in water uptake of the DCA beads. This may be due to a high porosity and a decrease of gel strength of calcium alginate beads after heating, leading to a higher water uptake especially at 60 min. However, the time of heat treatment had slightly affected

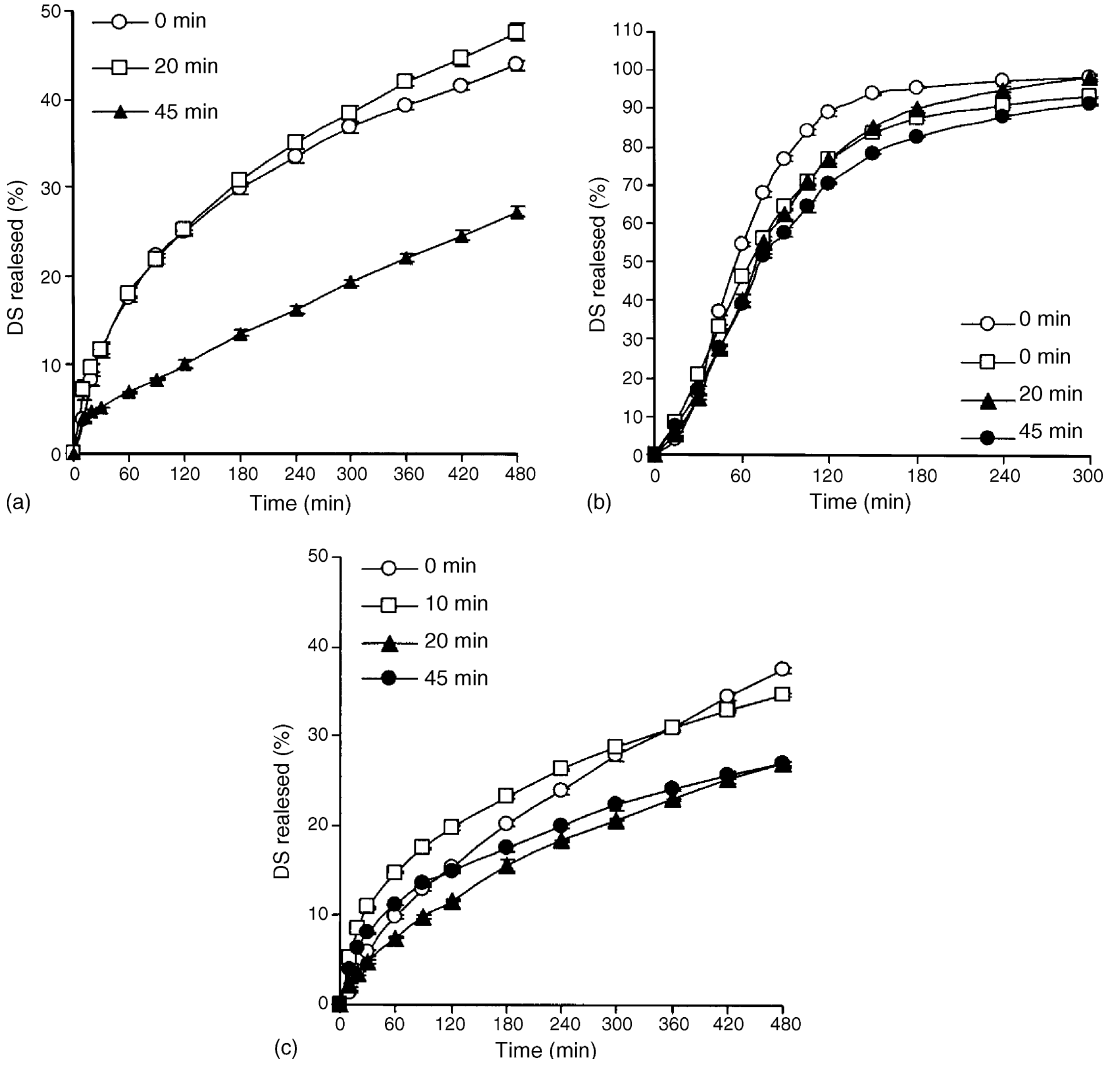

Fig. 6. Effect of heat treatment on the release of DS from the DCA beads in distilled water (a), and the 3%GPS–DCA beads in pH 6.8 phosphate buffer (b) and distilled water (c). Each point is the mean ± S.D., $n = 3$.

the water uptake of the 3%GPS–DCA beads because resolidified GPS dispersed in the calcium alginate matrix could retard the water uptake, although the gel strength of calcium alginate might be decreased after heating.

3.5. In vitro release studies

The release profiles of DS from the DCA beads in pH 6.8 phosphate buffer and distilled water are shown in Figs. 5 and 6. The DS release in both dissolution media showed a different behavior. The release of DS in pH 6.8 phosphate buffer gave a fast and complete release, whereas an incomplete release for 8 h was observed in distilled water. The effect of GPS on the release of DS from the DCA beads is present in Fig. 5. Incorporation of GPS into the DCA beads caused a decrease in release rate of DS in both dissolution media (Table 2), which the slowest release rate in distilled water was found in the case of the 3%GPS–DCA beads. The heat treatment did not affect the DS release from the DCA beads in pH 6.8 phosphate buffer (Table 2). On the other hand, the T_{20} value in distilled water of the DCA beads with heat treatment for 45 min was significantly longer ($P < 0.05$) than that without and with heat treatment for 20 min (Table 2, Fig. 6a). The heat treatment for 45 min of the 3%GPS–DCA beads gave a statistically longer T_{50} and T_{75} values ($P < 0.05$) in pH 6.8 phosphate buffer (Table 2, Fig. 6b). The drug release in distilled water showed a change in release behavior (Fig. 6c) and the T_{20} value obtained did not correlate with the time of heat treatment (Table 2). SEM photographs of the beads after release for 8 h in distilled water showed an erosion of the surface of the DCA beads without and with heat treatment (Fig. 7a and b). The surface morphology of the 3%GPS–DCA beads with heat treatment after release presented a rougher surface of the resolidified GPS (Fig. 7c) when compared with that before release (Fig. 1d). The erosion of the calcium alginate beads in distilled water can be observed because a residual alginate could release from the beads (Murata et al., 1993b), which occurred from a small amount of calcium ion released in distilled water (Østberg et al., 1994).

The DCA beads could be swollen in pH 6.8 phosphate buffer and the DS release profiles followed swelling controlled mechanism, whereas the release in distilled water could be explained using matrix diffusion-controlled mechanism (Sugawara et al., 1994; Puttipipatkhachorn et al., 2005). The higher water uptake of the beads and disintegration of the swollen beads during the release testing provided a fast and complete release of DS in pH 6.8 phosphate buffer. The GPS–DCA beads gave a slower DS release because GPS had a hydrophobic property that could retard the DS release from the beads. Although the effect of GPS on DS release was not observed clearly in pH 6.8 phosphate buffer because the DCA beads could be swollen. However, the effect of GPS was obviously observed in distilled water that the beads was more stable and could not disintegrate. Thus, the slowest release rate of the 3%GPS–DCA beads was obtained.

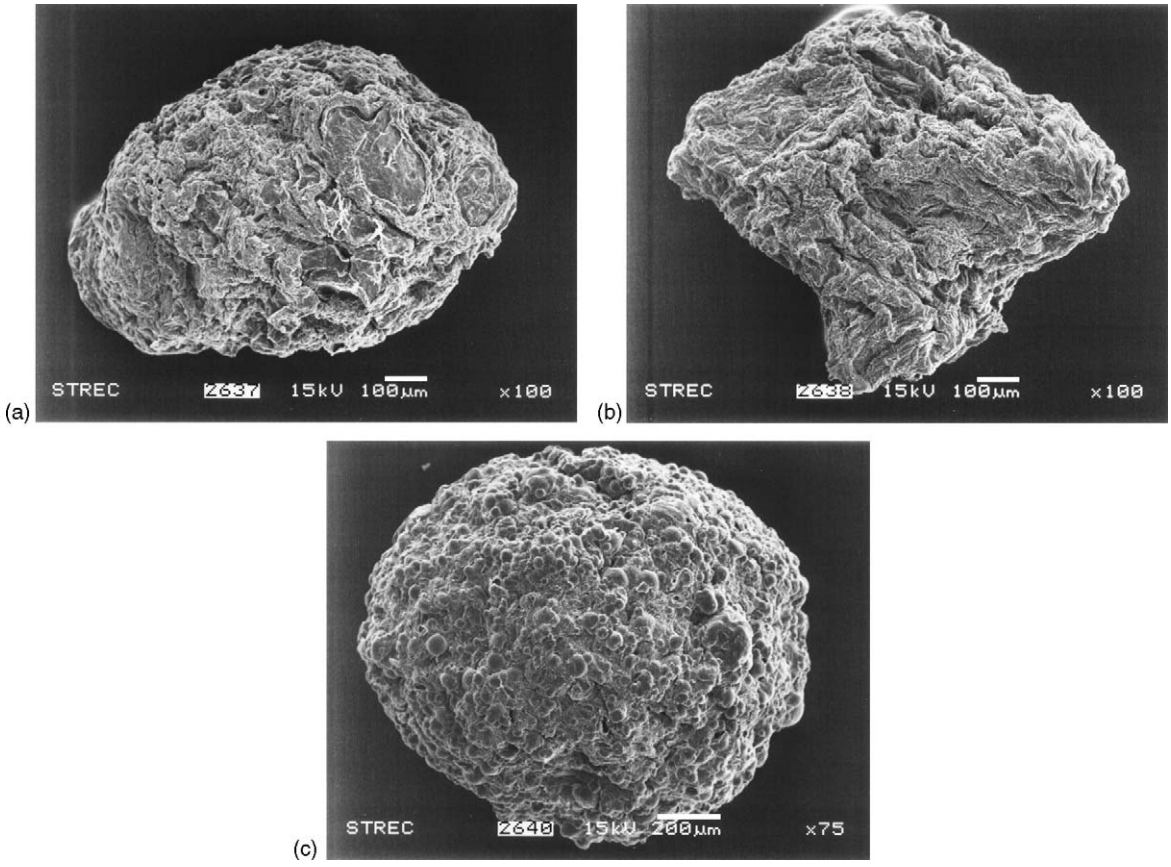

Fig. 7. Microscopic morphology of DCA bead (a), DCA bead (b) and 3%GPS–DCA bead heated for 45 min (c) after release testing in distilled water.

The heat treatment gave no change in DS release rate in the DCA beads when using pH 6.8 phosphate buffer, although the highest water uptake of the DCA beads heated for 45 min was found. This finding was similar to the previous report (Puttipipatkhachorn et al., 2005) that the release rate of the DCA beads loaded with some additives did not correlate with the water uptake results in this medium. This also suggested that water sorption process might not involve the DS release of this beads and heat treatment may induce more interaction of DS and alginate polymer in the DCA beads. On the other hand, a remarkably slower DS release in distilled water of the DCA beads after heat treatment for 45 min was found, although the beads had higher water uptake. This may be due to an interaction of DS and alginate polymer matrix, and a restriction of water sorption into the inside region of the beads that the shrinkage of the beads occurred.

A decrease of DS release rate of the 3%GPS–DCA beads with heat treatment in pH 6.8 phosphate buffer could be attributed to the controlled release of DS from resolidified GPS particles in the beads, which the DS was dispersed at higher extent. Besides, heating caused a change in internal structure of polymer matrix and interface property between resolidified GPS and calcium alginate matrix. These led to a change of release rate and release pattern in distilled water.

4. Conclusions

Incorporation of GPS into the DCA beads affected particle size, DS entrapment efficiency, water uptake, and DS released from the beads. The heat treatment caused a change in particle shape and the DS release in distilled water of the DCA beads. The 3%GPS–DCA beads with heat treatment showed a lower DS entrapment efficiency and an unchanged in particle shape. The slower release of DS from the 3%GPS–DCA beads with heat treatment in both pH 6.8 phosphate buffer and distilled water was found because DS release was controlled by not only the calcium alginate matrix, but also the resolidified GPS matrix in the alginate beads.

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