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ORIGINAL ARTICLE

Effects of microwave on drug-release responses of spray-dried alginate microspheres

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Abstract

Context: Microspheres prepared from rigid guluronic acid- (MG) and flexible mannuronic acid-rich (MC) alginate will undergo different drug release changes with respect to the influence of microwave on the matrix. An in-depth understanding of their differences in drug release changes is attainable through investigating cross-linking agent-free alginate microspheres prepared by spray-drying technique. *Objective*: The behavior of MG and MC alginate in controlling drug release responses of spray-dried microspheres against microwave was investigated. Sodium diclofenac was used as a model water-soluble drug. The formed microspheres were subjected to drug release, drug content, size, shape, surface morphology, Fourier transform infrared spectroscopy, differential scanning calorimetry, and X-ray diffractometry analysis. *Results*: MC microspheres required a shorter period of microwave irradiation to reduce drug release extent than MG microspheres. In response to microwave, the drug release profiles of 1:1 MG—MC microspheres resembled MC microspheres. *Discussion*: The state of polymer—polymer and drug—polymer interaction via O–H and/or N–H moiety of microspheres was affected by alginate chain flexibility under the influence of microwave. It then governed the drug release responses of microspheres. *Conclusion*: The drug release property of alginate microspheres can be modified by microwave irradiation, and its changes are a function of alginate conformation.

Key words: *Alginate; drug–polymer interaction; guluronic acid; mannuronic acid; microwave; polymer–polymer interaction*

Introduction

Alginate is a linear polysaccharide obtained from brown seaweed such as *Laminaria hyperborea*, *Macrocystis pyrifera*, and *Ascophyllum nodosum*[1–3]. It is made of 1,4 linked α-L-guluronic acid (*G*) and β-D-mannuronic acid (*M*) residues, forming homopolymeric *MM* or *GG* blocks that are interspersed with heteropolymeric *MG* or *GM* blocks. The use of alginate as the matrix material of drug delivery system has gained widespread interest over the years owing to its biodegradability, biocompatibility, and low oral toxicity[4].

Microwave is a high-frequency radiation (300 MHz to 300 GHz), which has both electrical and magnetic components[5]. It receives a growing interest in drying of pharmaceutical excipients, granules, gel, and film coats, as well as for the sterilization of injection ampoules and analysis of transdermal drug delivery system[5–8]. Microwave has also been employed to design controlled-release alginate, alginate-chitosonium, pectinate, chitosan-pectinate, pectinate-chitosonium, and poly(methyl vinyl ether-co-maleic acid) beads[5,9–14], as well as gelatin microspheres[15], albumin compact[16], Ispaghula husk tablet[17], silicon dioxide–felodipine physical mixture[18], polyvinylpyrrolidone/vinyl acetate 60/40, and hydroxypropyl-β-cyclodextrin solid dispersion[19].

Practically, the drug-release characteristics of polymeric matrices are dependent on the extent of polymer crosslinkage and complexation brought about by the microwave[5]. In the case of alginate matrix, it is commonly known that its drug-release property is strongly governed by the composition of the uronic acid sequences[1,3,20]. The mannuronic acid-rich alginate matrix gives lower drug-release rates in dissolution medium of pH 1.2, while the guluronic acid-rich alginate matrix gives lower drug-release rates in dissolution

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medium of pH 6.8[21–24]. The mannuronic acid-rich alginate matrix is deemed to hydrate faster under acidic condition and diffusion barrier is built up more rapidly, giving rise to a slower drug release in acidic dissolution medium. In the case of guluronic acid-rich alginate matrix, it tends to exhibit crack formation and lamination under acidic condition, thereby resulting in burst drug release. At near neutral pH, the guluronic acid-rich alginate matrix forms a more rigid gel upon hydration, which can be less susceptible to erosion and provides an effective barrier to retard drug release when compared to mannuronic acid-rich alginate matrix[21,25,26]. The drug-release profiles of matrices prepared from both grades of alginate are modifiable through varying type, load, and aqueous solubility of crosslinking agent and/or pH modifier incorporated into matrices[22–24,27]. However, there is no known study on the effects of microwave on drug-release behavior of alginate matrix of different uronic acid compositions. As such, the present study sets to investigate the drug-release property of alginate microspheres prepared from guluronic acid-rich and mannuronic acid-rich alginates in response to the irradiation of microwave. The alginate microspheres were prepared by spray-drying technique to avoid the need for crosslinking agent or binder to prepare the matrix. This granted a basic investigation on the changes of drug-release profiles of matrix prepared from different alginate conformers, following its treatment by microwave. The mannuronic acid-rich alginate has a more flexible conformation than guluronic acid-rich alginate[2,28]. In response to microwave, it is hypothesized that microspheres prepared from these alginates will undergo different drug-release changes, as the interactive profile of microwave with matrix is governed by physicochemical attributes of an object[5].

Materials and methods

Materials

Two grades of sodium alginate were employed as the matrix polymer of microspheres, namely guluronic acid-rich (MG, Manugel® DMB; ISP, San Diego, CA, USA) and mannuronic acid-rich (MC, Manucol® DMF; ISP, San Diego, CA, USA) alginates. These alginates had a similar viscosity value of 300 mPa.s and their mannuronic acid/guluronic acid ratios were 0.59 and 1.56 for MG and MC, respectively. The sodium diclofenac was selected as the model water-soluble drug. Other chemicals employed were sodium hydroxide (Merck, Darmstadt, Germany) and potassium dihydrogen phosphate (Merck, Darmstadt, Germany) for the preparation of phosphate buffer (pH 6.0) USP, which was used as the dissolution medium in drug-release study.

Methods

Equipment

A microwave oven (EM-G A; Sanyo, Osaka, Japan) equipped with a single magnetron emitter operating at 2450 ± 50 MHz was used. The microwave is generated through emission and acceleration of electrons from cathode to anode under the influence of applied electric and magnetic fields in a continuous manner[5]. The desired power setting and duration of irradiation were set using the electronic touch-control panel. The oven consisted of a turntable on which the samples were placed at an off-center position and rotated to achieve uniform irradiation.

Preparation of microspheres

The microspheres were prepared by spray-drying method using a spray dryer (Mini Spray Dryer B-290, Buchi, Switzerland). An aqueous dispersion containing 2% (w/w) of sodium alginate and 0.2% (w/w) of sodium diclofenac was prepared and spray-dried into drug-loaded microspheres under the following process conditions: inlet temperature = 65 ± 2°C, outlet temperature = 55 ± 2°C, and spray flow rate = 3 ml/min using a two-fluid nozzle with an internal tip diameter of 0.7 mm. A drug to sodium alginate ratio of 1:10 was selected, as it represented the highest processing load possible by means of spray-drying method without drug encapsulation efficiency negated via losses to walls of processing chamber and delivery tubing. The formed microspheres were collected and subjected to further drying in a hot-air oven at 40 ± 0.5°C for 3 days and subsequently equilibrated to a constant weight by storing in a desiccator at 25 ± 1°C. Blank microspheres were prepared in the same manner, except that no drug was incorporated.

Microsphere morphology

The size and shape of microspheres were determined using an image analyzer consisting of a computer system connected to a video camera (Coolpix 8400; Nikon, Tokyo, Japan) mounted on a compound microscope (Leica Microsystems Wezler GmBH, Wetzlar, Germany). The length and breadth were measured from the image of each microsphere and its size calculated from the average of these two dimensions. The median size and span values of microspheres were derived from the cumulative frequency plot of particle size against percentage population. The shape of the microspheres was represented by the elongation ratio, which is the quotient of its length to breadth. An elongation ratio of value unity represents a sphere while higher values represent greater elongation. For each batch of formulation, a total of 450 microspheres were randomly selected for measurement and the results averaged.

Scanning electron microscopy

The surface structure of microspheres was examined using the scanning electron microscopy (SEM) technique (JSM-6701F; Jeol, Tokyo, Japan). The microspheres were fixed with an aluminum tape onto studs and sputter-coated with gold (JFC-1600, Jeol, Tokyo, Japan). The prepared studs were viewed directly under a scanning electron microscope at a magnification level of 10,000×. Representative sections were photographed.

Microwave treatment of microspheres

An accurately weighed amount of microspheres was contained in a lidless glass Petri dish (internal diameter = 9 cm) and was subjected to microwave treatment at 80 W for 5, 10, and 20 minutes. Microwave power higher than 80 W was not employed so as to avoid the degradation of matrix[10]. The color and weight variation of microspheres were noted before and after the microspheres were treated with microwave.

Drug release and drug content

The drug-release and drug-content profiles of microspheres were determined using phosphate buffer (pH 6) USP in simulation of the pH of intestinal medium as previously described[11,12]. Acidic dissolution medium was omitted in the test, as an insignificant level of drug was expected to release from the matrices owing to drug precipitation via the acid–base reaction[13,14]. An accurately weighed amount of sample was placed in 500 ml of dissolution medium (sink condition) and was agitated at 50 strokes/min in a shaker bath (Memmert GmbH+Co., KG, Schwabach, Germany) at 37 ± 0.2°C. Aliquots were withdrawn at various time intervals and assayed spectrophotometrically for sodium diclofenac at a wavelength maxima of 275 nm (Cary 50 Conc; Varian Australia Pty Ltd., Melbourne, Australia). The percentage of drug released was calculated with respect to the drug content of microspheres. The drug content was expressed as the percentage of drug encapsulated in a unit weight of microspheres. The drug content was determined by subjecting the same sample of microspheres from the drug-release study for an additional 16 hours of magnetic stirring followed by ultrasonication for at least 3 consecutive periods of 10 minutes before assaying for sodium diclofenac. Each experiment was carried out in triplicates and the results averaged.

Kinetics of drug release

The drug content and percentage of drug released from the microspheres treated by microwave irradiation were compared to those of the untreated microspheres. The statistical significance of the effects of microwave irradiation on the drug-release property and drug content of the microspheres was assessed using Student's *t*-test, unless otherwise stated. The mechanism of drug release was investigated by fitting the drug-release data to Korsmeyer–Peppas dissolution model as expressed by

$$F = kt^n, \tag{1}$$

where F is the percentage of drug released at time t (min), k is the drug-release rate constant incorporating the properties of polymeric system and drug, and n is the release exponent indicative of drug-release mechanism. The n and k values were obtained from the plots of log F against log t and the goodness of fit of the drug-release data was evaluated by linear regression. The value of n = 0.45 represents Fickian diffusional (Case I) release, $0.45 < n < 0.89$ represents non-Fickian (Anomalous) release, n = 0.89 indicates Case II (Zero order) release, and $n > 0.89$ indicates Super Case II release. Case II release refers to transport of drug solute via the dissolution of polymeric matrix due to relaxation of polymer chains, whereas Anomalous release refers to the summation of both drug-diffusion- and polymer-dissolution-controlled drug release. Super Case II release denotes drug dissolution that is controlled by polymer relaxation and is characterized by a sigmoidal release pattern[11].

Fourier transform infrared spectroscopy

A total of 2% (w/w) of sample with respect to potassium bromide (KBr) disc was mixed with dry KBr (FTIR Grade, Sigma, St. Louis, MO, USA). The mixture was ground into a fine powder using an agate mortar before compressing into a disc. Each disc was scanned at a resolution of 4 cm^{-1} over a wavenumber region of 400–4000 cm^{-1} using a Fourier transform infrared (FTIR) spectrophotometer (Spectrum RX1 FTIR system; Perkin Elmer, Shelton, MO, USA). The characteristic peaks of infrared (IR) transmission spectra were recorded. At least triplicates were carried out for each batch of sample and the results averaged.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) thermograms were obtained using a differential scanning calorimeter (Pyris 6 DSC; Perkin Elmer, Shelton, MO, USA). Two milligrams of sample were crimped in a standard aluminum pan and heated from 30°C to 380°C at a heating rate of 10°C/min under constant purging of nitrogen at 40 ml/min. The characteristic peak temperature and enthalpy values of endotherm and exotherm were recorded. At least triplicates were carried out for each batch of sample and the results averaged.

X-Ray diffractometry

The crystalline state of samples was evaluated using X-ray diffractometer (MiniFlex II, Rigaku Corporation, Shibuya-ku, Japan) with Cu-K_α radiation generated at 30 kV

and 15 mA. The X-ray diffraction was operated at a scanning speed of 3°/min, ranging from 3° to 80° (2θ). At least triplicates were carried out for each batch of sample and the results averaged.

Results and discussion

The formed MG and MC microspheres had median sizes of 3.82 ± 0.31 and 6.22 ± 0.28 μm, spans of 2.01 ± 0.36 and 1.78 ± 0.05, as well as elongation ratios of 1.11 ± 0.07 and 1.07 ± 0.07, respectively. SEM analysis indicated that the MC microspheres tended to have an indented dense surface morphology when compared to MG microspheres, which exhibited a flaky surface appearance (Figure 1), similar to that reported by Takka and Acarturk[20]. Irradiation of these microspheres by microwave did not result in significant color and weight variations of microspheres under all the given experimental conditions. The observation of insignificant weight change in microspheres ≤0.02%, w/w indicated that all the microspheres used were appropriately dried and there was minimal loss of substances through volatilization. The drug contents of MG and MC microspheres amounted to 10.34 ± 0.08 and 10.91 ± 0.04% (w/w), respectively. The drug contents of both treated and untreated matrices were not significantly different from each other ($P > 0.05$).

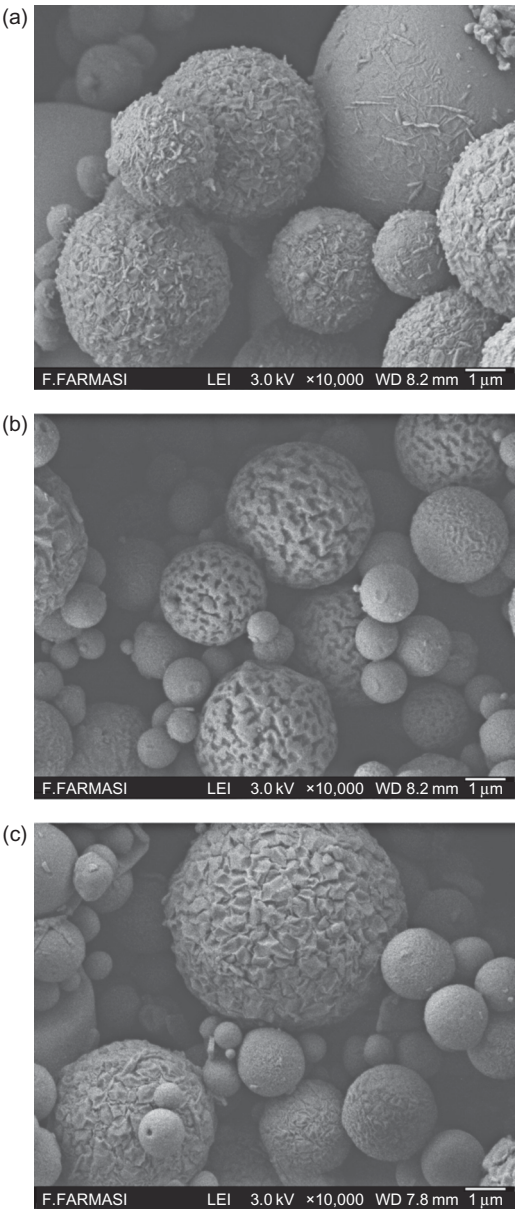

Figure 1. Surface morphology of (a) MG microspheres; (b) MC microspheres; (c) MG–MC microspheres.

Drug dissolution

An average of 33.39 ± 1.04% of sodium diclofenac was released from the untreated MG microspheres at 6 hours of dissolution (Table 1). The extent of drug released from the MG microspheres treated by microwave for 5 minutes was higher than that of the untreated counterpart at 6 hours of dissolution (Table 1). The percentage of drug released at 6 hours of dissolution decreased from 33.39 ± 1.04% to 30.76 ± 0.24% and 29.02 ± 1.74% when the MG microspheres were subjected to microwave treatment for 10 and 20 minutes, respectively (Table 1; $P < 0.05$). During the early phase of dissolution, similarly low extents of drug releases were observed in the case of these microwave-treated microspheres when compared to that of the untreated sample (Figure 2).

An average of 23.77 ± 2.00% of drug was released from the untreated MC microspheres at 6 hours of dissolution (Table 1). In contrast to findings which indicated that the mannuronic acid-rich alginate matrix exhibited a higher drug-release propensity in near-neutral dissolution medium than guluronic acid-rich alginate matrix[21–24], the MC microspheres provided a lower extent of drug release throughout the entire period of dissolution than MG microspheres (Figure 2). This was probably ascribed to the larger particle size of MC microspheres, which gave rise to a lower specific surface area for drug dissolution, in addition to their denser microstructure, as reported by Gombotz and Wee[1] as well as George and Abraham[3]. The preparation of microspheres by spray drying could produce flaky or dense matrix as a function of uronic acid composition of alginate. This dictated the drug-release profiles of these microspheres unlike the previous studies, which mainly evaluated dense alginate matrix produced by means of compaction or agglomeration technique.

Table 1. Drug-release kinetics of MG, MC, and MG–MC microspheres treated under various microwave irradiation conditions.

	Condition of microwave irradiation		Drug-release kinetics			
			Korsmeyer–Peppas model			
Type of microspheres	Power (W)	Time (min)	k	n	r^2	Drug released at 6 hours (%)
MG	0	0	1.37 ± 0.29	0.60 ± 0.04	0.90	33.39 ± 1.04
	80	5	1.46 ± 0.19	0.60 ± 0.04	0.93	37.16 ± 1.04
	80	10	0.86 ± 0.18	0.66 ± 0.03	0.94	30.76 ± 0.24
	80	20	1.08 ± 0.07	0.61 ± 0.04	0.97	29.02 ± 1.74
MC	0	0	0.86 ± 0.03	0.58 ± 0.01	0.98	23.77 ± 2.00
	80	5	0.75 ± 0.08	0.54 ± 0.03	0.94	18.82 ± 0.08
	80	10	0.87 ± 0.17	0.59 ± 0.03	0.96	25.33 ± 0.28
	80	20	0.67 ± 0.07	0.62 ± 0.02	0.94	25.06 ± 0.35
MG–MC	0	0	0.88 ± 0.02	0.59 ± 0.01	0.93	29.21 ± 2.92
	80	5	0.76 ± 0.10	0.60 ± 0.01	0.98	27.82 ± 0.55
	80	10	0.99 ± 0.20	0.58 ± 0.03	0.97	28.20 ± 0.30
	80	20	0.81 ± 0.15	0.60 ± 0.03	0.92	28.81 ± 0.79

Conflicting drug-release outcomes with respect to the influences of uronic acid composition of alginate were similarly reported by Martinsen et al.[29] and Amsden and Turner[30]. Unlike MG microspheres, the extent of drug released from the MC microspheres became lower when they were treated by microwave for a short duration of 5 minutes (Figure 2; $P < 0.05$). The extent of drug released from the MC microspheres treated by microwave for 5 minutes at 6 hours of dissolution was 18.82 ± 0.08% (Table 1). The extents of drug released from MC microspheres treated by microwave for 10 and 20 minutes at 6 hours of dissolution were 25.33 ± 0.28 and 25.06 ± 0.35%, respectively. Nonetheless, the MC microspheres treated by microwave for 20 minutes exhibited a delayed release at the first 30 minutes of dissolution with respect to that of the untreated sample (Figure 2; $P < 0.05$).

Differential scanning calorimetry analysis

Differential scanning calorimetry analysis showed that the sodium diclofenac melted at 297.4 ± 1.5°C with a melting enthalpy of 119.0 ± 19.8 J/g (Figure 3). Further heating of sodium diclofenac beyond 300°C resulted in drug decomposition via oxidation reaction between the drug and the oxygen in air[31]. The thermogram of unprocessed MG was characterized by two endotherms at melting peak temperatures of 144.5 ± 0.4°C and 153.5 ± 4.4°C, and an exothermic peak at 244.7 ± 1.7°C (Figure 3). Spray drying of MG into blank microspheres resulted in an increase in the endothermic peak temperature of MG from 144.5 ± 0.4°C to 147.5 ± 1.3°C, as well as an increase in the corresponding enthalpy values of endotherm and exotherm of MG at 144.5 ± 0.4°C and 244.7 ± 1.7°C to 22.7 ± 4.2 and –304.1 ± 13.0 J/g, but a decrease in the endothermic enthalpy and exothermic peak temperature of MG at 153.5 ± 4.4°C and 244.7 ± 1.7°C, respectively (Figure 3). The results suggested that the transformation of MG into blank microspheres modified the strength and extent of polymer–polymer interaction at different domains of the same matrix. At domain characterized by low melting peak temperature, both strength and extent of polymer–polymer interaction of blank MG microspheres were higher than MG. At domain characterized by high melting peak temperatures, both strength and extent of polymer–polymer interaction of blank MG microspheres were lower than MG. The spray-dried alginate microspheres exhibited a lower interaction propensity of matrix when compared to alginate matrix prepared through crosslinking of polymer with calcium ions, where the exothermic peak of unprocessed alginate was completely lost upon its transformation into a crosslinked matrix[31].

The incorporation of drug into blank MG microspheres was accompanied by drug–polymer and/or polymer–polymer interaction, which was denoted by an increase in the exothermic peak temperature and a reduction in the exothermic enthalpy value of blank microspheres at 240.2 ± 0.4°C to 245.3 ± 1.3°C and –202.3 ± 23.8 J/g, respectively (Figure 3). The event of drug–polymer and/or polymer–polymer interaction in one domain negated the strength of matrix interaction of another, thereby resulting in the coalescence of endotherms of blank MG microspheres at 147.5 ± 1.3°C and 161.8 ± 7.4°C into a single peak at the lower temperature region of 145.8 ± 2.5°C in the case of drug-loaded MG matrix. Similar to MG counterpart, the formation of blank microspheres from MC by means of spray-drying technique brought about varying changes in the

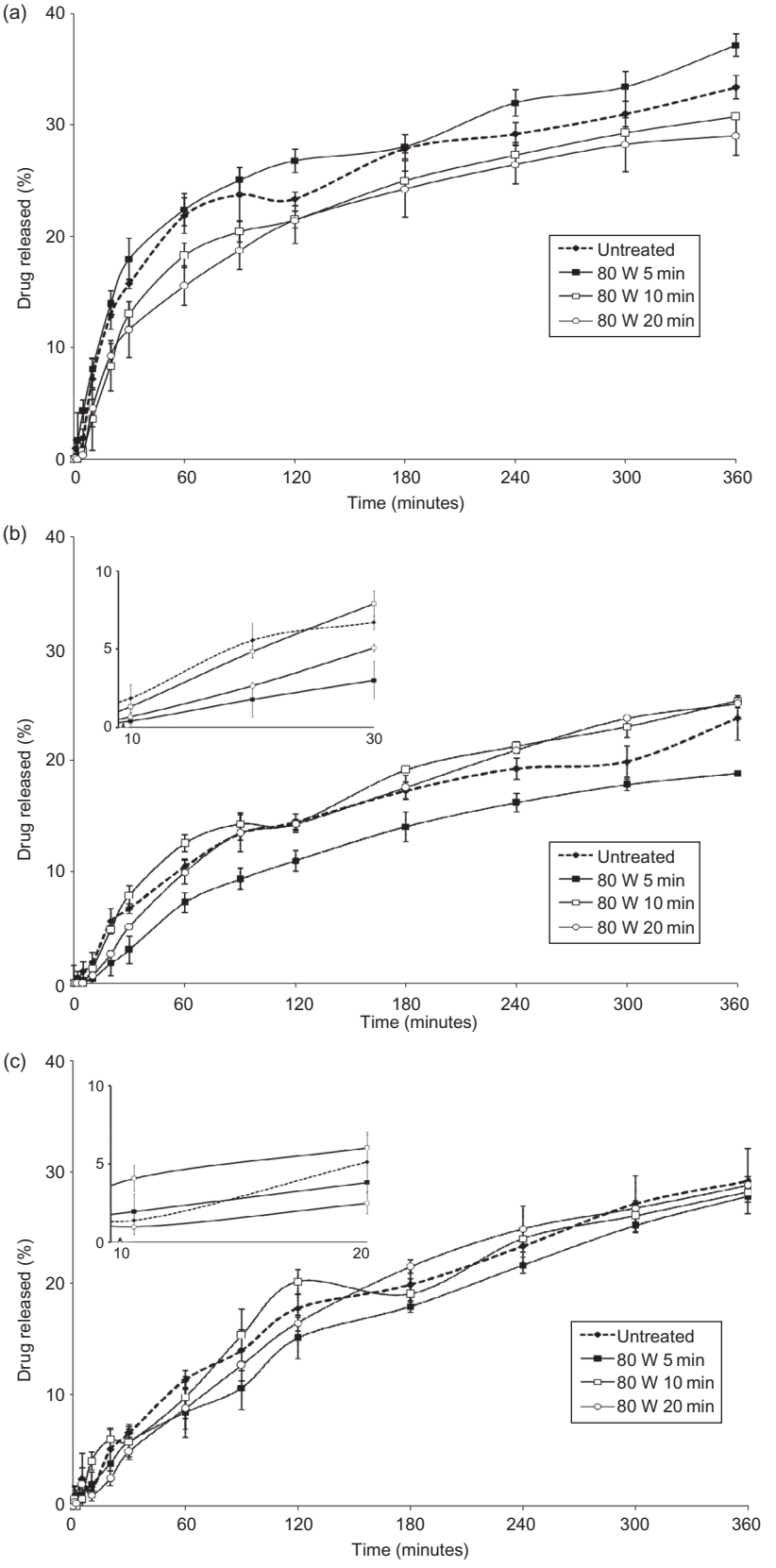

Figure 2. Drug-release profiles of (a) MG microspheres; (b) MC microspheres; (c) MG–MC microspheres subjected to various microwave irradiation conditions.

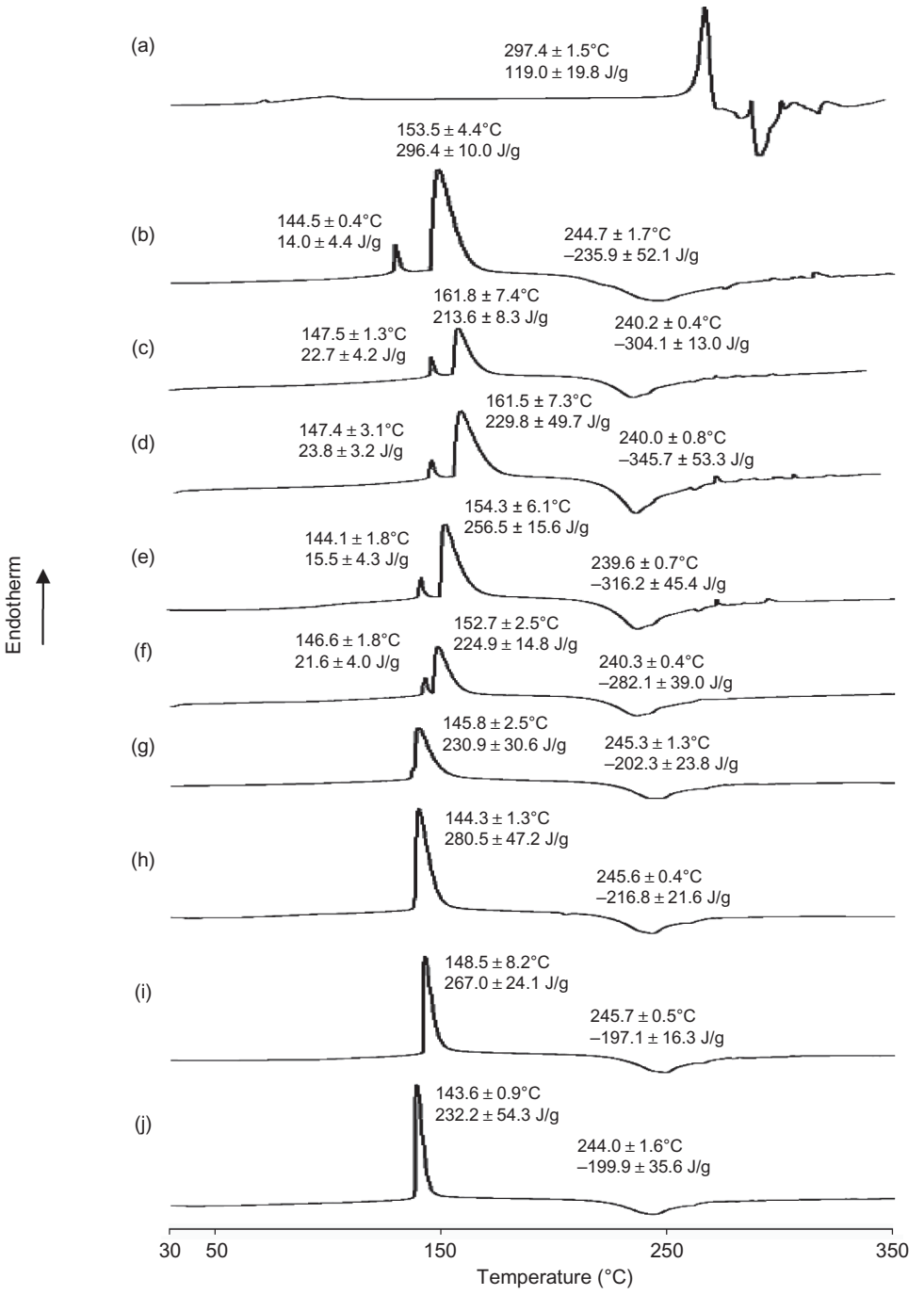

Figure 3. DSC thermograms of (a) sodium diclofenac; (b) unprocessed MG; (c) blank MG microspheres and microspheres treated at 80 W for (d) 5 minutes; (e) 10 minutes; (f) 20 minutes; (g) drug-loaded MG microspheres and microspheres treated at 80 W for (h) 5 minutes; (i) 10 minutes; (j) 20 minutes.

strength and extent of polymer–polymer interaction as indicated by the differences in peak temperatures and enthalpy values of endotherms and exotherms between MC and the formed matrix (Figure 4). The incorporation of drug into blank MC microspheres was accompanied by drug–polymer and/or polymer–polymer interaction in specific domain at the expense of the strength of matrix interaction at other domains.

The endotherm of untreated drug-loaded MC microspheres at 148.7 ± 1.6°C was transformed into two melting peaks at 147.9 ± 1.4°C and 164.7 ± 4.2°C when the sample was subjected to microwave treatment for 5 minutes (Figure 4). The formation of endotherm at a higher melting peak temperature of the latter indicated that the treatment of drug-loaded MC microspheres by microwave for 5 minutes induced the formation of

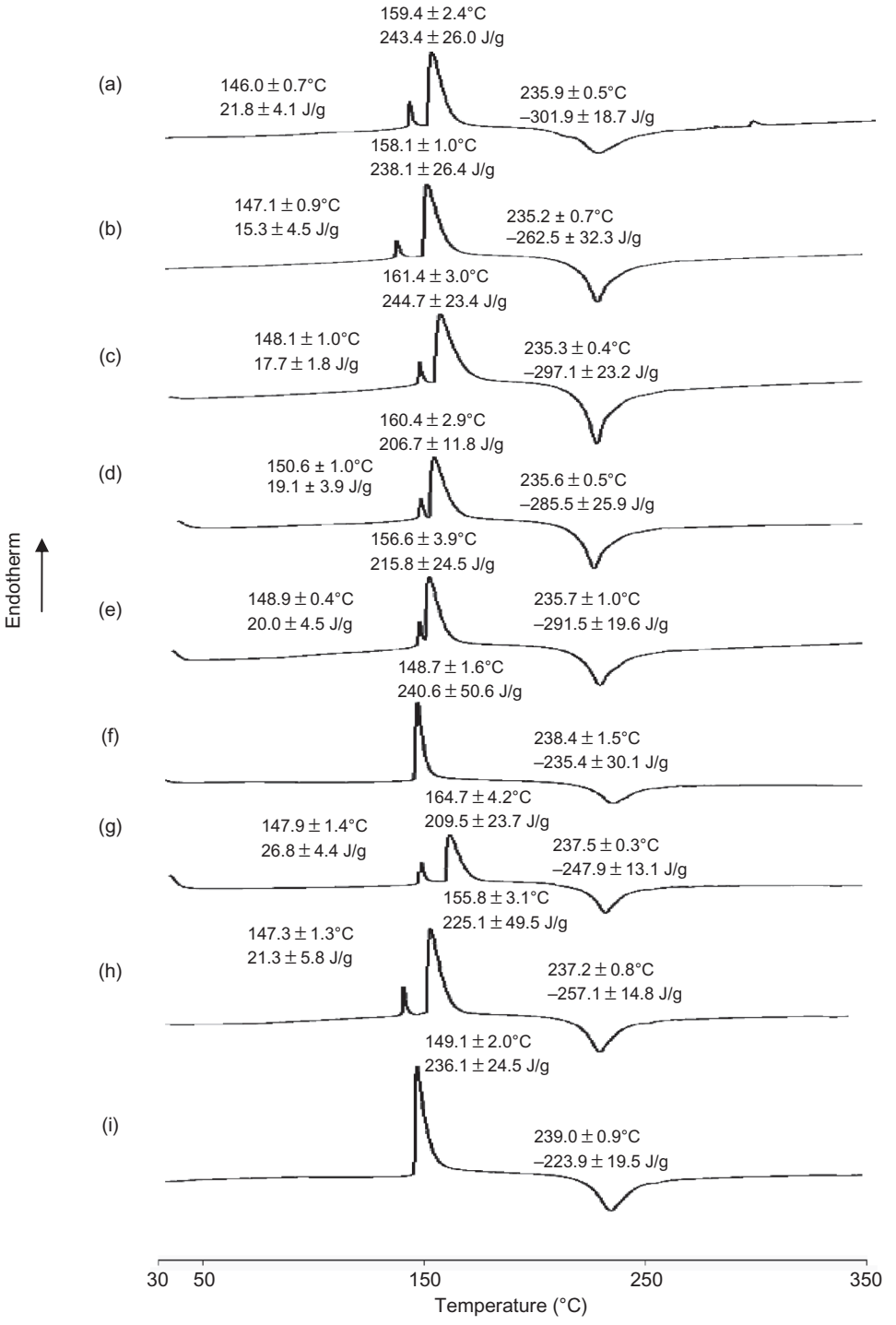

Figure 4. DSC thermograms of (a) unprocessed MC; (b) blank MC microspheres and microspheres treated at 80 W for (c) 5 minutes; (d) 10 minutes; (e) 20 minutes; (f) drug-loaded MC microspheres and microspheres treated at 80 W for (g) 5 minutes; (h) 10 minutes; (i) 20 minutes.

domain with a greater strength of polymer–polymer and/or drug–polymer interaction than untreated sample. The DSC analysis of blank MC microspheres similarly suggested that the strength and extent of polymer–polymer interaction in the matrix was higher in sample treated by microwave for 5 minutes. The melting peak temperature and enthalpy value of endotherm at 161.4 ± 3.0°C of these microspheres tended to be higher than the untreated microspheres or samples treated by microwave for 10 and 20 minutes (Figure 4). This provided the basis of MC microspheres treated by microwave for 5 minutes for showing a lower extent of drug

dissolution when compared to untreated sample and samples treated by microwave for 10 and 20 minutes. Unlike drug-loaded MC microspheres treated by microwave for 5 and 10 minutes, the treatment of the same batch of microspheres by microwave for 20 minutes did not lead to the formation of domain of a greater strength with an endotherm between 150°C and 170°C. Nonetheless, the treatment of drug-loaded MC microspheres by microwave for 20 minutes brought about a reduction in the enthalpy value of exotherm at 239.0 ± 0.9°C to –223.9 ± 19.5 J/g (Figure 4). The drug–polymer and/or polymer–polymer interaction at a different domain was enhanced to a great extent via treating the microspheres by microwave for 20 minutes, and this aptly explained the lower extent of drug released from these microwave-treated microspheres than the untreated sample within the first 30 minutes of dissolution. On the other hand, the drug-loaded MC microspheres treated by microwave for 10 minutes exhibited two endothermic domains but at a lower temperature regime than the sample treated for 5 minutes and had an exotherm with the largest rise in its enthalpy with respect to untreated microspheres and samples treated for 5 and 20 minutes (Figure 4). Consequently, the MC microspheres failed to reduce their extent of drug release at both initial and late phases of dissolution when they were treated by microwave for 10 minutes.

Unlike MC microspheres, the treatment of drug-loaded MG microspheres by microwave did not lead to the conversion of endotherm at 145.8 ± 2.5°C into dual melting peaks as characterized by a higher temperature regime (Figure 3). The treatment of blank and drug-loaded MG microspheres brought about a rise followed by a reduction in the enthalpy values of endotherms at 161.8 ± 7.4°C and 145.8 ± 2.5°C, as well as exotherms at 240.2 ± 0.4°C and 245.3 ± 1.3°C respectively with an increase in the irradiation time of microwave (Figure 3). Generally, the MG microspheres treated by microwave for 10 and 20 minutes exhibited higher endothermic but lower exothermic enthalpy values than the untreated samples. The extent of drug–polymer and/or polymer–polymer interaction was higher in these microwave-treated microspheres, thereby leading to a lower extent of drug release. The MG microspheres treated by microwave for 5 minutes, though exhibited higher endothermic temperature and enthalpy with respect to specific domains of the blank and drug-loaded matrices than samples treated by microwave for a longer duration of irradiation, had larger exothermic enthalpy values in both blank and drug-loaded matrices than the untreated sample (Figure 3). The propensity of drug–polymer and/or polymer–polymer interaction was markedly reduced at the specific domain of MG microspheres subjected to microwave treatment for 5 minutes. This brought about a higher extent of drug release from these microwave-treated MG microspheres at 6 hours of dissolution than the untreated sample.

The MG and MC microspheres had different alginate composition with respect to the contents of guluronic acid and mannuronic acid. The treatment of MG and MC microspheres by microwave involved dissimilar changes of peak temperature and enthalpy values of endotherms and exotherms, representing different domains of the matrix. The changes in the state of drug–polymer and/or polymer–polymer interaction of MC microspheres could be effected over a wider range of domain than MG microspheres. The treatment of drug-loaded MC microspheres by microwave led to the conversion of endotherm at 148.7 ± 1.6°C into dual melting peaks, whereas similar endotherm of MG microspheres at 145.8 ± 2.5°C was not convertible into multiple melting peaks when these microspheres were subjected to microwave irradiation. The MC microspheres contained mannuronic acid-rich alginate as the matrix material. The mannuronic acid of alginate had $^4C_1$ conformation, whereas the guluronic acid of alginate had $^1C_4$ conformation[2]. The mannuronic acid-rich alginate had an extended conformation and a higher level of flexibility than guluronic acid-rich alginate of MG microspheres[2,28]. As a result, the treatment of MC microspheres by microwave generated different changes in physicochemical and drug-release properties from MG microspheres.

Fourier transform infrared analysis

The FTIR analysis of MG and MC indicated that additional FTIR peaks were found in the spectra of MG at the wavenumbers of 622.5 ± 2.7 and 1125.7 ± 1.5 cm^{-1} denoting C–H and C–O moieties, respectively (Figures 5 and 6). The results suggested that there was a lower propensity of polymer–polymer interaction via the C–H and C–O moieties of alginate chains of MG than MC owing to probable conformational differences between the MG and MC with the latter having an extended chain structure, thereby providing a higher level of flexibility to interact with the adjacent polymers. The transformation of MG and MC into blank microspheres without the use of crosslinking agent by means of spray-drying technique brought about less marked changes to the FTIR characteristics of alginate than the dosage form prepared from alginate using the cationic crosslinking agent[9,10]. The loading of sodium diclofenac into blank MG and MC microspheres gave rise to additional FTIR peaks at 1506.3 ± 2.3 and 1506.3 ± 1.6 cm^{-1} respectively, which denoted the aromatic moiety of the drug (Figures 5 and 6).

The O–H moiety of alginate was represented by FTIR peak at wavenumber between 3200 and 3500 cm^{-1} [31–33]. The treatment of blank MC microspheres by microwave

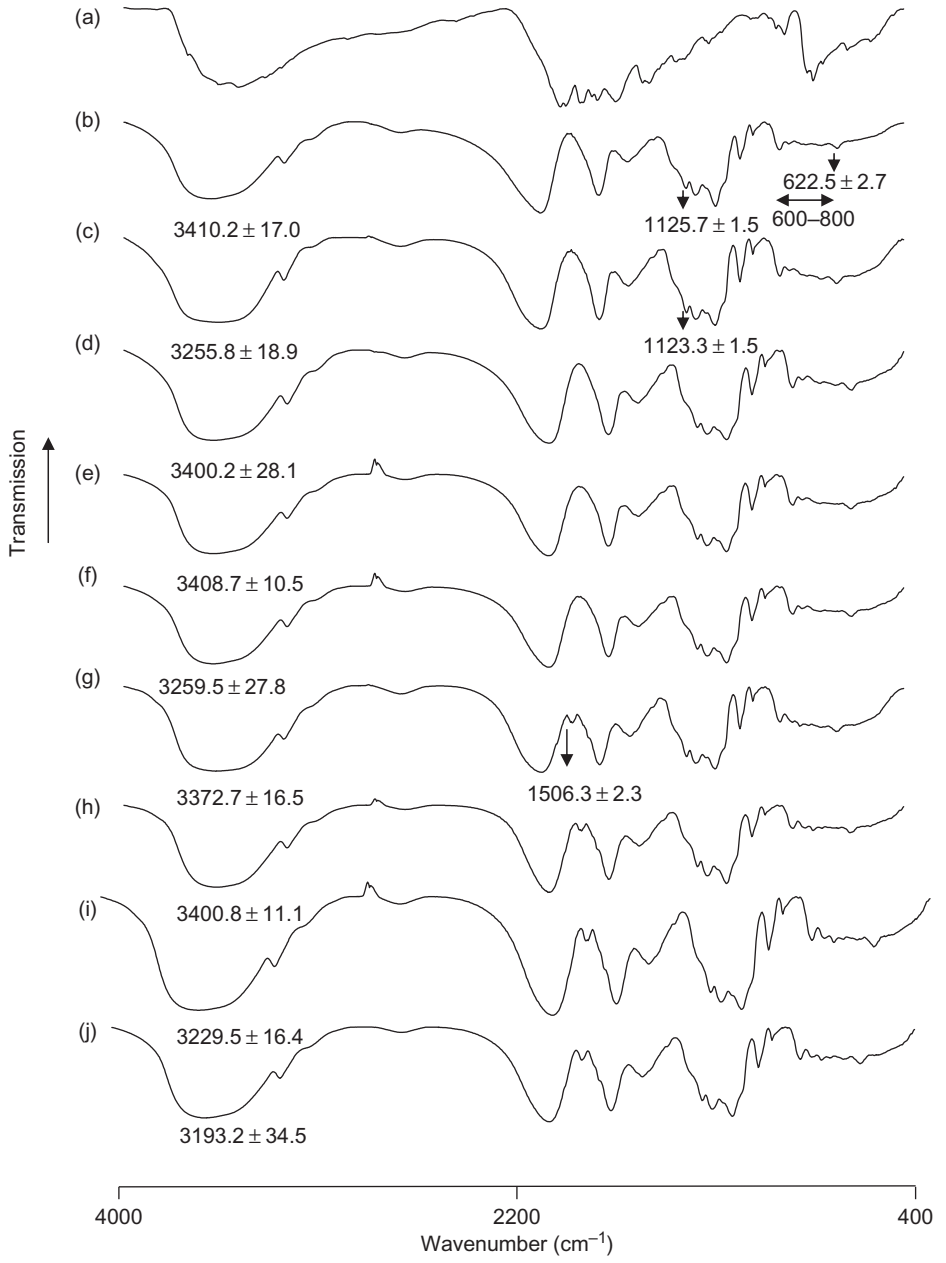

Figure 5. FTIR spectra of (a) sodium diclofenac; (b) unprocessed MG; (c) blank MG microspheres and microspheres treated at 80 W for (d) 5 minutes; (e) 10 minutes; (f) 20 minutes; (g) drug-loaded MG microspheres and microspheres treated at 80 W for (h) 5 minutes; (i) 10 minutes; (j) 20 minutes.

for 5 minutes reduced the FTIR peak wavenumber of untreated sample from 3397.8 ± 15.8 to 3222.4 ± 36.8 cm^{-1} (Figure 6). The treatment of MC microspheres by microwave for 5 minutes brought about polymer–polymer interaction via the O–H moiety of alginate, thereby reducing the extent of drug release. In the case of drug-loaded MC microspheres, it was noted that the FTIR peak wavenumber of untreated sample at 3393.9 ± 23.4 cm^{-1} was reduced to 3260.1 ± 21.7 cm^{-1} when these microspheres were treated by microwave for 20 minutes (Figure 6). The treatment of MC microspheres induced matrix interaction involving both domains of polymer and drug through the O–H and/or N–H moiety. This could probably account for a lower extent of drug released from the MC microspheres treated by microwave for 20 minutes at the first 30 minutes of dissolution when compared to untreated sample. Similar to MC microspheres, the treatment of drug-loaded MG microspheres by microwave involved changes of polymeric and drug domains containing the O–H and/or N–H functional group. The FTIR peak wavenumbers of untreated blank and drug-loaded MG microspheres

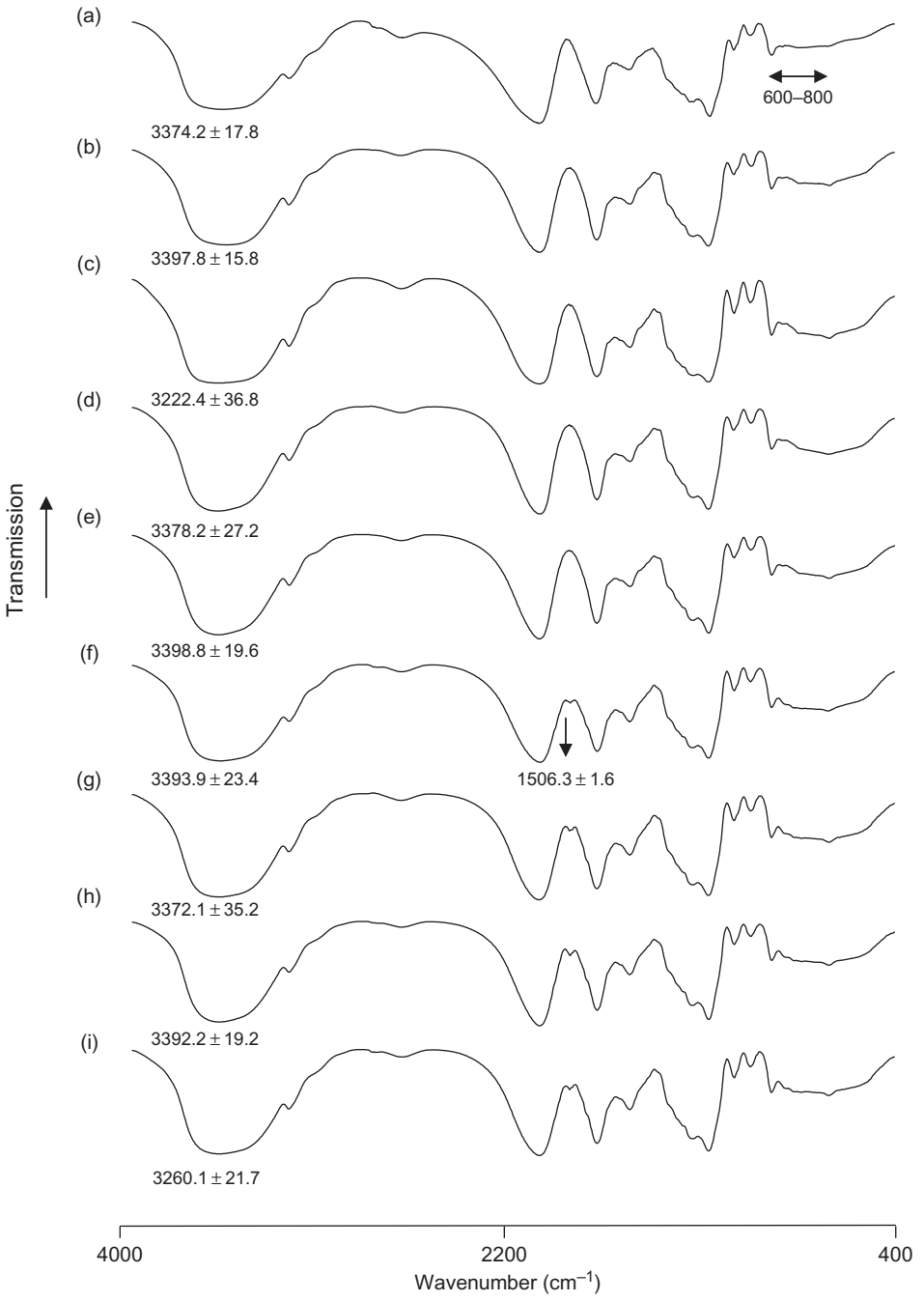

Figure 6. FTIR spectra of (a) unprocessed MC; (b) blank MC microspheres and microspheres treated at 80 W for (c) 5 minutes; (d) 10 minutes; (e) 20 minutes; (f) drug-loaded MC microspheres and microspheres treated at 80 W for (g) 5 minutes; (h) 10 minutes; (i) 20 minutes.

at 3255.8 ± 18.9 and 3372.7 ± 16.5 cm^{-1} were increased to 3400.2 ± 28.1 and 3400.8 ± 11.1 cm^{-1}, respectively, when these microspheres were treated by microwave for 5 minutes (Figure 5). Under the influence of microwave, the propensity of matrix interaction of MG microspheres treated for 5 minutes was lower than the untreated sample, thereby leading to an increase in the extent of drug release at 6 hours of dissolution. The FTIR wavenumber of drug-loaded MG microspheres at 3372.7 ± 16.5 cm^{-1} was decreased to 3229.5 ± 16.4 and 3193.2 ± 34.5 cm^{-1} when the same microspheres were subjected to microwave treatment for 10 and 20 minutes, respectively (Figure 5). The interaction propensity between polymer and/or drug of MG microspheres treated by microwave for 10 and 20 minutes was higher than the untreated microspheres and they exhibited a

lower extent of drug release throughout the entire period of dissolution.

MG–MC microspheres

The drug-release properties of MG and MC microspheres were affected by the interplay between drug–polymer and polymer–polymer interactions via the O–H and N–H moieties of matrix. Preparation of alginate microspheres using a mixture of MG and MC in a weight ratio of 1:1 [median size = 5.13 ± 0.51 μm; span = 1.69 ± 0.11; elongation ratio = 0.98 ± 0.02; drug content = 10.32 ± 0.19% (w/w); less flaky surface structure (Figure 1)] brought about matrix a lower level of response against microwave with respect to its drug-release retardation capacity when compared to MG or MC microspheres alone (Figure 2). Apparently, the extent of drug released from the MG–MC microspheres tended to be lower than that of the untreated sample throughout the entire period of dissolution when they were treated by microwave for 5 minutes and exhibited an inclination to have a lower extent of drug release at the early phase of dissolution than the untreated sample when they were treated by microwave for 20 minutes (Figure 2). Incidentally, it appeared that the drug-release characteristics of MC microspheres prevailed in MG–MC sample in response to microwave, though it had been negated substantially by the presence of MG. The MC contained mannuronic acid-rich alginate chains, which had a higher level of flexibility than MG and was more susceptible to the vibrating motion brought about by microwave. This in turn led to the drug-release property of MG–MC microspheres being governed by MC to a greater extent than MG in response to microwave irradiation.

The DSC analysis indicated that there was a rise in the endothermic enthalpy and a reduction in the exothermic enthalpy values of peaks at 160.8 ± 3.6°C and 236.7 ± 0.8°C of untreated blank and at 160.4 ± 5.8°C and 238.8 ± 0.3°C of untreated drug-loaded MG–MC microspheres respectively when they were treated by microwave for 5 minutes (Figure 7). This suggested that the treatment of MG–MC microspheres by microwave for 5 minutes gave rise to polymer–polymer and/or drug–polymer interaction in matrix thereby leading to a reduced tendency of drug release. The treatment of blank MG–MC microspheres by microwave for 10 and 20 minutes was accompanied by a marked decrease in the endothermic enthalpy values of these microspheres at the respective melting peaks of 163.7 ± 5.8°C and 161.4 ± 1.5°C (Figure 7). Nonetheless, the exothermic enthalpy values ascribing to both blank and drug-loaded microspheres treated by microwave for 10 and 20 minutes were similarly reduced as in the case of samples treated for 5 minutes. In addition, the drug-loaded MG–MC microspheres treated by microwave for 20 minutes exhibited a higher rise in endothermic enthalpy at the melting peak of 157.2 ± 1.3°C than that of samples treated for 10 minutes. It was envisaged that the interaction propensity at the specific domain of matrix was promoted to a greater extent in MG–MC microspheres treated by microwave for 20 minutes than that of untreated sample or sample treated for 10 minutes. Consequently, the former demonstrated a lower extent of drug release during the early phase of dissolution than the untreated sample and sample treated by microwave for 10 minutes.

The FTIR analysis indicated that the blending of MG and MC in the form of microspheres was characterized by spectra resembling that of the MC microspheres. The FTIR peaks of blank MG microspheres at 1123.3 ± 1.5 cm^{-1} and between 600 and 800 cm^{-1} were less evident in the corresponding spectra of MG–MC microspheres (Figure 8). The observation denoted that the formed MG–MC microspheres were likely to demonstrate polymer–polymer interaction via the C–O and C–H moieties of the alginate chains at the specific domains of matrix, as the flexible MC could extend its chain interaction over the rigid MG molecules. The treatment of blank and drug-loaded MG–MC microspheres by microwave did not translate to marked changes in the state of chemical association between the alginate chains and/or alginate and drug molecules. The treatment of blank MG–MC microspheres by microwave for 5 minutes led to a reduction in the FTIR wavenumber of untreated sample from 901.9 ± 3.1 cm^{-1} to 897.9 ± 5.4 cm^{-1} (Figure 8). An increase in the propensity of polymer–polymer interaction of these microwave-treated microspheres via the C–H moiety was responsible for their tendency to have a reduced extent of drug release when compared to the untreated sample. Unlike drug-loaded MG–MC microspheres treated by microwave for 5 and 10 minutes, the treatment of the same batch of microspheres for 20 minutes led to a reduction in the FTIR wavenumber of untreated sample from 3367.8 ± 37.8 cm^{-1} to 3339.0 ± 17.6 cm^{-1} (Figure 8). This in turn aptly explained the tendency of MG–MC microspheres treated by microwave for 20 minutes to have a lower extent of drug release during the early phase of dissolution, following an increase in the interaction propensity of matrix involving both polymer and drug via the O–H and/or N–H moiety.

X-Ray diffractometry analysis

The diffractograms of MG and MC exhibited two crystalline peaks at 2θ = 12.73° and 23.69° (Figure 9), similar to alginate samples examined by Dong et al.[34]. X-Ray Diffractometry (XRD) analysis of drug, MG, MC, and their physical mixtures indicated that the drug was

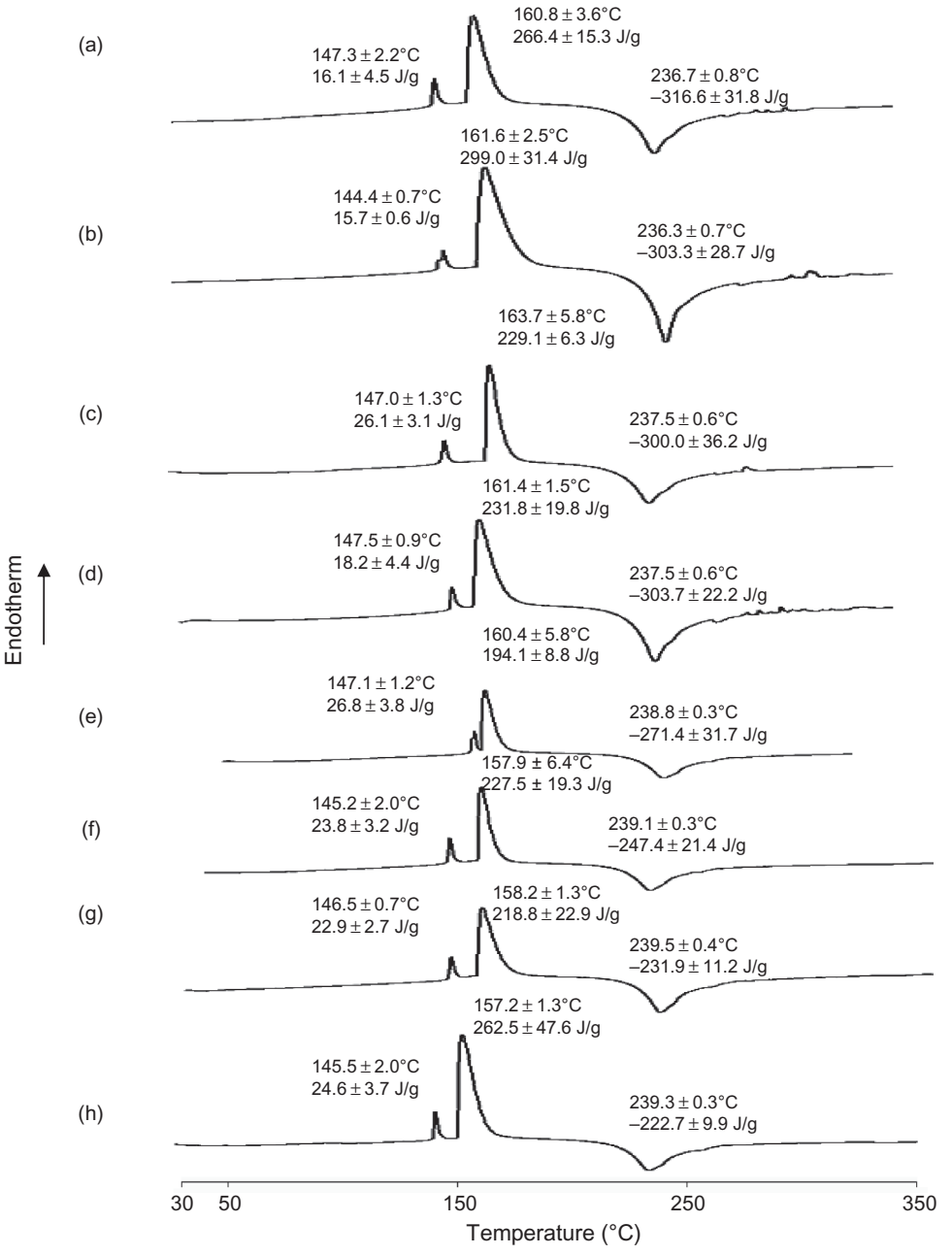

Figure 7. DSC thermograms of (a) blank MG–MC microspheres and microspheres treated at 80 W for (b) 5 minutes; (c) 10 minutes; (d) 20 minutes; (e) drug-loaded MG–MC microspheres and microspheres treated at 80 W for (f) 5 minutes; (g) 10 minutes; (h) 20 minutes.

highly crystalline when compared to alginates (Figure 9). The transformation of drug and alginates into microspheres brought about the formation of amorphous matrix. The amorphousness of microspheres was imparted by reducing the crystallinity of both drug and alginates. The reduction of drug crystallinity was inferred from the loss of sharp XRD peaks in the diffractograms of physical mixtures against drug-loaded microspheres. The reduction in alginate crystallinity was indicated by a decrease in crystallinity count in diffractograms of blank microspheres against unprocessed polymers. The MC tended to have a more amorphous and flexible microstructure than MG. This was suggested by a lower crystallinity count of the former in the diffractograms of unprocessed polymers. Loading of drug into blank microspheres further decreased the level of matrix crystallinity as drug could possibly act as a plasticizer, thereby enhancing the amorphousness of alginate microspheres, apart from its own defection of crystals[35]. With reference to MG, MC, and MG–MC microspheres, the treatment of these matrices by microwave did not provide clear changes in their state of crystallinity. Gain

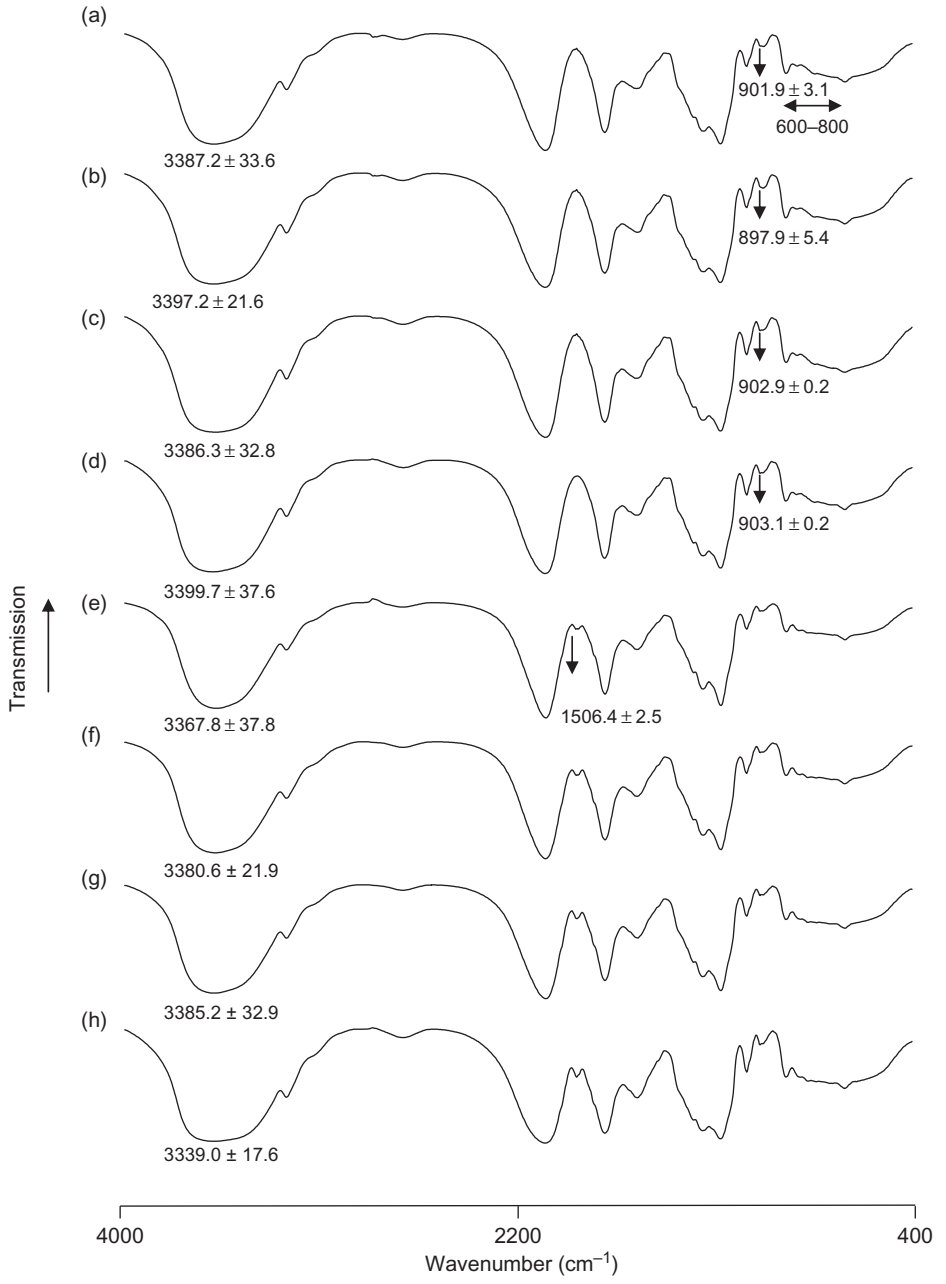

Figure 8. FTIR spectra of (a) blank MG–MC microspheres and microspheres treated at 80 W for (b) 5 minutes; (c) 10 minutes; (d) 20 minutes; (e) drug-loaded MG–MC microspheres and microspheres treated at 80 W for (f) 5 minutes; (g) 10 minutes; (h) 20 minutes.

and loss of polymer–polymer and drug–polymer interaction could have effected in various amorphous and crystalline domains of microspheres.

Mechanism of drug dissolution

The treatment of MG microspheres by microwave for 5 minutes led to an increase in rate of drug release whilst the treatment of MC or MG–MC microspheres by microwave for 5 and 20 minutes resulted in a reduction in the rate of drug release (Table 1). A prolonged duration of microwave irradiation was needed to reduce the rate of drug released from the MG microspheres. The rate of drug released from MC and MG–MC microspheres was reduced by subjecting these microspheres to a short duration of microwave treatment. The reduction in the rate of drug released from MC and MG–MC microspheres treated by microwave for 20 minutes was an attribute of delay in the initial phase of drug dissolution. The kinetics of

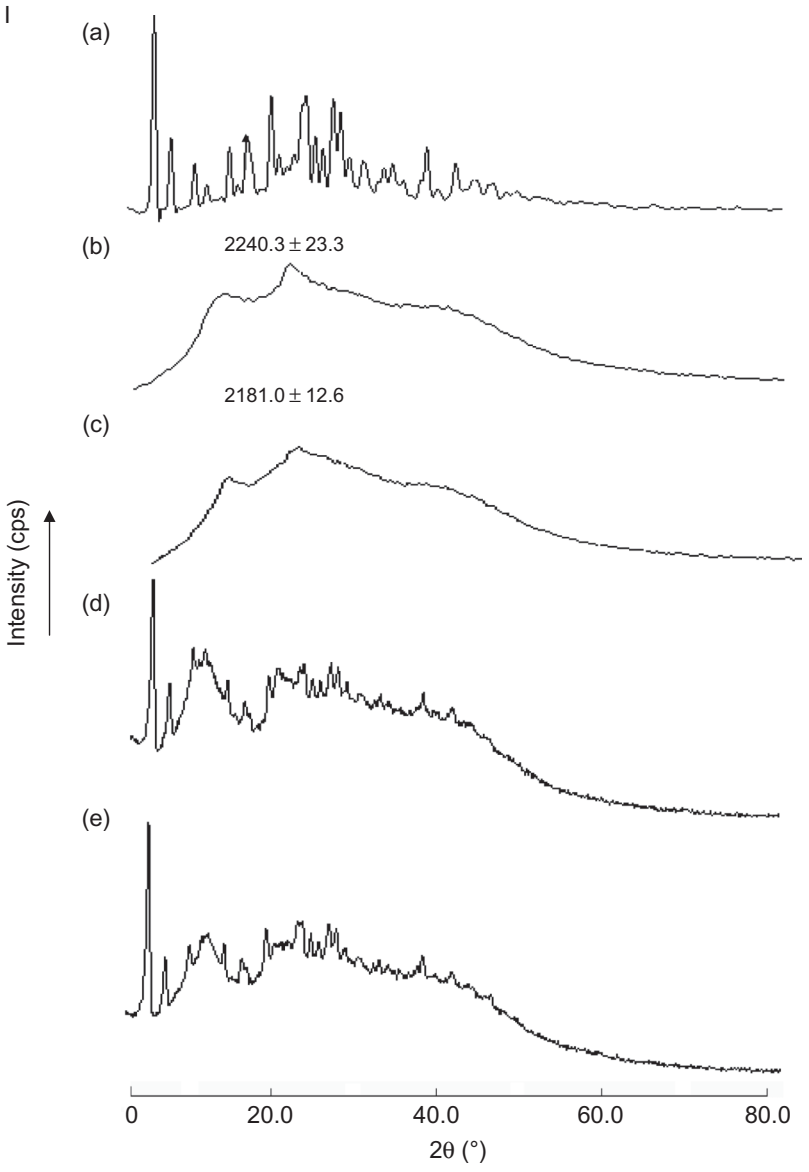

Figure 9. XRD diffractograms of I. (a) sodium diclofenac; (b) unprocessed MG; (c) unprocessed MC, physical mixtures of (d) unprocessed MG and sodium diclofenac; (e) unprocessed MC and sodium diclofenac. Physical mixture contained 10% (w/w) drug. II. (a) blank MG microspheres and microspheres treated at 80 W for (b) 5 minutes; (c) 10 minutes; (d) 20 minutes; (e) drug-loaded MG microspheres and microspheres treated at 80 W for (f) 5 minutes; (g) 10 minutes; (h) 20 minutes. III. (a) blank MC microspheres and microspheres treated at 80 W for (b) 5 minutes; (c) 10 minutes; (d) 20 minutes; (e) drug-loaded MC microspheres and microspheres treated at 80 W for (f) 5 minutes; (g) 10 minutes; (h) 20 minutes. IV. (a) blank MG–MC microspheres and microspheres treated at 80 W for (b) 5 minutes; (c) 10 minutes; (d) 20 minutes; (e) drug-loaded MG–MC microspheres and microspheres treated at 80 W for (f) 5 minutes; (g) 10 minutes; (h) 20 minutes.

drug released from both microwave-treated and untreated MG, MC, and MG–MC microspheres followed the anomalous release behavior (Table 1; $r^2 \geq 0.90$). The good fit of drug-dissolution data into these models suggested that drug release was markedly governed by the state of drug diffusion and polymer relaxation of the matrix, which can be affected by the state of polymer–polymer and/or drug–polymer interaction in microspheres.

Conclusions

MC microspheres had their release extent of embedded drug reduced following a short period of microwave treatment (5 minutes) when compared to MG microspheres (10–20 minutes). Under the influence of microwave, the drug-release characteristics of microspheres prepared from 1:1 MG–MC polymer blend tended to resemble that of MC microspheres.

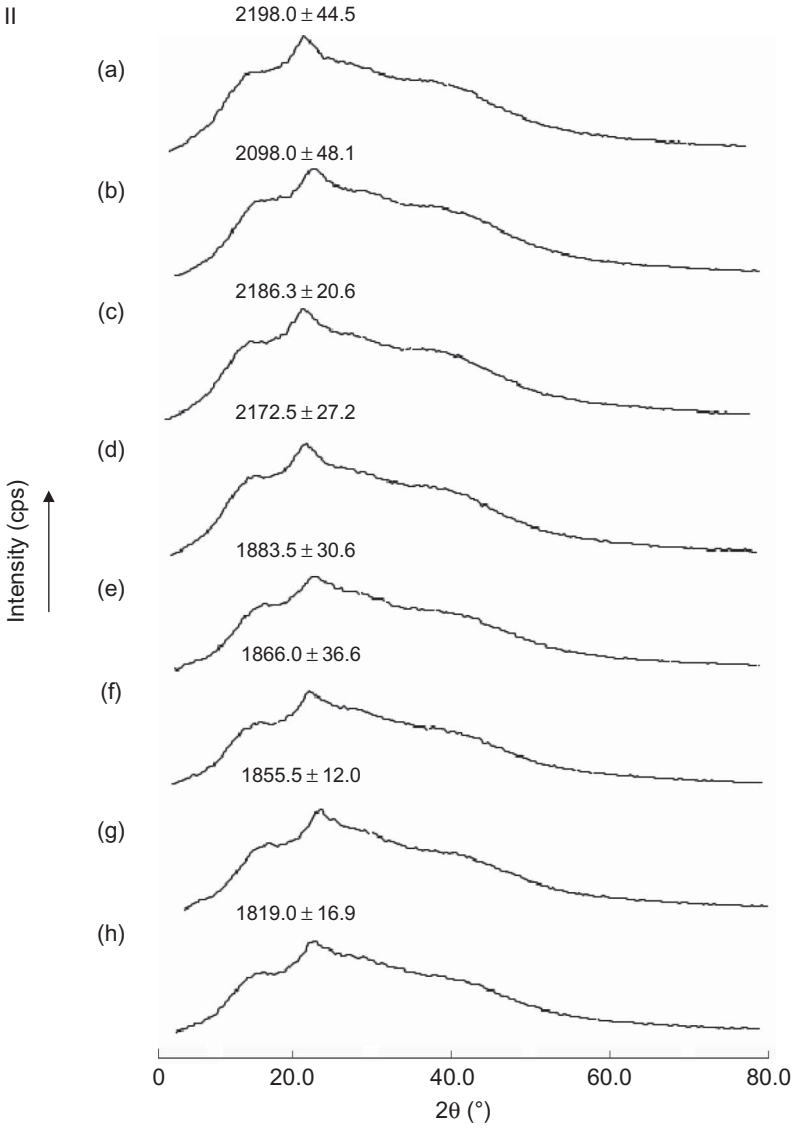

Figure 9. (Continued)

These observations of drug-release responses of MG, MC, and MG–MC microspheres were ascribed to MC alginate that had a more flexible conformation than MG alginate. Under the influence of microwave, the alginate chain flexibility affected the state of polymer–polymer and drug–polymer interaction via the O–H and/or N–H moiety of microspheres. This in turn brought about a difference in drug-release profiles of MG and MC microspheres against the influence of microwave.

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Declaration of interest

The authors report no conflicts of interest.

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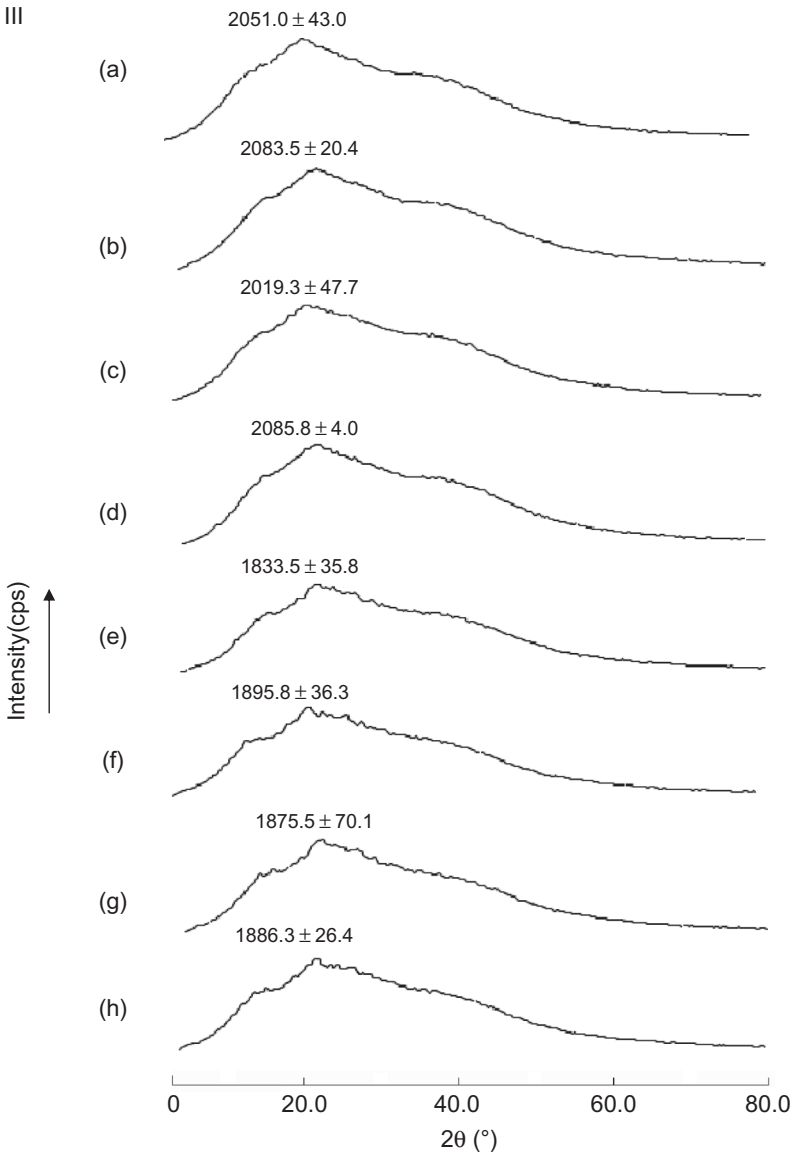

Figure 9. (Continued)

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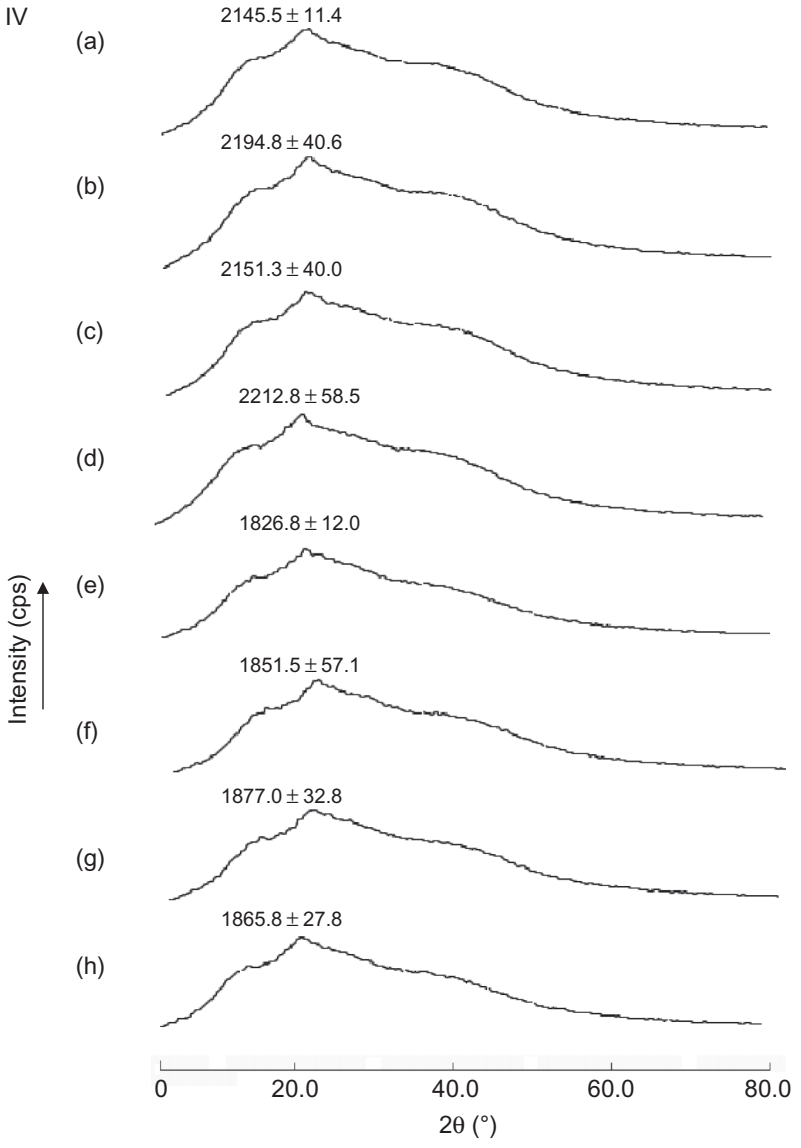

Figure 9. (Continued)

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