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CHITOSAN SPHEROIDS WITH MICROWAVE MODULATED DRUG RELEASE

Z. Zabliza and T.-W. Wong

Particle Design Research Group, Faculty of Pharmacy
Non-Destructive Biomedical and Pharmaceutical Research Centre
Universiti Teknologi MARA Malaysia
Puncak Alam, Selangor 42300, Malaysia

Abstract—The interplay effects of matrix formulations with microwave on drug release were investigated using an agglomerate system. Chitosan spheroids were formulated with stearic acid and/or sodium chloride by extrusion-spheronization technique, and chlorpheniramine maleate as water-soluble model drug. The spheroids were treated by microwave at 80 W for 5 to 40 min. The profiles of drug dissolution, drug content, drug-polymer interaction, polymer-polymer interaction, sodium leaching, matrix morphology and integrity were determined. Unlike chitosan matrix prepared by ionotropic gelation method, the retardation of drug release from chitosan spheroids by microwave required a more complex formulation containing both stearic acid and sodium chloride unless a high stearic acid fraction was used. These spheroids demonstrated a high resistance to disintegration during dissolution owing to salt-induced bridging by sodium chloride. In response to microwave, sodium chloride aided stearic acid spread and effected domain interaction via $C = O$ moiety over a matrix with reduced specific surface area thereby reducing drug dissolution. The drug release of spheroids can be retarded by microwave through promoting the layering of hydrophobic stearic acid in a matrix structure sustained by sodium chloride.

1. INTRODUCTION

Microwave is a high frequency radiation (300 MHz to 300 GHz) which possesses both electrical and magnetic properties [1, 2]. The transmission of microwave to an object results in vibration of molecules by induced or permanent dipoles. The microwave technology has been

Corresponding author: T.-W. Wong (wongtinwui@salam.uitm.edu.my).

used to design controlled release solid matrices of pectin, alginate, chitosan, poly(methylvinylether-co-maleic acid) and gelatin prepared by ionotopic gelation, coacervation and denaturation processes [3–8]. The drug release property of these solid matrices is modified through subjecting the formed products to microwave treatment in solid or liquid state. The drug release can be further retarded using microwave through changing the profiles of polymer crosslinkage and complexation as well as drug-polymer interaction.

Extrusion-spheronization technique has been adopted to prepare multi-particulate pharmaceutical oral dosage form. The formed spheroids have a high degree of roundness, a low level of friability, good flow and uniform packing characteristics [9]. However, the spheroids often require an additional film coat to modulate the profiles of drug release [10–13]. This introduces additional processing steps and a long period of operation [9, 14]. Utilization of microwave technology is deemed to be a simpler approach to modulate the drug release property of matrix spheroids.

The formation of spheroids by extrusion-spheronization processes involves nucleation, coalescence, densification and breakage of agglomerates. The dynamics and mechanism of solid particle assembly are different from matrices formed via ionotropic gelation, coacervation and denaturation processes whereby the former produces spheroids via wetting of solid powder by binding liquid whereas the latter involves the transformation of polymer solution into solid polymeric matrices. Under the influence of microwave, the changes in interaction propensity between polymer and/or drug of an object is known to be affected by its physicochemical attributes which in turn are a function of its assembly conditions [8]. The ease of modifying drug release property of spheroids by microwave can be lower than matrices prepared by gelation, coacervation and denaturation methods as polymer and/or drug particles in a spheroid are assembled with a larger inter-particulate distance and their interaction propensity can be less inducible by microwave. Thus, the present study sets to investigate the responses of spheroids prepared by extrusion-spheronization technique to microwave with respect to the outcome of drug release and identify a new formulation strategy to produce spheroids with drug release property susceptible to be modulated by microwave.

2. MATERIALS AND METHODS

2.1. Materials

Microcrystalline cellulose (MCC, M101 D+, Mingtai Chemical Co. Ltd.,Taiwan) and chitosan (Degree of deacetylation = 86%, Zulat

Pharmacy, Malaysia) were employed as matrix polymers for spheroids, with stearic acid (Hesego Industry Sdn Bhd, Malaysia) and sodium chloride (Fluka, Switzerland) as additives and chlorpheniramine maleate (Supriya Chemicals, India) as water-soluble model drug.

2.2. Preparation of Spheroids

Three types of spheroids were prepared using the extrusion-spheronization technique namely chitosan, chitosan-stearic acid (CS) and chitosan-stearic acid-sodium chloride (CSSC) spheroids with MCC as extrusion and spheronization aids. The total amount of chitosan, MCC, stearic acid and/or drug was kept at 100 g. The ratio of MCC to chitosan in all batches of spheroids was 3 : 2. Twelve %w/w drug as well as 12 and 24 %w/w stearic acid, expressed with respect to the total amount of processing materials, were used. Whenever necessary, 0.2 or 0.5 g of sodium chloride was employed as additive for each batch of spheroids.

The mixture of processing materials was first pre-mixed for 5 min in a blender (Waring Blender, Waring Products, USA). The powder mixture was then transferred to a planetary mixer (Kenwood Chef KM 300, Kenwood Ltd, UK) for further mixing and wetting with deionized water (chitosan spheroids: 105–120 g/batch; CS12% spheroids: 70–105 g/batch; CS12%SC0.2 g spheroids: 66–97 g/batch; CS12%SC0.5g: 65.8–94 g/batch; CS24%: 52–90 g/batch) added dropwise to the rotating mass. The duration of wetting was kept at 30 min. The wet mass was subsequently extruded through an orifice of 1 mm diameter by means of screen type extruder (Extruder 20, Caleva Process Solutions Ltd, UK) at $25\pm1^{\circ}$C and a constant speed of 30 rpm. The formed extrudates were spheronized at 600 rpm for 10 min using a spheronizer (Spheronizer 250, Caleva Process Solutions Ltd, UK) equipped with a polytetrafluoroethylene-coated square pitch friction disc. The spheroids were then collected and dried in an oven at $40\pm0.5^{\circ}$C for 6 days and subsequently equilibrated to a constant weight by storing in a desiccator at $25 \pm 1^{\circ}$C. Blank spheroids were similarly prepared except that no drug was incorporated. Practically, a lower load of deionized water was needed in wetting when sodium chloride and chlorpheniramine maleate were added into spheroids as partial solvation of these substances might aid particle binding. The addition of stearic acid resulted in a lesser amount of binding liquid needed for its hydrophobicity brought about a lower propensity of interaction between the binding liquid and processing mass.

2.3. Spheroid Morphology

The size and shape of the spheroids were determined using a digimatic vernier caliper system (Mitutoyo, Japan). The length and breadth were measured from each spheroid and its size calculated from the average of these two dimensions. The shape of spheroid was represented by elongation ratio which is the quotient of its length to breadth. An elongation ratio of value unity represents a perfect sphere while higher values represent greater elongation. For each formulation, 20 spheroids were randomly selected for measurement and the results averaged.

2.4. Microwave Treatment of Spheroids

An accurately weighed amount of spheroids was placed in a lidless petri dish (internal diameter = 9 cm) and was subjected to microwave treatment at 80 W for 5, 10, 20 and 40 min using a microwave oven (EM-G A, Sanyo, Japan) equipped with a single magnetron emitter operating at 2450 ± 50 MHz in accordance to the previously reported protocols [4–7]. The color and weight variation of spheroids were noted before and after the spheroids were treated with microwave.

2.5. Drug Release and Drug Content

The drug release profiles of the spheroids were determined using the USP buffer pH 2 in simulation of gastric medium which represented the first site of spheroid entry. An accurately weighed amount of spheroids was placed in 500 ml of dissolution medium (sink condition) and was agitated at 50 strokes/min (Memmert GmbH + Co. KG, Germany) at $37 \pm 1^{\circ}$C. Aliquots were withdrawn at various time intervals and assayed spectrophotometrically for chlorpheniramine maleate at 261 nm (Cary 50 Conc, Varian Australia Pty Ltd, Australia). The percentage of drug released was calculated with respect to the drug content of spheroids. The drug content was expressed as the percentage of drug encapsulated in a unit weight of spheroids. The drug content was determined by subjecting the same sample of spheroids from the drug release study for an additional 24 h of magnetic stirring followed by ultrasonication for at least three consecutive periods of 10 min before assaying for chlorpheniramine maleate. Each experiment was carried out in triplicates and the results averaged.

2.6. Photography of Drug Dissolution Process

The morphological changes of spheroids during the process of drug dissolution were determined using the same dissolution conditions as

stated under Section 2.5. At a specified time, the images of spheroids immersing in dissolution medium were captured undisturbed by means of a digital camera (Nikon E8400, Nikon Corp., Japan) from a fixed distance with reference to the dissolution bath. Triplicates were carried out and the results averaged.

2.7. Atomic Absorption Spectrophotometry (AAS)

The amount of sodium released from the selected batches of spheroids was determined using the same dissolution conditions as stated under Section 2.5. except that the withdrawn aliquots were assayed spectrophotometrically for sodium using AAS technique (Z-2000 series, Hitachi Hi-Technologies Corporation, USA). Triplicates were carried out and the results averaged.

2.8. Fourier Transform Infra-red Spectroscopy (FTIR)

Two %w/w of sample, with respect to the potassium bromide (KBr) disc, was mixed with dry KBr (FTIR grade, Aldrich, Germany). 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 $4\,\mathrm{cm}^{-1}$ over a wavenumber region of 400 to $4000\,\mathrm{cm}^{-1}$ using a FTIR spectrophotometer (Spectrum RX1 FTIR system, Perkin Elmer, USA). The characteristic peaks of IR transmission spectra were recorded. At least triplicates were carried out for each batch of sample and the results averaged.

2.9. Differential Scanning Calorimetry (DSC)

DSC thermograms were obtained using a differential scanning calorimeter (Pyris 6 DSC, Perkin Elmer, USA). Five mg of sample were crimped in a standard aluminium pan and heated from 30 to 380°C at a heating rate of 10°C/min under a constant purging of nitrogen at 40 ml/min. The characteristic peak temperature and enthalpy values of the melting endotherms and exotherms of samples were recorded. At least triplicates were carried out for each batch of sample and the results averaged.

2.10. Scanning Electron Microscopy (SEM)

The surface structure of spheroids was examined using the SEM technique (FEI Quanta 200F, Holland). The spheroids were fixed with a carbon tape onto studs, and the prepared studs were viewed directly

Table 1. Size, shape and drug content of spheroids.

Spheroid type	Size (mm)	Elongation ratio	Drug content (%w/w)
Chitosan	1.17 ± 0.16	0.96 ± 0.04	8.85 ± 0.19
CS12%	1.08 ± 0.08	0.99 ± 0.06	9.60 ± 0.05
CS12%SC0.2g	1.02 ± 0.06	1.02 ± 0.08	9.48 ± 0.08
CS12%SC0.5g	0.99 ± 0.06	1.01 ± 0.05	9.58 ± 0.15
CS24%	1.07 ± 0.05	1.01 ± 0.08	9.57 ± 0.03

under a scanning electron microscope at a magnification level of 1000×. Representative sections were photographed.

2.11. Interactive Effects of Stearic Acid with Microwave and Sodium Chloride on Drug Dissolution of Spheroids

Both blank and drug loaded spheroids containing chitosan, MCC and sodium chloride were prepared without the addition of stearic acid (CSC) in accordance to previously described protocol under Section 2.2. The formed spheroids were treated by microwave at 80 W for 40 min, and both the microwave-treated and untreated spheroids were subjected to drug dissolution study. The drug release profiles of CSC spheroids were analyzed against those of CSSC and CS matrices to indicate the interplay effects of microwave and sodium chloride with stearic acid on the drug dissolution property of spheroids.

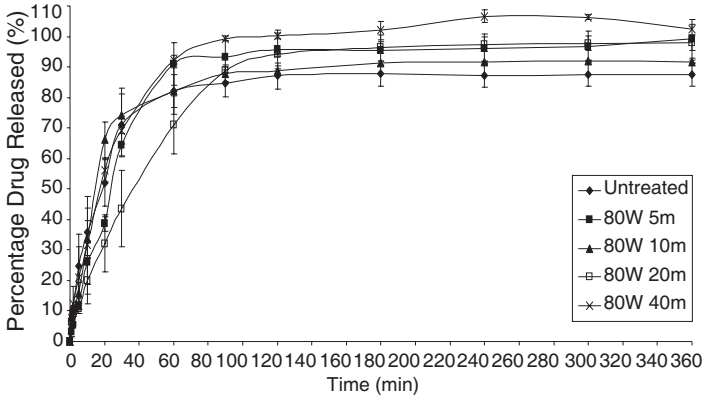
3. RESULTS AND DISCUSSION

The size, shape and drug content of chitosan, CS12%, CS12%SC0.2g, CS12%SC0.5g and CS24% were shown in Table 1. Irradiation of these spheroids by microwave did not result in marked color variation and weight loss ($< 0.02\%$) under all the given experimental conditions. The drug contents of both untreated and treated spheroids were not significantly different from each other (Student's t-test, $P > 0.05$).

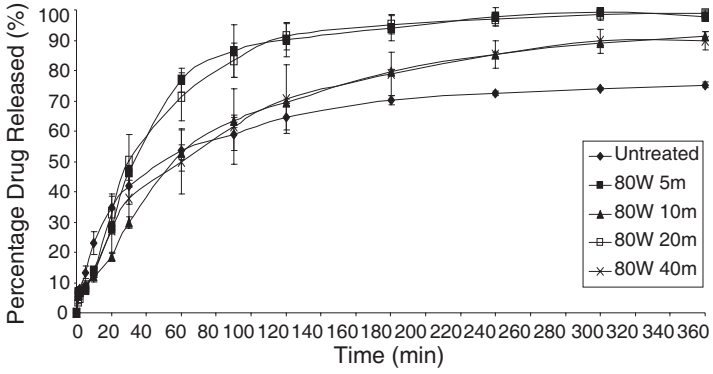
3.1. Chitosan Spheroids

3.1.1. Drug Dissolution

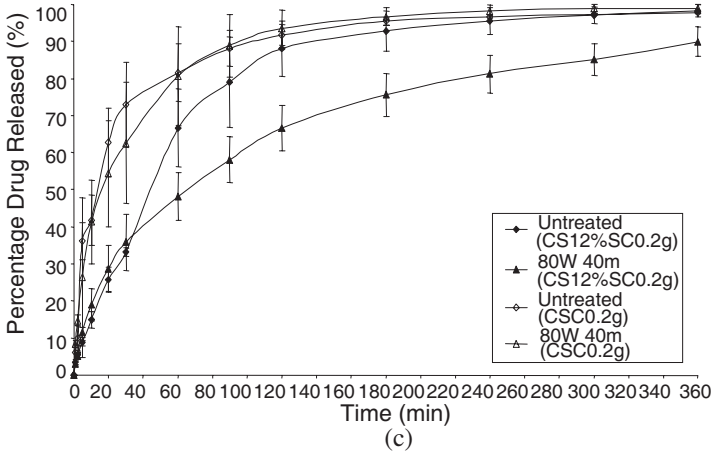
An average of 87.53 ± 3.75 % of chlorpheniramine maleate was released from the untreated chitosan spheroids at 6 h of dissolution (Fig. 1(a)). The treatment of chitosan spheroids by microwave increased the


Percentage Drug Released (%)
Time (min)
Untreated
80W 5m
80W 10m
80W 20m
80W 40m

(a)


Percentage Drug Released (%)
Time (min)
Untreated
80W 5m
80W 10m
80W 20m
80W 40m

(b)


Percentage Drug Released (%)
Time (min)
Untreated (CS12%SC0.2g)
80W 40m (CS12%SC0.2g)
Untreated (CSC0.2g)
80W 40m (CSC0.2g)

(c)

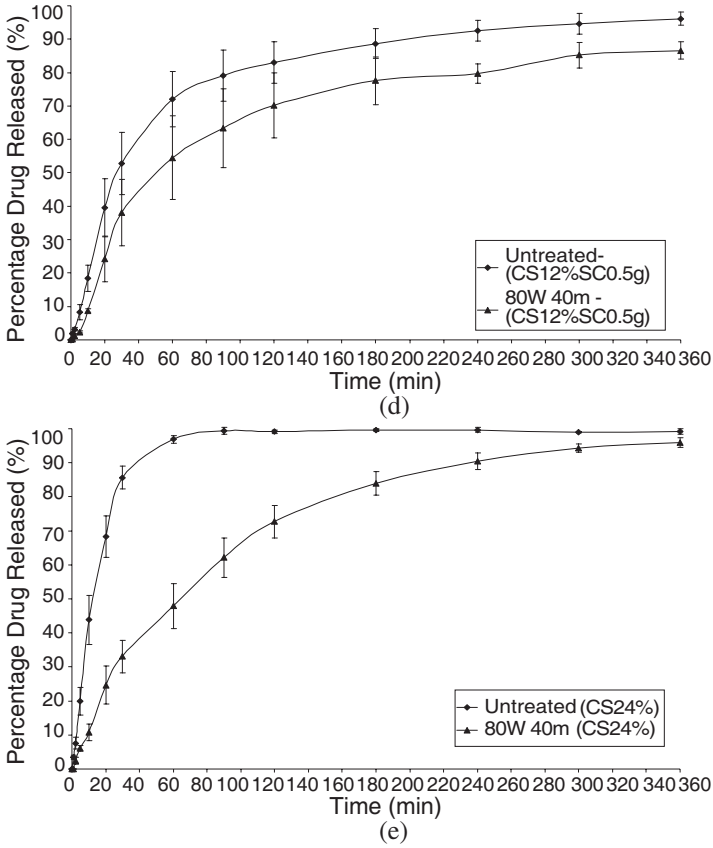


Figure 1. Drug release profiles of (a) chitosan, (b) CS12%, (c) CS12%SC0.2g and CSC0.2g, (d) CS12%SC0.5g and (e) CS24% spheroids, subjected to various microwave irradiation conditions.

percentage of drug released at 6 h of dissolution particularly those treated for 5, 20 and 40 min (Fig. 1(a); Student's t-test, $P < 0.05$). Unlike chitosan matrix prepared by means of ionotropic gelation technique [4], the treatment of chitosan spheroids by microwave did not retard the drug release. Among all batches of microwave treated chitosan spheroids, spheroids treated for 10 min demonstrated the lowest extent of drug released at 6 h of dissolution. Interestingly, the treatment of chitosan spheroids by microwave for 20 min resulted in a lower extent of drug released at the first hour of dissolution when compared with the untreated matrix (Fig. 1(a); Student's t-test, $P < 0.05$).

3.1.2. DSC Analysis

The treatment of blank chitosan spheroids by microwave for 10, 20 and 40 min brought about a reduction in the endothermic peak temperatures and the formation of dual bands at specific endotherms of samples treated for 20 and 40 min, but an increase in the endothermic enthalpy values of these peaks (Fig. 2). Similarly, there was an increase in the endothermic enthalpy values of blank chitosan spheroids when the samples were subjected to microwave treatment for 5 min (Fig. 2). Nonetheless, its exothermic enthalpy was greatly enhanced by microwave. The extent of drug dissolution was generally higher in microwave treated chitosan spheroids as the strength and extent of polymer-polymer interaction at specific domains of matrix were reduced. Among all batches of microwave treated blank chitosan spheroids, the endotherm at 162.0 ± 3.1°C of samples treated for 10 min denoted the largest rise in the enthalpy value (Fig. 2). The extent of polymer-polymer interaction was relatively high and this aptly explained that the drug dissolution extent of chitosan spheroids treated by microwave for 10 min tended to be lower than other treated samples at 6 h of dissolution.

The addition of chlorpheniramine maleate in the blank chitosan spheroids resulted in an increase in the exothermic peak temperature of blank spheroids at 315.9 ± 0.5°C to 318.4 ± 0.3°C in drug loaded samples, as well as, a reduction in the corresponding exothermic enthalpy of drug loaded chitosan spheroids, beyond that could be accounted by the inaccuracy derived from the enthalpy computation owing to the introduction of drug mass in matrix (Fig. 2). The observation suggested that drug-polymer interaction had taken place in the formed matrix. The treatment of drug loaded chitosan spheroids by microwave for 5 to 40 min brought about similar thermal responses as blank spheroids (Fig. 2). The treatment of spheroids by microwave reduced both strength and extent of polymer-polymer and/or drug-polymer interaction at specific domains of matrix thereby giving rise to a higher extent of drug released at 6 h of dissolution than the untreated sample. In drug loaded chitosan spheroids treated by microwave for 10 min, there was a lack of tendency to exhibit dual bands at the specific domain which ascribed its higher propensity of polymer-polymer and/or drug-polymer interaction and lower dissolution extent of drug at 6 h of dissolution than other microwave treated samples. The endothermic enthalpy of drug loaded chitosan spheroids at the melting peak temperature of 176.7 ± 1.6°C was raised when these spheroids were treated by microwave for 20 min (Fig. 2). It was envisaged that a rise in the extent of drug-polymer interaction in specific domain of matrix might be responsible for the delayed release characteristics

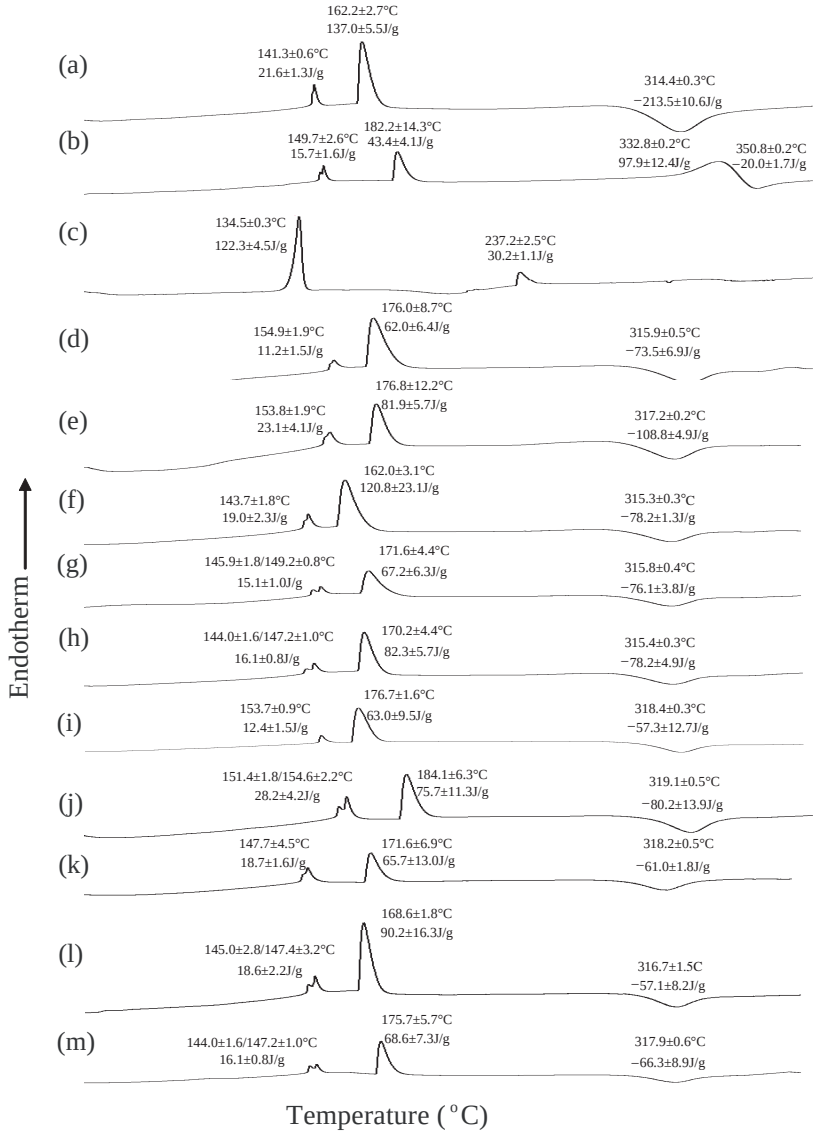


Figure 2. DSC thermograms of (a) chitosan, (b) MCC, (c) chlorpheniramine maleate, (d) blank chitosan spheroids and spheroids treated at 80 W for (e) 5 min, (f) 10 min, (g) 20 min, and (h) 40 min, (i) drug-loaded chitosan spheroids and spheroids treated at 80 W for (j) 5 min, (k) 10 min, (l) 20 min and (m) 40 min.

of these microwave treated spheroids at the initial period of drug dissolution.

3.1.3. FTIR Analysis

The treatment of blank chitosan spheroids by microwave for 5 to 40 min brought about an increase in FTIR wavenumber of $C = O$ moiety at $1644.5 \pm 1.7\,\mathrm{cm}^{-1}$ (Fig. 3). The FTIR peak of blank chitosan spheroids at $3344.5 \pm 6.4\ \mathrm{cm}^{-1}$ exhibited dual crest characteristics following the treatment of samples by microwave for 20 and 40 min (Fig. 3). There was a lower level of polymer-polymer interaction via $C = O$ moiety in all batches of microwave treated samples, and O-H and/or N-H moiety in samples treated for 20 and 40 min thereby resulting in the tendency of chitosan spheroids, particularly the latter, to have a higher extent of drug release at 6 h of dissolution. The dissolution extent of drug from microwave treated chitosan spheroids at 6 h tended to be greater than untreated samples, though the blank matrices undergoing microwave treatment for 10, 20 and 40 min demonstrated polymer-polymer interaction via C-O and/or C-N moiety as marked by an increase in the transmission intensity of FTIR peaks between 1000 and $1400\,\mathrm{cm}^{-1}$ (Fig. 3). With reference to blank chitosan spheroids treated by microwave for 10 min, a broader FTIR peak at the lower wavenumber of $3324.8 \pm 2.4\,\mathrm{cm}^{-1}$ than the untreated sample was noted (Fig. 3). The observation denoted polymer-polymer interaction via O-H and/or N-H moiety of these spheroids was promoted by microwave. Thus, the chitosan spheroids treated by microwave for 10 min would have a relatively small increment of drug dissolution when compared to other samples in response to microwave irradiation.

The incorporation of drug into blank chitosan spheroids was accompanied by drug-polymer interaction in matrix. This was suggested by the transformation of FTIR peaks ascribing to O-H and/or N-H as well as $C = O$ moieties of drug loaded chitosan spheroids at the lower wavenumbers of 3341.0 ± 2.8 and $1586.0 \pm 2.6\,\mathrm{cm}^{-1}$ respectively, and the disappearance of dual crest characteristics in the FTIR spectra of drug loaded chitosan spheroids treated by microwave for 20 and 40 min (Fig. 3). Nonetheless, the treatment of drug loaded chitosan spheroids by microwave induced the dissociation between the drug and polymers via $C = O$, C-O and/or C-N moieties as inferred from the formation of dual band features in association with the FTIR peak ascribing to $C = O$ moiety of untreated samples at $1586.0 \pm 2.6\,\mathrm{cm}^{-1}$, and a decrease in the transmission intensity of FTIR peaks at the wavenumber between 1300 and $1400\,\mathrm{cm}^{-1}$ (Fig. 3). In response to microwave irradiation, the loss of matrix interaction via $C = O$, C-O and/or C-N moieties of

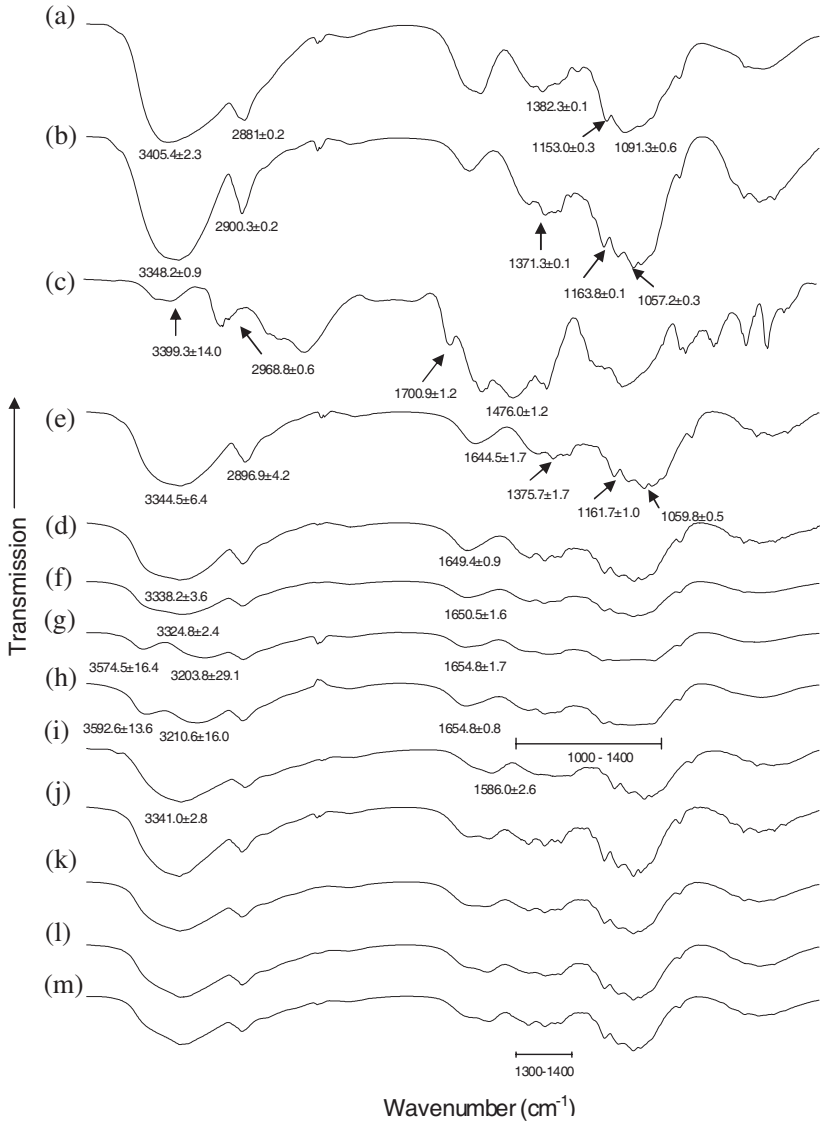


Figure 3. FTIR spectra of (a) chitosan, (b) MCC, (c) chlorpheniramine maleate, (d) blank chitosan spheroids and spheroids treated at 80 W for (e) 5 min, (f) 10 min, (g) 20 min and (h) 40 min, (i) drug-loaded chitosan spheroids and spheroids treated at 80 W for (j) 5 min, (k) 10 min, (l) 20 min and (m) 40 min.

drug loaded chitosan spheroids resulted in an enhancement of drug dissolution. The degree of losses in matrix interaction, inferring from the respective FTIR bands of $C = O$ as well as C-O and/or C-N moieties, was comparatively low in drug loaded chitosan spheroids treated by microwave for 10 and 20 min respectively (Fig. 3). This was translated to a lower increment in the extent of drug dissolution and initial delayed release of drugs from the corresponding batches of spheroids.

3.2. CS12% Spheroids

3.2.1. Drug Dissolution

The incorporation of 12 %w/w stearic acid in chitosan spheroids aided to reduce the extent of drug release at 6 h of dissolution to $75.19 \pm 0.85\%$, owing to the hydrophobic nature of stearic acid (Fig. 1). Similar to that of chitosan spheroids, the treatment of CS12% spheroids by microwave resulted in an increase in the extent of drug dissolution at 6 h (Fig. 1(b); Student's t-test, $P < 0.05$) though it was predicted that the microwave could deform the stearic acid, promote its spreading over the matrix and retard drug release. The microwave treated CS12% spheroids exhibited a short period of slow release at the first 20 min of dissolution when compared to the untreated samples (Student's t-test, $P < 0.05$). Among all microwave treated CS12% spheroids, batches treated for 10 and 40 min demonstrated the lowest extent of drug release at 6 h of dissolution.

3.2.2. DSC Analysis

The thermogram of unprocessed stearic acid was characterized by a single endotherm (Fig. 4(a)). Apparently, the addition of stearic acid in chitosan spheroids resulted in an increase in their melting peak temperature from 176.0 ± 8.7 to 192.9 ± 3.4°C as well as the formation of dual band endotherm of higher melting peak temperatures at 153.4 ± 3.6°C/157.2 ± 1.3°C (Fig. 2(d); Figs. 4(a) and (b)). This denoted stearic acid-polymer interaction was effected in the specific domains of the matrix. In spite of interaction between polymers and stearic acid, the melting peak of unprocessed stearic acid remained detectable in the thermogram of untreated blank CS12% spheroids. It was envisaged that a fraction of free fatty acid was available without interacting with the matrix materials of CS12% spheroids.

The treatment of blank CS12% spheroids by microwave for 5 to 40 min brought about a reduction in the melting peak temperatures of endotherms at $153.4 \pm 3.6/157.2 \pm 1.3$°C and 192.9 ± 3.4°C but

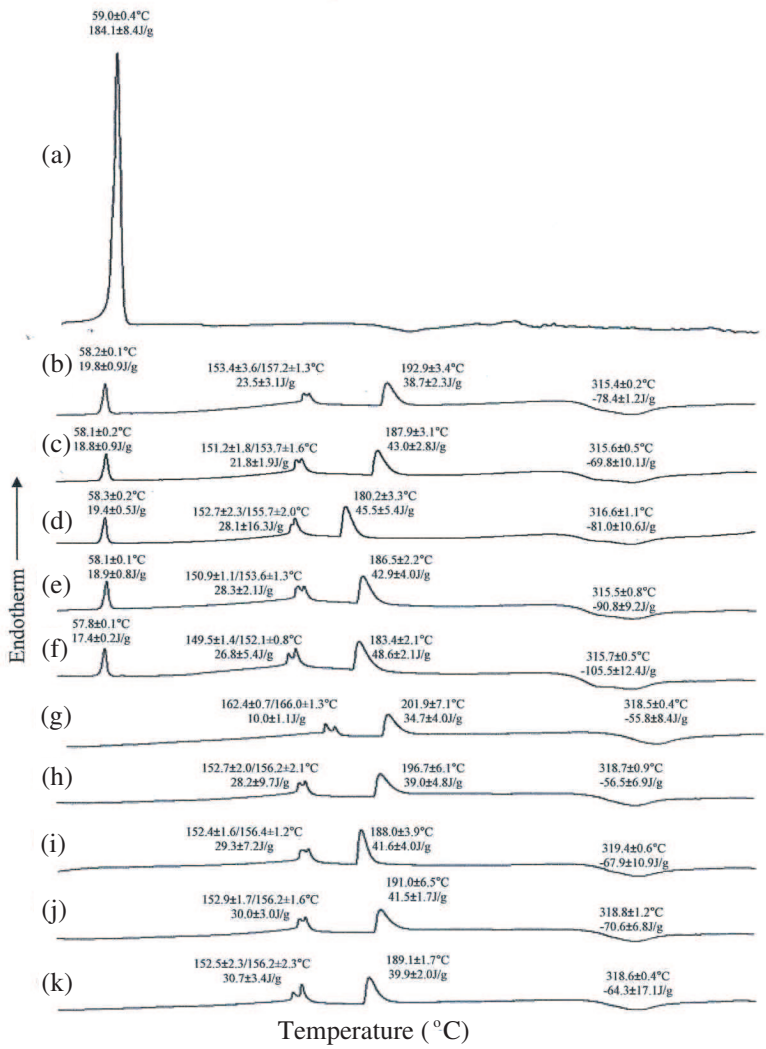


Figure 4. DSC thermograms of (a) stearic acid, (b) blank CS12% spheroids and spheroids treated at 80 W for (c) 5 min, (d) 10 min, (e) 20 min and (f) 40 min, (g) drug-loaded CS12% spheroids and spheroids treated at 80 W for (h) 5 min, (i) 10 min, (j) 20 min and (k) 40 min.

with minimal increment in the melting enthalpy of the same spheroids at 192.9 ± 3.4°C unlike chitosan spheroids (Fig. 4). An increase in exothermic enthalpy of blank CS12% spheroids was also noted in the thermogram of samples treated by microwave for 10, 20 and 40 min

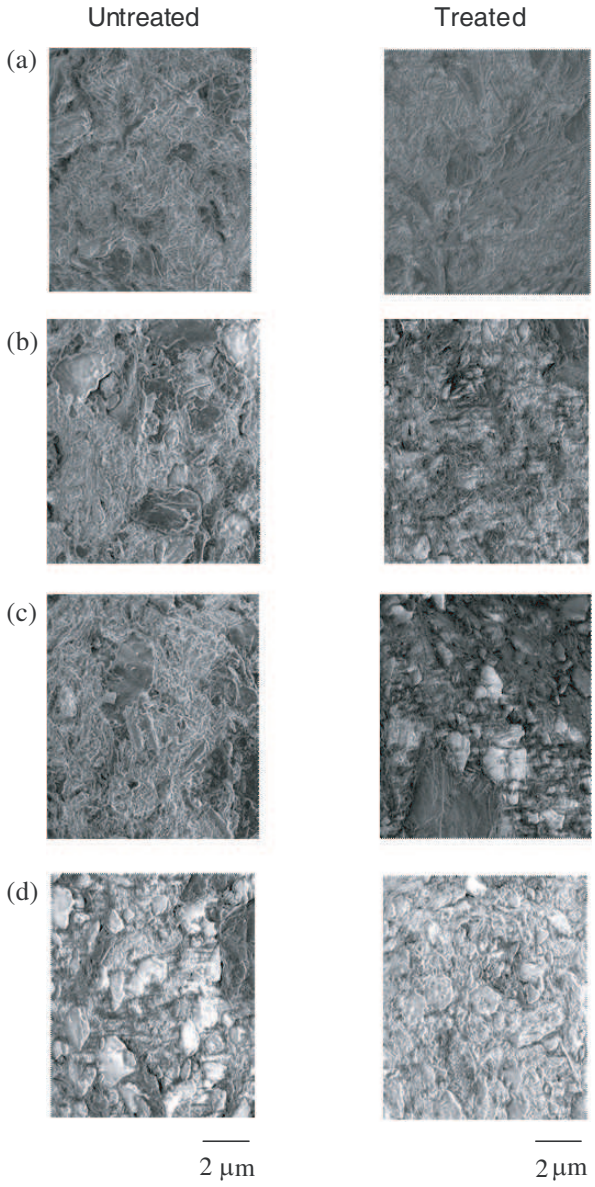


Figure 5. SEM profiles of (a) chitosan, (b) CS12%, (c) CS12%SC0.2g and (d) CS24% spheroids, subjected to microwave irradiation for 40 min.

(Fig. 4). It was expected that a loss of stearic acid-polymer and/or polymer-polymer interaction was effected at the specific domains of microwave treated spheroids and the extent of drug dissolution at 6 h of these spheroids was enhanced. Nevertheless, such losses of matrix interaction could translate to a greater mobility of polymer and specifically stearic acid molecules which had a high degree of deformability and a low melting point. The stearic acid molecules could lose their original physical shape (Fig. 5), deform over the matrix and aid to repel the incoming dissolution medium towards the matrix. Consequently, a slower drug release was observed at the early phase of dissolution when CS12% spheroids were treated by microwave. The deformed stearic acid molecules would have been those largely confined to regions near the vicinity of polymers as the endothermic melting peak temperature and enthalpy profiles of free stearic acid at 58.2 ± 0.1°C in blank CS12% spheroids were unaffected by the irradiation of microwave (Fig. 4). Under the influence of microwave, the melting peak, onset and end temperatures of untreated blank CS12% spheroids at 192.9 ± 3.4, 190.5 ± 3.1 and 200.0 ± 3.8°C were found to be markedly reduced to 180.2 ± 3.3, 177.7 ± 3.3 and 186.8 ± 3.6°C in samples treated by microwave for 10 min with no remarkable rise in its enthalpy (Fig. 4). Among all batches of microwave treated samples, the CS12% spheroids attained the lowest extent of drug release at 6 h of dissolution when they were treated by microwave for 10 min, in addition to that of treated for 40 min which entailed a large rise in the exothermic enthalpy of blank spheroids. Nonetheless, the extent of drug dissolution at 6 h of these microwave-treated spheroids remained higher than the untreated samples. The capacity of deformed stearic acid was not adequate to inhibit drug release, beyond 20 min of dissolution.

The DSC analysis indicated that interaction of drug with stearic acid and/or polymers was effected in matrix incorporating the drug. The endothermic peak ascribing to stearic acid was lost in the thermogram of drug loaded spheroids and the peak temperatures of endotherms and exotherm were higher in drug loaded spheroids than the blank samples (Fig. 4). Similar to blank CS12% spheroids, the treatment of drug loaded spheroids brought about a decrease in the melting peak temperatures of endotherms (Fig. 4). The exothermic enthalpy values of drug loaded CS12% spheroids treated by microwave for 10, 20 and 40 min tended to be higher than untreated samples. The net effect was a rise in the extent of drug dissolution at 6 h with a brief initial delayed release when CS12% spheroids were treated by microwave.

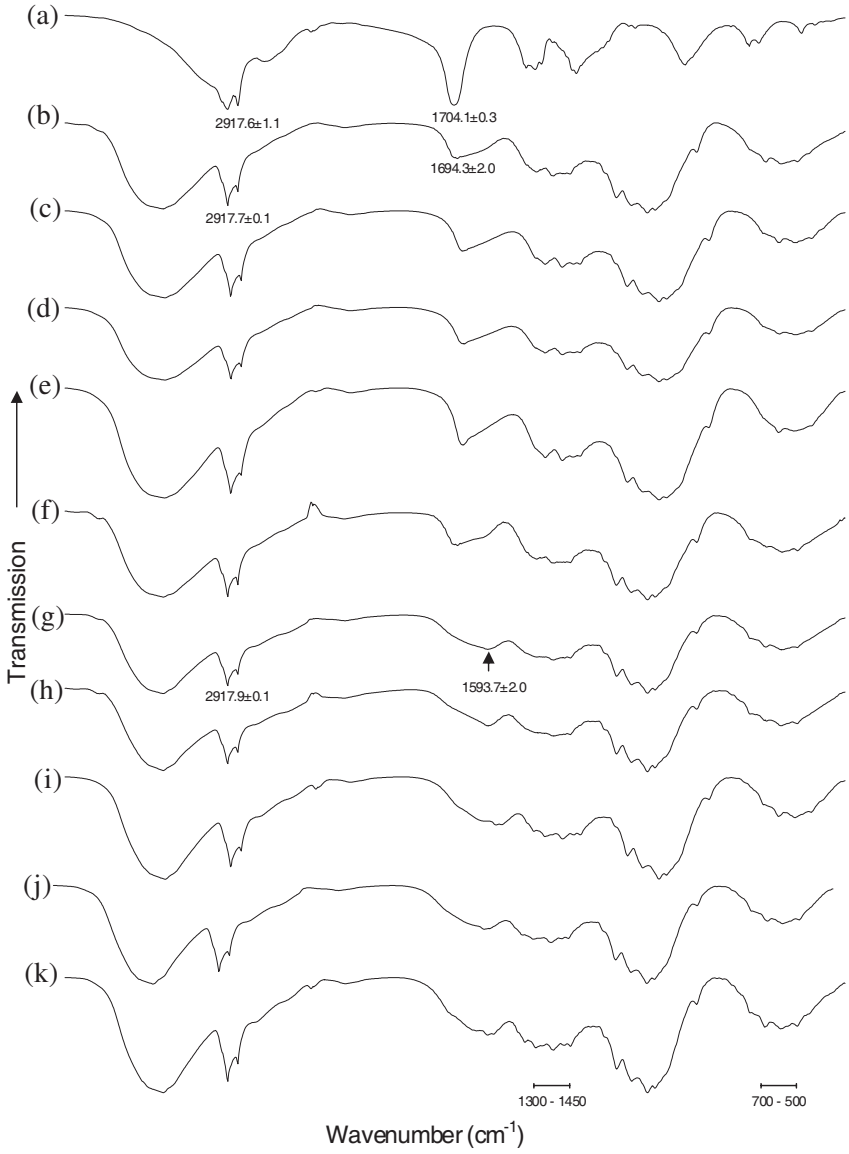


Figure 6. FTIR spectra of (a) stearic acid, (b) blank CS12% spheroids and spheroids treated at 80 W for (c) 5 min, (d) 10 min, (e) 20 min and (f) 40 min, (g) drug-loaded CS12% spheroids and spheroids treated at 80 W for (h) 5 min, (i) 10 min, (j) 20 min and (k) 40 min.

3.2.3. FTIR Analysis

The FTIR analysis of blank CS12% spheroids indicated that the level of chemical interaction between stearic acid and chitosan spheroids was low except at $C = O$ domain of which exhibited a wavenumber of $1694.3 \pm 2.0\,\mathrm{cm}^{-1}$, intermediate to those of found in the spectra of stearic acid and blank chitosan spheroids (Fig. 3(d); Figs. 6(a) and (b)). The FTIR peaks ascribing to C-H moiety of stearic acid at $2917.6 \pm 1.1\,\mathrm{cm}^{-1}$ were found in the spectra of blank CS12% spheroids at the expense of FTIR interference from the C-H moiety of matrix polymers. This observation suggested that a fraction of free fatty acid was available in blank CS12% spheroids as indicated previously by the DSC study. The addition of drug into blank CS12% spheroids did not give rise to any variation in the FTIR C-H peak characteristics of stearic acid, but a reduction in the wavenumber of FTIR peak ascribing $C = O$ moiety of the matrix from 1694.3 ± 2.0 to $1593.7 \pm 2.0\,\mathrm{cm}^{-1}$ (Fig. 6). The results indicated that the chemical interaction between the drug and blank CS12% matrix was effected via $C = O$ moiety.

The treatment of blank CS12% spheroids by microwave did not bring about marked changes to the FTIR spectra of untreated matrix (Fig. 6). The treatment of drug loaded CS12% spheroids by microwave brought about a decrease in the transmission intensity of FTIR peaks between 1300 and 1450 as well as 500 and $700\,\mathrm{cm}^{-1}$ denoting mobilization of C-O, C-N and/or C-H moieties when these matrices were subjected to irradiation (Fig. 6). With reference to DSC study which indicated both physical and chemical changes of matrix, a low degree of FTIR changes and a marked variation in DSC thermograms would mean that the drug dissolution profiles of CS12% spheroids were largely governed by physical changes of matrix substances towards microwave.

3.3. CSSC Spheroids

3.3.1. Drug Dissolution

In response to the influence of microwave irradiation, the extent of drug release from CS12% spheroids was reduced at the initial phase of dissolution but it was greatly enhanced at the late phase of the same process when compared to untreated samples. It was envisaged that the responses of CS12% matrix particularly stearic acid to microwave were not adequate to reduce the extent of drug dissolution over a period of 6 h. Among the various batches of microwave treated CS12% spheroids, the samples treated at 80 W for 40 min demonstrated the lowest extent of drug release at 6 h of dissolution. From the

preliminary study on unprocessed stearic acid and its mixture with sodium chloride, it was found that the solid particles of stearic acid can be melted or softened using the supplied microwave energy of 81 to 192 kJ in the presence of sodium chloride. On the note of the possibility of using sodium chloride to increase the deformability of stearic acid using microwave, the CSSC spheroids were prepared and their drug dissolution profiles were examined with respect to the treatment conditions of microwave at 80 W for 40 min which constituted a supplied energy of 192 kJ.

At 6 h of dissolution, an average of $98.27 \pm 1.74\%$ of drug was released from the untreated CS12%SC0.2g spheroids (Fig. 1(c)). The extent of drug dissolution from CS12%SC0.2g spheroids was higher than untreated chitosan and CS12% spheroids as sodium chloride can act as channeling agent owing to its high aqueous solubility attribute (36 g/100 g water) and fast release upon placement of spheroids in dissolution medium (Fig. 7). The treatment of CS12%SC0.2g spheroids

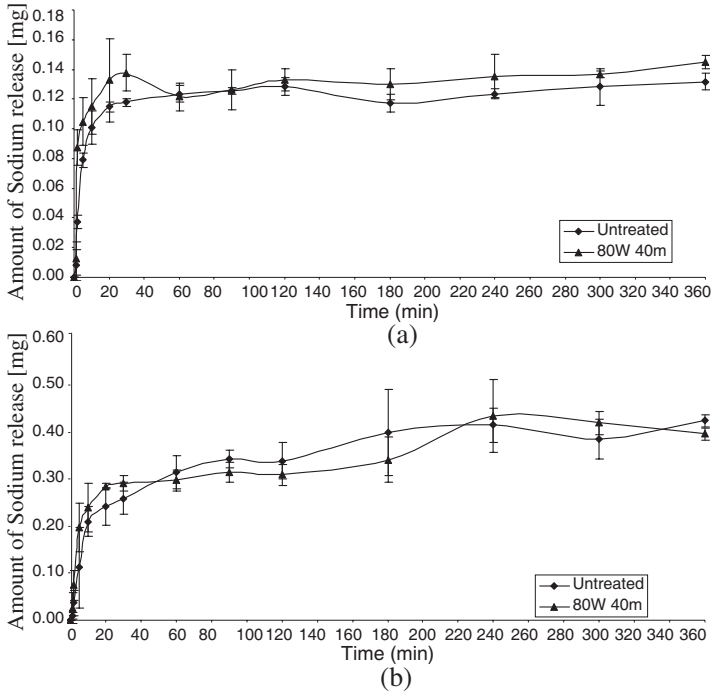


Figure 7. Sodium leaching profiles of (a) CS12%SC0.2g and (b) CS12%SC0.5g spheroids, subjected to microwave irradiation for 40 min.

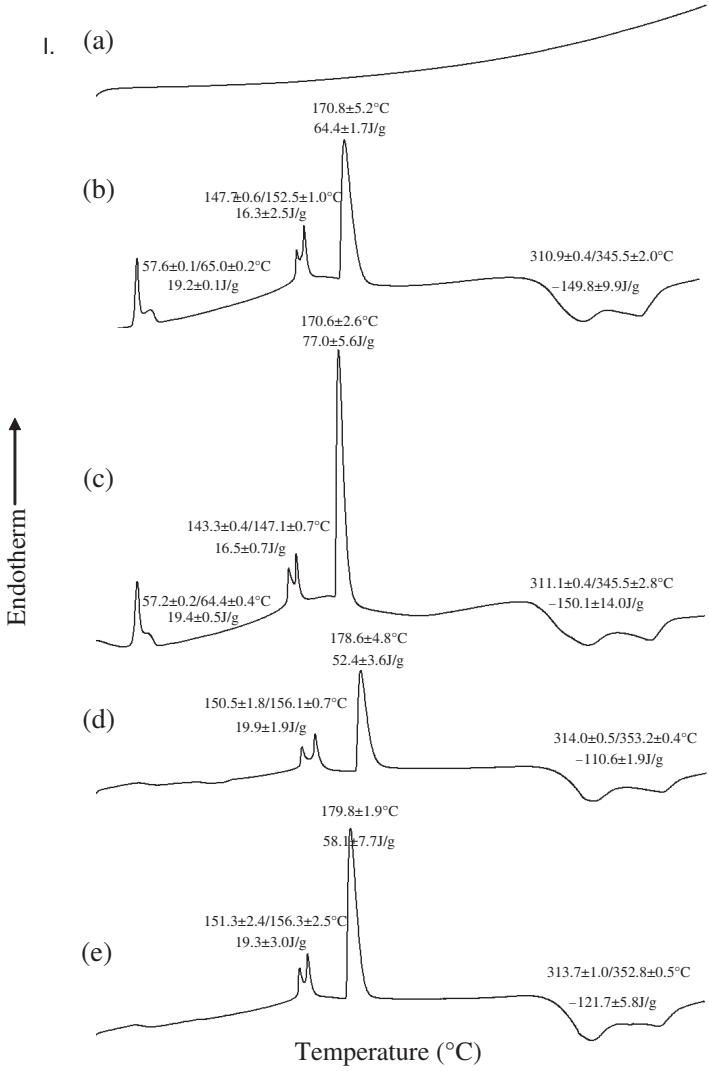
by microwave brought about a reduction in the extent of drug release (Fig. 1(c)). The extent of drug released from microwave treated CS12%SC0.2g spheroids at 6 h of dissolution was lower than untreated samples (Student's t-test, $P < 0.05$). The reduction in drug release of spheroids was not ascribed by reduced channeling effect (Fig. 7). The profiles of sodium leaching from both untreated and microwave treated spheroids were similar.

3.3.2. DSC Analysis

The addition of sodium chloride into blank CS12% spheroids resulted in a reduction of the endothermic and exothermic peak temperatures at various domains of CS12% spheroids (Figs. 4(b) and 8I(b)). In addition, it transformed the single peak characteristics of endotherm ascribing to stearic acid at 58.2 ± 0.1°C and exotherm at 315.4 ± 0.2°C to dual band feature with the second band of endotherm and exotherm ascribing by higher peak temperatures of 65.0 ± 0.2 and 345.5 ± 2.0°C, as well as, an increase in the enthalpy value of endotherm at 170.8 ± 5.2°C in the case of CS12%SC0.2g spheroids (Figs. 4(b) and 8I(b)). The results indicated that sodium chloride interacted with matrix involving the domain of free fatty acid. Its interaction with matrix led to the formation of specific sites with a high interactive strength. In the presence of sodium chloride, the formation of interacted matrix was also reflected by the ease of particle binding into spheroids and thus a lower requirement on binding liquid level as described under Section 2.2. The incorporation of drug into CS12%SC0.2g spheroids resulted in the loss of endothermic peak ascribing to stearic acid at $57.6 \pm 0.1/65.0 \pm 0.2$°C and an increase in the endothermic as well as exothermic peak temperatures of blank CS12%SC0.2g spheroids, following drug-polymer interaction in the formed matrix (Figs. 8I(b) and (d)). The summative effect provided CS12%SC0.2g spheroids a higher level of resistance to disintegration in dissolution medium over a period of 6 hours than chitosan and CS12% spheroids (Fig. 9).

Unlike CS12% spheroids, the treatment of both blank and drug loaded CS12%SC0.2g spheroids by microwave for 40 min did not further reduce the melting peak temperature of endotherms at 170.8 ± 5.2 and 178.6 ± 4.8°C respectively (Fig. 8I). The treatment of these spheroids by microwave for 40 min was accompanied by a corresponding rise in the enthalpy value of endotherms. In addition, a marked loss of chitosan/MCC structure and emergence of stearic acid particulate matters was noted over the surfaces of microwave treated CS12%SC0.2g spheroids (Fig. 5). The addition of a low amount of sodium chloride enhanced stearic acid spread as well as matrix binding. Keeping the matrix robust against disintegration, the spread

of stearic acid over drug/polymer domain of CS12%SC0.2g spheroids would lead to the formation of hydrophobic surfaces with reduced specific surface area for drug release. This in turn decreased the extent of drug dissolution of microwave treated spheroids. Further examination on the concomitant effect of stearic acid and sodium chloride with microwave on drug release indicated that mere CSC0.2g spheroids without stearic acid or CS12% spheroids without sodium chloride did not give rise to a marked reduction in the extent of



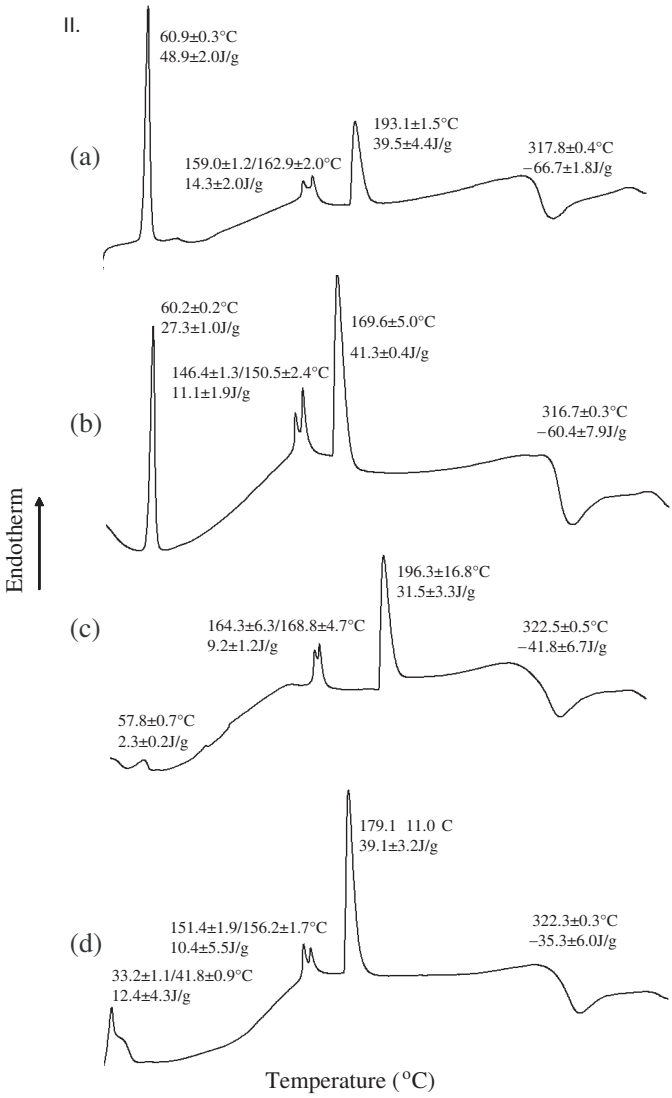


Figure 8. DSC thermograms of I: (a) Sodium chloride, (b) blank CS12%SC0.2g spheroids and spheroids treated at 80 W for (c) 40 min, (d) drug-loaded CS12%SC0.2g spheroids and spheroids treated at 80 W for (e) 40 min. II: (a) Blank CS24% spheroids and spheroids treated at 80 W for (b) 40 min, (c) drug-loaded CS24% spheroids and spheroids treated at 80 W for (d) 40 min.

drug dissolution following their treatment by microwave for 40 min (Figs. 1(b) and (c)). The induction of drug release retardation by microwave required both stearic acid spread over drug/polymer domain as well as reduced tendency of spheroid disintegration aided by the use of sodium chloride. A similar drug release retardation profile was attained by means of microwave when an additional amount of sodium chloride was introduced to prepare CS12%SC0.5g spheroids (Fig. 1(d)). A lower extent of drug was released from microwave treated CS12%SC0.5g spheroids than untreated samples throughout 6 h of dissolution. The difference in drug release characteristics of these spheroids was not ascribed by variation in sodium leaching profile (Fig. 7).

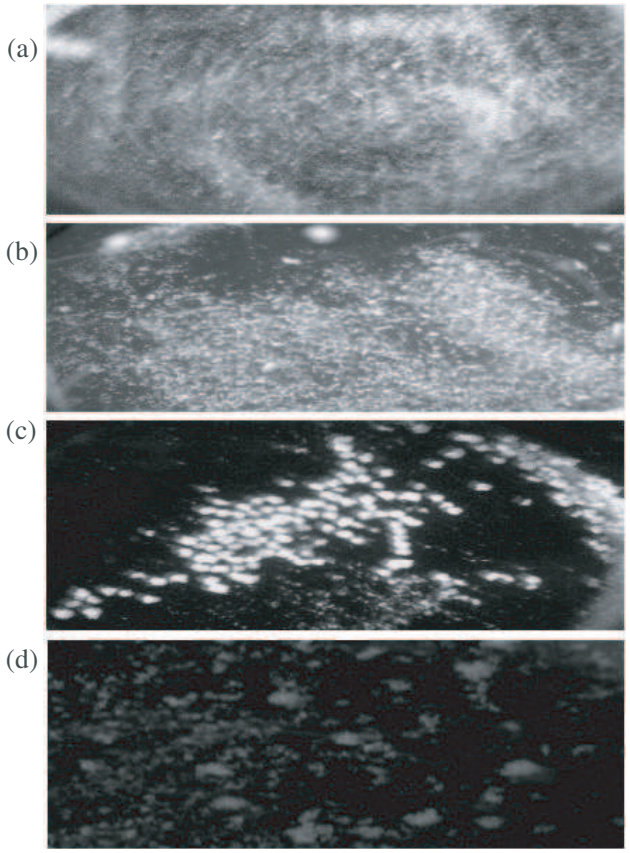


Figure 9. Morphology images of (a) chitosan, (b) CS12%, (c) CS12%SC0.2g and (d) CS24% spheroids undergoing process of dissolution at 6 h.

3.3.3. FTIR Analysis

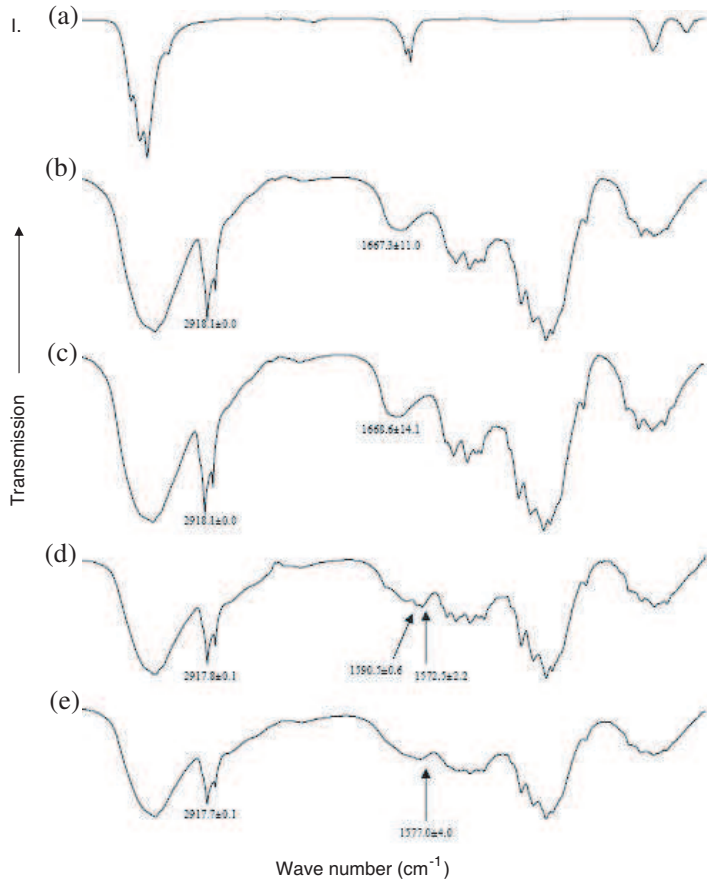
The FTIR spectra of the untreated blank as well as drug loaded CS12%SC0.2g spheroids indicated that the addition of sodium chloride strengthened the matrix via $C = O$ moiety. The wavenumbers ascribing $C = O$ functional group at 1667.3 ± 11.0 and $1572.5 \pm 2.2/1590.5 \pm 0.6\,\text{cm}^{-1}$ were lower in the respective blank and drug loaded CS12%SC0.2g spheroids than the corresponding CS12% spheroids (Figs. 6 and 10I). The treatment of blank CS12%SC0.2g spheroids by microwave for 40 min did not give rise to marked changes to the state of chemical interaction between various moieties of the matrix (Fig. 10I). Nonetheless, the treatment of drug loaded CS12%SC0.2g spheroids brought about the coalescence of FTIR peaks ascribing to $C = O$ moiety at 1590.5 ± 0.6 and $1572.5 \pm 2.2\,\text{cm}^{-1}$ to a single peak at $1577.0 \pm 4.0\,\text{cm}^{-1}$ (Fig. 10I). The observation denoted that the reduced extent of drug released from CS12%SC0.2g spheroids, following their treatment by microwave for 40 min, was partly an attribute of drug-stearic acid-polymer interaction via the $C = O$ moiety. Inferring from DSC and FTIR studies, the retardation of drug release from CS12%SC0.2g spheroids was concluded to be mediated by microwave through both physical and chemical changes of matrix.

3.4. CS24% Spheroids

The use of sodium chloride was able to sensitize the CS12% spheroids to sustained drug release induction effect of microwave. Nonetheless, the overall drug release propensity of these spheroids was high with an increase in the amount of sodium chloride employed. This was suggested by the drug release profiles of CS12%SC0.2g and CS12%SC0.5g matrices (Figs. 1(c) and (d)). One major reason was that the channeling effect was greater with spheroids containing a higher level of sodium chloride (Fig. 7). As such, CS24% spheroids were formulated with no sodium chloride added, and instead, a higher fraction of stearic acid was introduced to acquire a larger summative effect of microwave-stearic acid interaction in the subsequent study.

Untreated CS24% spheroids exhibited a higher rate of drug release than CS12% spheroids in spite of the former contained a higher fraction of stearic acid and a lower level of disintegration due to it had a reduced fraction of dispersible chitosan/MCC (Figs. 1(b), 1(e) and 9). One possibility was that the hydrophobic stearic acid particles might not be tightly interspersed within the hydrophilic domain of untreated spheroids (Fig. 5). The formation of gap provided channels for drug released from core to exterior dissolution medium. Unlike CS12% spheroids, the treatment of CS24% spheroids by microwave for

40 min reduced the propensity of drug release remarkably (Figs. 1(b) and (e)). The reduction extent of drug release within the first 120 min of dissolution was greater even than those of CS12% spheroids with sodium chloride added (Figs. 1(c), (d) and (e)). Examination of SEM profile of CS24% spheroids indicated that the stearic acid particles of these spheroids were deformed and a greater extent of stearic acid spread over the surfaces of matrix was noted than CS12% spheroids due to a larger summative response to microwave of a greater fraction of fatty acid (Fig. 5). With higher stearic acid content, the matrix was characterized by a higher level of domain mobility in response to microwave. This was supported by a remarkable reduction in endothermic peak temperatures ascribing drug/polymer as well as free stearic acid domains of spheroids in the DSC study (Fig. 8II). Nonetheless, the disintegration resistance of CS24% spheroids was



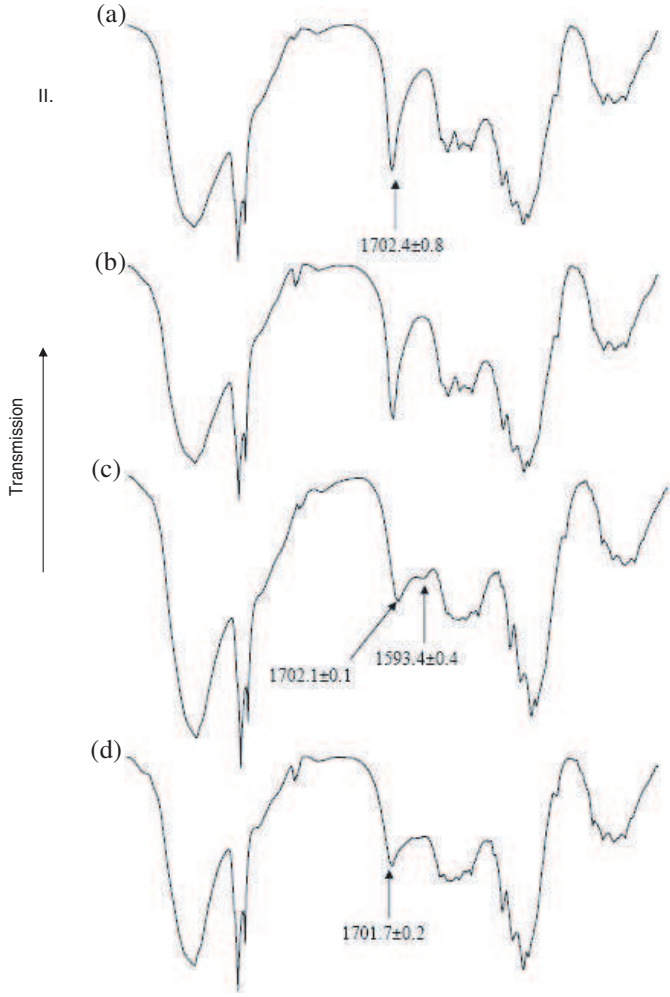


Figure 10. FTIR spectra of I: (a) Sodium chloride, (b) blank CS12%SC0.2g spheroids and spheroids treated at 80 W for (c) 40 min, (d) drug-loaded CS12%SC0.2g spheroids and spheroids treated at 80 W for (e) 40 min. II: (a) Blank CS24% spheroids and spheroids treated at 80 W for (b) 40 min, (c) drug-loaded CS24% spheroids and spheroids treated at 80 W for (d) 40 min.

maintained by the use of a lower fraction of dispersible chitosan/MCC. The drug released from CS24% spheroids was retarded by microwave through physical modification as well as chemical changes of matrix at $C = O$ moiety (Figs. 8II and 10II).

4. CONCLUSION

The sustained-release behavior of chitosan spheroids can be induced by microwave when these spheroids were prepared with a high weight ratio of stearic acid to dispersible chitosan/MCC. Using a low level of stearic acid, sodium chloride was needed to maintain matrix integrity as well as enhance the deformation and spread of stearic acid over spheroids thereby reducing their drug release propensity in response to microwave.

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