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Simultaneous X-ray Diffraction-Differential Scanning Calorimetry and Physicochemical Characterizations of Spray Dried Drugs and Chitosan Microspheres

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Abstract

The aim of this study was to characterize the morphology and physicochemical properties of drug-chitosan spray dried particles (SDPs) which have been wildly used as drug carriers. The SDP systems were composed of chitosan (CS) loaded with diclofenac sodium (DS) or theophylline anhydrous (TH) as model drugs. In the SDPs preparation process, the model drug was dissolved in distilled water and then dropped into a solution of CS dissolved in 0.5 % acetic acid. The drug-CS suspension was spray dried then the products were collected and characterized. Scanning electron microscopy (SEM) and particle size analysis demonstrated that DS-CS SDPs were irregular in shape with sizes between 3.28 - 6.72 µm. On the other hand, TH-CS SDP were spherical and the size of TH-CS microspheres were between 3.64 - 4.28 µm. SEM images also showed that TH was completely encapsulated by CS at a ratio of over 1:8. From simultaneous DSC-XRD analysis, it was found that crystallinity of the drugs decreased when increasing the temperature. TH was altered from the crystalline to amorphous state when CS portion was greater. Fourier transformed infrared (FTIR) spectroscopy indicates that some DS (salt form) changed to acid form after the spray drying process. Proper portions of CS delayed the release of DS and TH.

Keywords: Chitosan, microspheres, microparticles, spray drying, simultaneous XRD-DSC measurement

Introduction

Recently drugs and chitosan (CS) microspheres were applied in several pharmaceutical dosage forms such as inhalation powders [1,2], as an excipient in drug control released tablet [3,4] and as a core of the oral drug delivery by coating with enteric materials [5]. This is because the LD_{50} of CS is considerably high (16 g/kg body weight) [6]; therefore, it is considered as non-toxic and biocompatible. Moreover, its bioadhesive properties can increase drug retention time in gastrointestinal tract [7]. Besides that, a positive charge from the primary amine of protonated CS can interact with the tight junction of gastrointestinal cells [8] culminating in greater drug absorption though the paracellular pathway [9-11]. However, there are only a few studies conducted with characterized researches the physicochemical properties of the drug-CS microspheres. Physicochemical characterization, particle size analysis and morphology studies can improve understanding of drug release behavior, thermal drug stability and interaction of the drug and polymer.

Drug-chitosan microspheres have been extensively prepared using a spray drying technique [12,13] since it is a simple system whereby the particle size of the product can be controlled in a single step. Furthermore, the spray drying technique can be scaled up to ton quantities. In addition, amorphous forms of crystalline drugs were produced by this technique thus the poor solubility of the drug could be improved [14,15].

In this present study chitosan was spray dried with model drugs in various drug-polymer ratios to examine the effect of CS portion, type of drug and temperature on the drug-CS spray dried system. Diclofenac sodium (DS) and anhydrous theophylline (TH) were selected as the model drugs to be representative of acid and basic drugs, respectively. Morphology, thermal behavior, physicochemical properties and drug release profile of the drugs-CS spray dried particles (SDPs) in several ratios were investigated using particle size analyzer, scanning electron microscopy (SEM), simultaneous measurements of X-ray diffraction-differential scanning calorimetry (XRD-DSC), Fourier transformed infrared (FTIR) and *in vitro* drug release study.

Materials and methods

Chitosan (with 87 % degree of deacetylation and a molecular weight of 45 kDa) was purchased from Seafresh Co. Ltd (Lot No. COA050507, Bangkok, Thailand). DS was obtained from Amoli Organics Ltd (Lot No. DS/0501/588A, Mumbai, India). TH was purchased from P.C. Drug Center Co., Ltd. (Batch No. A000725, Bangkok, Thailand). Glacial acetic acid was purchased from Carlo Erba (Lot No. 6M387197A, Val de Reuil, France). All other chemicals were of reagent grade.

Preparation of drug-chitosan microspheres

To prepare DS-CS SDP, DS was dissolved in purified water then the DS solution was dropped in the CS solution which previously dissolved in 0.5 % acetic acid. The drug-CS dispersions were spray dried (model SD-60, Labplant, UK) under the following conditions: inlet temperature was 140 ºC, feed rate was 5 mL/min and outlet temperature was around 80 - 90 ºC. The TH-CS SDP were prepared by dissolving TH and CS in 0.5 % acetic acid aqueous solution. Then the TH-CS solutions were spray died under similar conditions. Both drugs-polymer mixtures were prepared in several drug:polymer ratios including 1:2, 1:4, 1:6, 1:8 and 1:10, respectively. For purposes of comparison, the DS-CS and TH-CS physical mixtures (PM) were also prepared by agitation for 5 min using a vortex mixer (VX-100, Labnet, USA). The obtained powders were collected and kept in a desiccator for further investigation.

Drug-chitosan microspheres morphology

SEM (MX2000, Camscan, UK) analyses were performed on the products obtained from the spray-drying process. The SDP were gold sputtered under high vacuum and photographs were taken at a magnification range of 1000 - 20,000×.

Particle size and span

Dynamic light scattering analysis was carried out using a particle size distribution analyzer (LA-950, Horiba, Japan). The samples were prepared by dispersing 10 mg of the spray dried products in 10 ml of distilled water. The suspensions were sonicated for 10 min and analyzed. The polydispersity, i.e. the width of the particle size distribution was measured by span following the equation below. A small span indicates a narrow size distribution.

$$Span = \frac{(particle\ diameter\ at\ 90\ \%\ cumulative\ size) - (particle\ diameter\ at\ 10\ \%\ cumulative\ size)}{particle\ diameter\ at\ 50\ \%\ cumulative\ size}$$

Simultaneous measurement of powder X-ray diffraction and differential scanning calorimetry

A simultaneous XRD-DSC measurement instrument which combined a heat-flux type DSC (Rigaku/ThermoPlus DSC8230) with a X-ray diffractometer (Rigaku/Rint-Ultima+) was used to investigate the thermal behavior and crystallinity of the spray dried drug-CS microspheres. The XRD

patterns were collected using a step scan: 0.01° 2θ/step, 2 s/step and 2θ start from 5º to 40º. Simultaneously, differential scanning calorimetry (DSC) was carried out. The operating conditions in the close pan system were 3 mg; heating rate, 10 ºC/min. The analysis temperature range was between 20 and 300 ºC.

The starting materials, the physical mixtures, and the drug-CS spray dried powders of 6 - 9 mg were packed in an aluminum square-shaped container without mechanical grinding.

FTIR spectrum

To monitor drug-polymer interactions, absorption spectra of the starting materials and the drug-CS microspheres were recorded on a FTIR spectrophotometer (Niclolet 4700, Thermo Electron Corporation, USA). Samples were prepared with drug-polymer spray dried powders dispersed in KBr pellets. All powders were dried at 100 °C for 24 h before tableting.

***In vitro* drug release of the spray dried mixtures**

The *in vitro* dissolution of the SDPs and the physical mixtures as well as the pure drugs were determined using the paddle method, USP apparatus II (PTWS3C, Pharma Test, Germany) on line with a UV-spectrophotometer (Lamda2, Perkin-Elmer, USA), in 900 mL of pH 6.8 tris-HCl buffer, under non-sink conditions. The paddle rotation was set at 200 rpm. The temperature was maintained at 37 ± 0.5 °C. The pure drugs, the spray dried mixtures and the physical mixtures containing an equivalent 25 mg of the model drugs were tested for their dissolution in a pH 6.8 tris-HCl buffer. The dissolved solution samples were collected, and the amount of drug measured at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10 and 12 h dissolution time. The drug concentrations were determined at λ 273 nm for DS and 276 nm for TH. For each sample, the dissolution test was done 3 times.

Results and discussions

Morphology of drug-chitosan microspheres

Figure 1 depicts the morphology of the DS-CS SDPs in various ratios examined by SEM. As presented in **Figures 1a** and **1b**, it was found that DS was uncoated by CS at 1:2 and 1:4 DS-CS ratios. DS was in crystal rod shape while CS was irregular in shape. When the ratio reach 1:6, 1:8 and 1:10 (**Figures 1C, 1D** and **1E**, respectively) the DS crystals were enveloped by CS. However, the SDPs were of an irregular shape. DS is only slightly soluble in acetic acid and thus the spray dried particle shape relies on the crystal shape of the drug.

Compared to the TH-CS spray dried microspheres presented in **Figure 2**, the CS particles were spherical with TH flakes attached on the surfaces of the microspheres at the TH:CS ratio, 1:2, 1:4 and 1:6 (**Figures 2A, 2B** and **2C**, respectively). However at a ratio 1:8 and 1:10 (**Figures 2D** and **2E**), the TH flakes were encapsulated by CS. At ratio 1:10 of TH-CS, the microsphere surfaces were not smooth. Their surfaces were shrinking and the shapes were less spherical. This could be because of a high amount of CS solution in the mixture prepared by spray drying caused of high evaporation of water during the spray drying process [13].

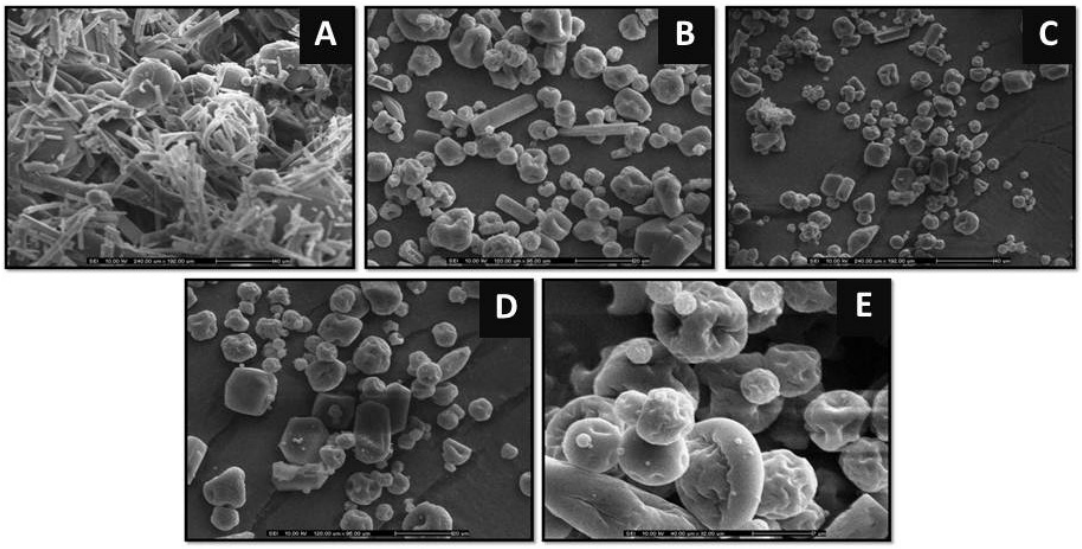

Figure 1 SEM images of the DS-CS SDP in various ratios ((A) 1:2, (B) 1:4, (C) 1:6, (D) 1:8 and (E) 1:10).

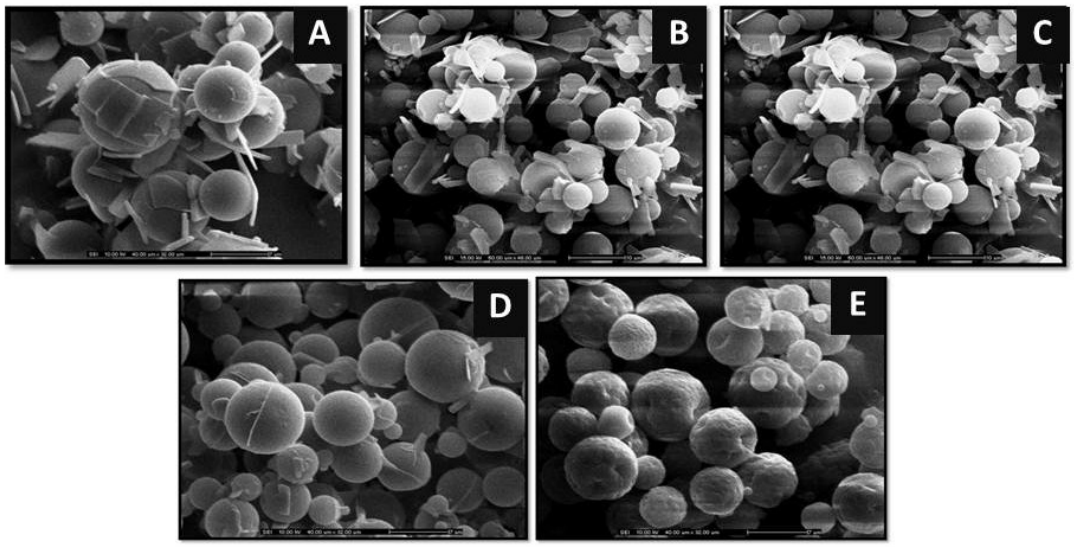

Figure 2 SEM images of the TH-CS SDP in various ratios ((A) 1:2, (B) 1:4, (C) 1:6, (D) 1:8 and (E) 1:10).

Particle size and size distribution

The average sizes and span of DS-CS and TH-CS SDP in various ratios including, 1:2, 1:4, 1:6, 1:8 and 1:10. CSA SDP size and span were 3.59 µm, 1.15, respectively (**Table 1**). The size of DS-CS and TH SDP were in the range of 3.28 - 6.72 µm and 3.64 - 4.28 µm, respectively. The size of CSA SDP was around 5.42 µm in diameter. The SDP at a ratio of 1:2 (drug:CS) gives the smallest particle. In drug:CS ratios of 1:4, 1:6, 1:8 and 1:10, the TH-CS microspheres were significantly smaller than that of DS-CS.

Furthermore, most of span of DS-CS SDP were larger that of TH-CS SDP (except 1:2 ratio, DS:CS). This is because DS precipitates in acid conditions (chitosan solution) causing particle aggregation in various sizes. However, at 1:2 ratio of the DS-CS SDP, the amount of the active drug was lower than the other ratios. Consequently, precipitation of DS was also less than the other ratios resulting in smaller particles. In addition, most of the drug-CS SDP were smaller than CSA SDP. This result is similar to the previous study by Alhalaweh *et al*. [15] which explained that drug molecules might reduce intermolecular forces between chitosan in the spray dry solution affecting lower drop size of the spray dry solution.

Table 1 Particle sizes and span of the DS-CS SDPs and TH-CS SDPs in various ratios.

Drug:Polymer ratio	DS-CS SDP		TH-CS SDP	
	Size (µm)	Span	Size (µm)	Span
1:2	3.28	0.47	3.64	1.11
1:4	6.40	1.68	3.39	1.07
1:6	6.72	1.60	4.28	1.44
1:8	5.18	1.52	3.88	1.33
1:10	4.49	1.34	3.76	1.27

Simultaneous measurement of powder XRD and DSC

The powder XRD patterns and DSC thermograms of the DS-CS and TH-DS microspheres and their PMs were simultaneously examined to monitor the effect of temperature on drug state in the microspheres at different drug polymer ratios.

DS-CS spray dried particles

The XRD-DSC scans of the DS-CS SDPs at a 1:2 ratio is illustrated in **Figure 3**. The XRD crystalline peaks were found at 15.28°, 23.62°, 24.44° and 28.54° under ambient temperature. All crystalline peaks disappeared when the temperature reached 140°C. This changing temperature was similar to the temperature of the DSC endothermic peak. It showed that DS crystals melted and showed amorphous properties at 140 °C and above. The DSC endothermic peaks were also found at 240 °C followed by an endothermic peak which were close to the decomposition temperatures of DS [16]. The XRD patterns of all ratios of CSA, DS and the DS-CS spray dried particles (1:2, 1:4, 1:6, 1:8 and 1:10) are shown in **Figure 4**. The XRD patterns of CSA exhibited signs of being amorphous, as a diffraction peak was not observed. On the contrary, DS expressed diffraction peaks at 15.3° and 23.58° which were typical of a crystalline material. Diclofenac in acid form also exhibits peaks of crystalline drug at 10.84°, 15.24°, 21.52° and 28.12°. The PXRD peaks of DS-CS PM were observed in the same region as DS. The XRD diagram of the spray-dried particles in all ratios showed dominant crystalline peaks at 23.62° which were similar to DS and at 15.28°, 24.44° and 28.54° which were similar diclofenac in acid form. These reveal that increasing portion of CS could not change DS from crystalline state to amorphous state. Some DS in the SDPs transformed to acid form during spray drying process. The XRD-DSC result of spray dried DS-CS was in agreement with the morphology results which confirmed that DS was precipitated into crystals in acetic acid and remained crystalline during the spray drying process. Increasing portion of CS only warped DS crystals inside. DS did not molecularly disperse in CS.

As presented in **Table 2**, there were 2 DSC endothermic peaks for DS-CS SDPs in every drug-polymer ratio which are around 140 and 220 °C. These indicate that there were 2 forms of diclofenac, acid and salt form, in the DS-CS SDPs. The endothermic peak at 140 °C referred to acid from of diclofenac and the endothermic peak at 220 °C came from the salt form [17].

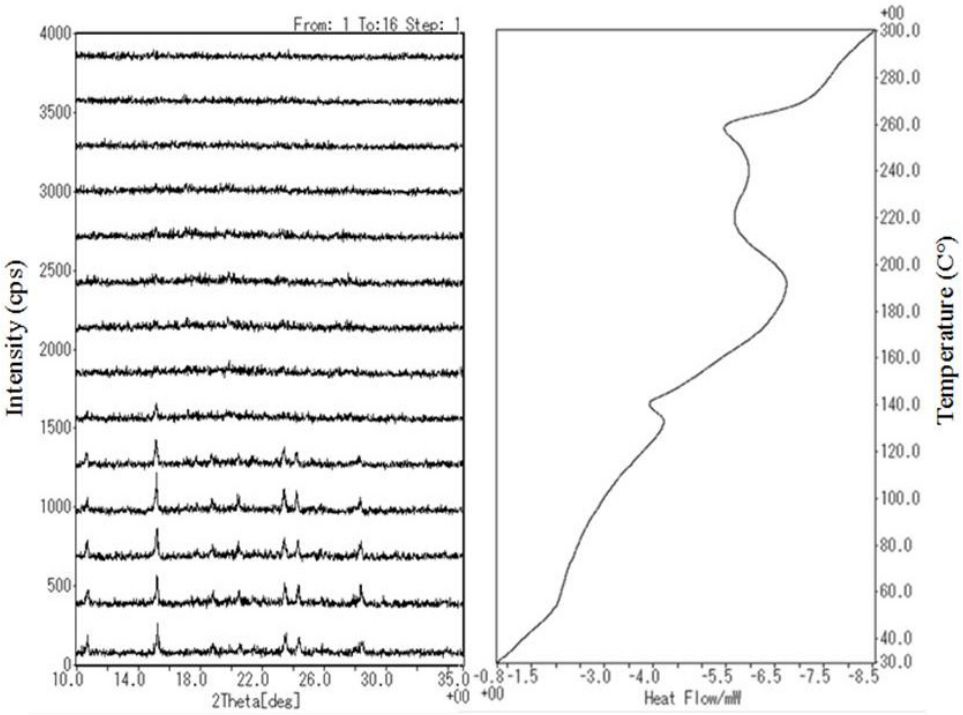

Figure 3 XRD-DSC scans of DS-CS SDPs at 1:2 ratio.

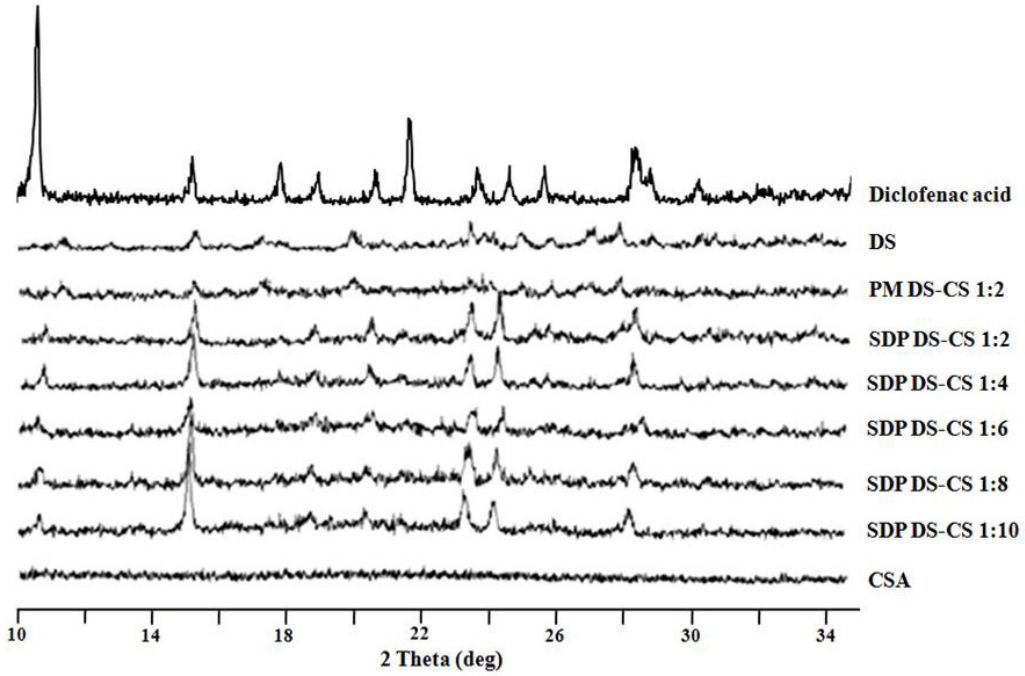

Figure 4 XRD patterns of DS, diclofenac acid, PM of CS-DS at 1:2 ratio, DS-CS SDPs at various ratios and spray dried CSA.

Table 2 Temperature of DSC peaks (Exothermic: ex, Endothermic: en) and range of changing temperature from crystalline to amorphous state from XRD patterns of CSA, DS, diclofenac, the PM and the SDPs of DS-CS.

Samples	Temperature of DSC peaks	Range of changing temperature to amorphous state from XRD
CSA	215.0 °C (en)	-
DS	272.4 °C (en), 276.2 °C (ex)	260 - 280 °C
Diclofenac (acid form)	141.6 °C (en), 155.1 °C (en)	140 - 160 °C
PM DS-CS (1:2)	277.1 °C (en)	260 - 280 °C
SDP DS-CS (1:2)	142.3 °C (en), 258.9 °C (en)	120 - 140 °C
SDP DS-CS (1:4)	139.8 °C (en), 221.7 °C (en)	120 - 140 °C
SDP DS-CS (1:6)	135.3 °C (en), 219.5 °C (en)	120 - 140 °C
SDP DS-CS (1:8)	138.5 °C (en), 220.1 °C (en)	120 - 140 °C
SDP DS-CS (1:10)	138.0 °C (en), 219.0 °C (en)	120 - 140 °C

TH-CS spray dried particles

Figure 6 shows the XRD pattern at different temperature and DSC thermogram of the TH-CS SDPs at 1:2 ratio. Sharp and dominant XRD peaks of the spray died TH-CS particles under ambient temperature were observed at 12.66°. The crystalline peaks disappeared around 230 °C which was in the similar temperature as DSC endothermic peak. This peak corresponds to the melting point of the active drug. The XRD patterns at room temperature of the TH, CSA and the TH-CS SDPs at various ratios (1:2, 1:4, 1:6, 1:8 and 1:10) are depicted in **Figure 6**. The XRD pattern of CSA exhibited typical halo patterns of amorphous structure while TH revealed sharp crystalline peaks at 12.68°, 14.44°, 24.08° and 26.50°. The PM of TH and CS at a ratio of 1:2 exhibited sharp crystalline peaks located in a similar region as pure TH. The crystalline peaks of TH-CS SDPs emerged in 3 ratios which were 1:2, 1:4 and 1:6. If the CS portions reach 1:8 and 1:10, the crystalline XRD peaks vanish. This indicates that TH was mono-molecularly dispersed by the spray drying process in a sufficient amount of CS [14]. This result agrees with the SEM images that the TH crystals disappeared from surface of the spray dried TH-CS particles at 1:8 and 1:10 ratios.

The temperature of the DSC peaks and range of changing temperature from crystalline to amorphous state from XRD patterns of stating materials, the PM of TH-CS and the TH-CS SDPs were displayed in **Table 3**. TH expressed endothermic peak at 270.6 °C and exothermic peak at 275.6 °C which were melting and decomposition temperatures, respectively. The endothermic peak of the PM between TH and CS was 251.1 °C. Increasing part of CS in the SDPs, reduced melting temperatures of TH compared to pure TH. At 1:8 and 1:10 ratios of the spray dried TH-CS particles, the endothermic peaks were broad and the crystalline XRD peaks were not observed. This demonstrates that TH converted to amorphous state using an adequate amount of CS (1:8 and 1:10, TH:CS). Furthermore, CS interfered in the formation of TH crystals which can lower the melting temperature [17].

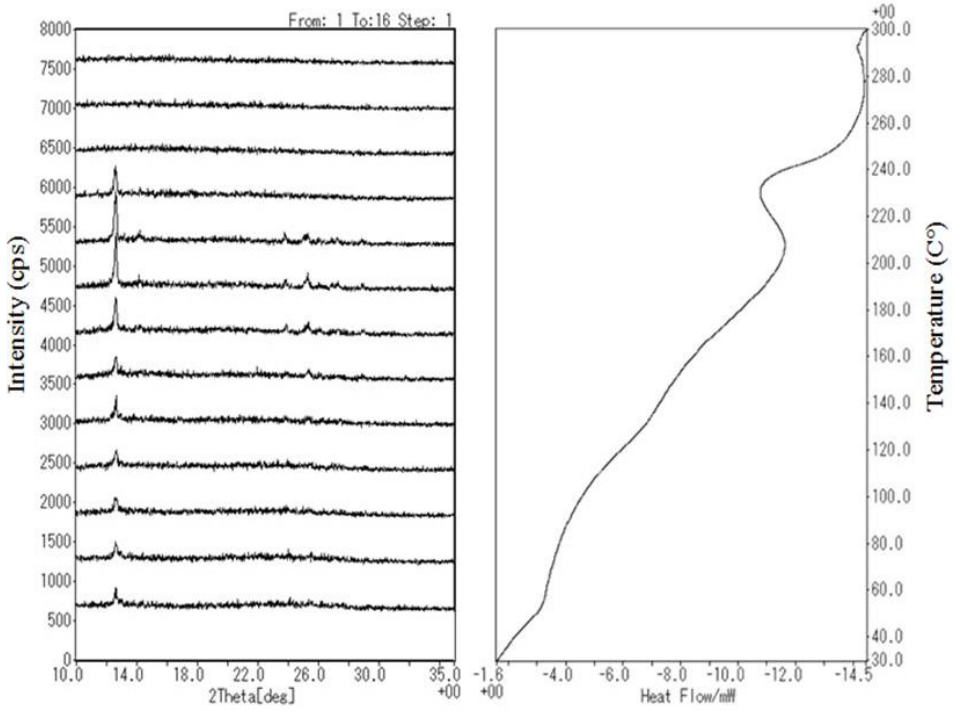

Figure 5 XRD-DSC scans of TH-CS SDPs at 1:2 ratio.

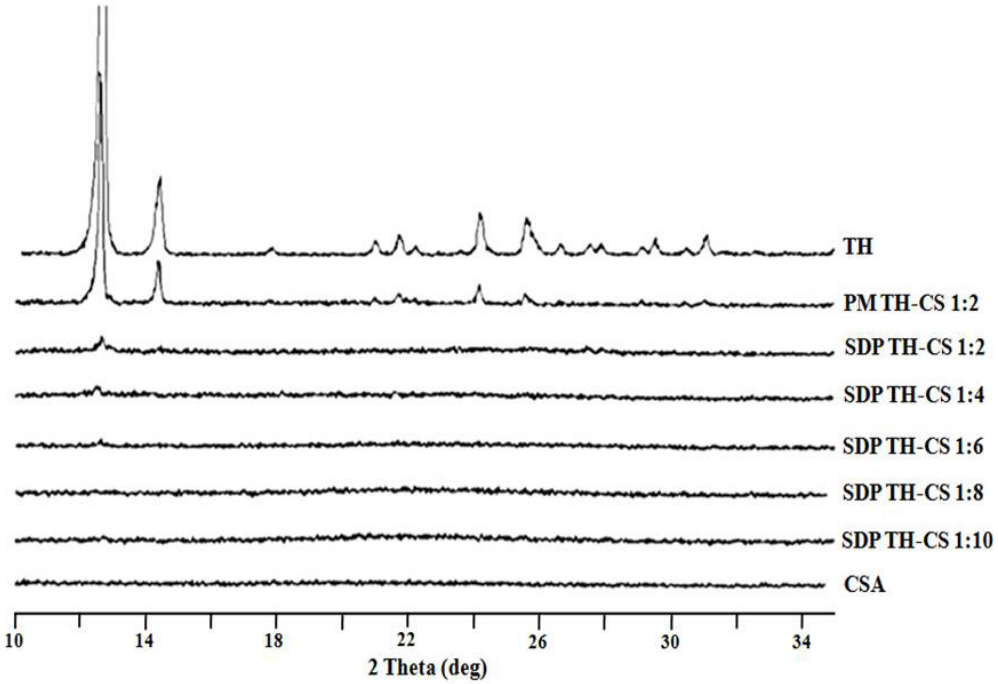

Figure 6 XRD patterns of TH, PM of CS-TH at 1:2 ratio, TH-CS SDPs at various ratios and spray dried CSA.

Table 3 Temperature of DSC peaks (Exothermic: ex, Endothermic: en) and range of changing temperature from crystalline to amorphous state from XRD patterns of CSA, TH, the PM and SDPs of TH-CS.

Samples	Temperature of DSC peaks	Changing temperature range to amorphous state from XRD
CSA	215.0 °C (en)	-
TH	270.6 °C (en), 275.6 °C (ex)	260 - 280 °C
PM TH-CS (1:2)	251.1 °C (en)	220 - 240 °C
SDP TH-CS (1:2)	231.7 °C (en)	220 - 240 °C
SDP TH-CS (1:4)	224.9 °C (en)	220 - 240 °C
SDP TH-CS (1:6)	228.1 °C (en)	220 - 240 °C
SDP TH-CS (1:8)	196.1 °C (en, broad)	-
SDP TH-CS (1:10)	196.0 °C (en, broad)	-

FTIR spectrum

FTIR analysis is proposed in many references as the possible way to investigate the interaction between substances [18]. In this study, the drug-chitosan SDPs were analyzed by FTIR to observe the possible interaction of the functional groups of both molecules.

DS-CS spray dried particles

FITR spectra of DS, diclofenac (acid form), CSA, the PM of DS and CSA at ratio of 1:2 and the DS-CS SDPs at various ratios (1:2, 1:4 1:6, 1:8 and 1:10) are presented in **Figure 7**. The FTIR spectrum of pure DS showed characteristic peaks at 1574 and 1399 cm^{-1} due to in C=O stretching of carboxylate anion in salt form. Diclofenac in acid form showed sharp peak at 1693 cm^{-1} due to the carbonyl group. In the FTIR spectra of the DS-CS SDPs the peak due to the drug carboxylic group was shifted to 1700 cm^{-1} due to carboxylic acid group which indicates that DS changed to acid form in the DS-CS ratios of 1:2 to 1:8 after spray drying process. These FTIR spectra confirm the XRD that the salt form of the active drug was altered to the acid form.

TH-CS spray dried particles

FITR spectra of CSA, TH, the PM of TH and CSA at ratio of 1:2 and the TH-CS SDPs at various ratios (1:2, 1:4 1:6, 1:8 and 1:10) are displayed in **Figure 8**. TH exhibits main characteristic bands of carbonyl groups near 1716 and 1666 cm^{-1}. The TH-CS SDP also demonstrated peaks of TH around 1716 and 1666 cm^{-1} in all drug polymer ratios. Those peaks were shaper when increasing of CS portion which indicates that moisture in SDPs product might diminish from the pure drug [19].

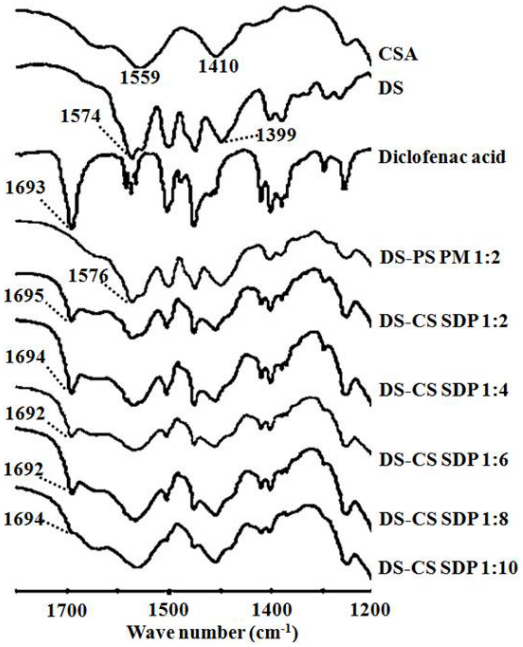

Figure 7 FTIR spectrum of CSA, DS, diclofenac (acid form), PM at 1:2 ratio of DS:CS and the DS-CS SDPs at various ratios (1:2, 1:4 1:6, 1:8 and 1:10).

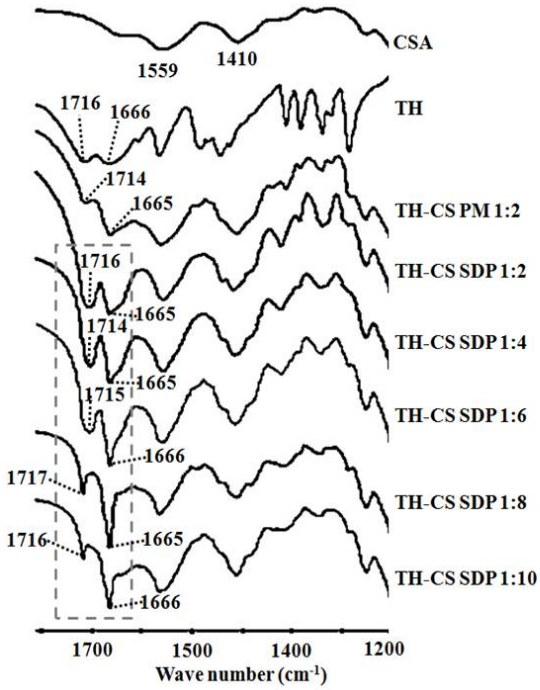

Figure 8 FTIR spectra of CSA, TH, the PM of TH:CS at 1:2 ratio and the TH-CS SDPs at various ratios (1:2, 1:4 1:6 1:18 and 1:10).

***In vitro* drug release of the spray dried microspheres**

Figure 9 shows the result of the release of DS, the PM of DS-CS at ratio of 1:2 and the DS-CS SDPs at various ratios in Tris-HCl buffer medium, pH 6.8. There was a burst effect during the first stage of dissolution, and most of the drug was released in 50 min. The DS-CS SDPs at 1:2 and 1:4 ratios demonstrated slow drug releases while drug release from DS-CS SDPs at 1:6, 1:8 and 1:10 ratios were faster. These could be because a high amount of CS in 1:6, 1:8 and 1:10 (DS:CS) ratios dispersed crystals of DS well compared to SDP with low CS portions. Consequently, the release of DS was enhanced in the dispersed formulations [20].

In vitro drug release behaviors of TH, TH-CS PM at 1:2 ratio and TH-CS SDP at 1:2 to 1:10 ratios are summarized in the cumulative percentage releases as shown in **Figure 10**. All mixtures produced an initial burst effect in 20 min. After that, constant slow TH releases were observed. The cumulative drug release at 20 min of TH, PM and SDPs of TH-CS at 1:2 ratio were around 95 %. The slower drug releases were obtained from the TH-CS SDP at 1:6, 1:8 and 1:10. This might be due to a gel barrier from CS retarding drug release [21]. However, the drug release profiles of TH, TH-CS of PM and SDP were not dramatically different because TH is freely soluble in aqueous medium and CS formed a thin layer in the SDP; therefore, the CS layer could slightly retard drug release.

For TH, a soluble drug, CS swelled after immersion in the dissolution medium. Gel from CS could retard drug diffusion and drug release. On the other hand, the releasing mechanism of insoluble drug (DS) is erosion. Therefore, the DS-CS SDPs containing a low portion of CS could release drugs better than those with a high portion of CS.

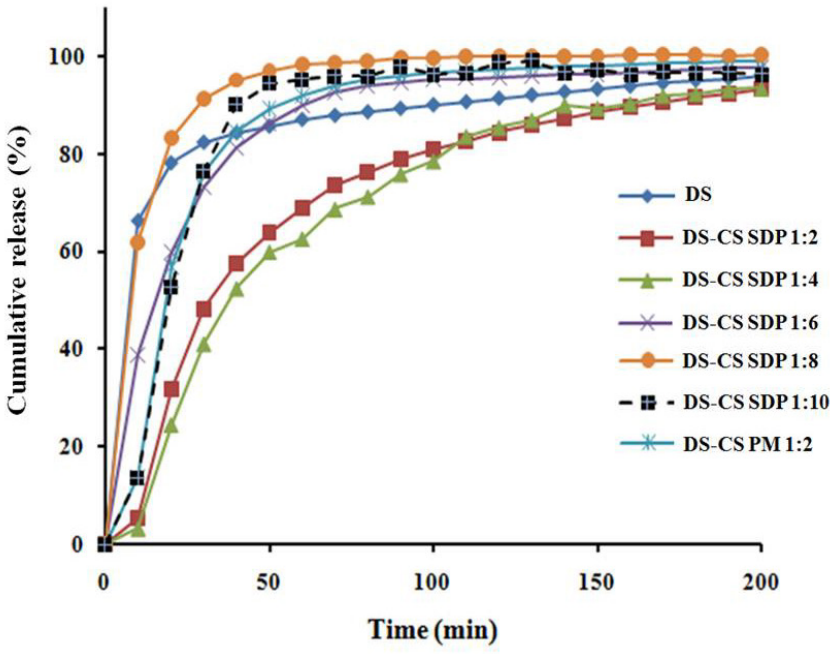

Figure 9 *In vitro* drug release profiles of DS, PM of DS:CS at ratio of 1:2 and DS-CS SDPs at various ratios (1:2, 1:4 1:6 1:18 and 1:10).

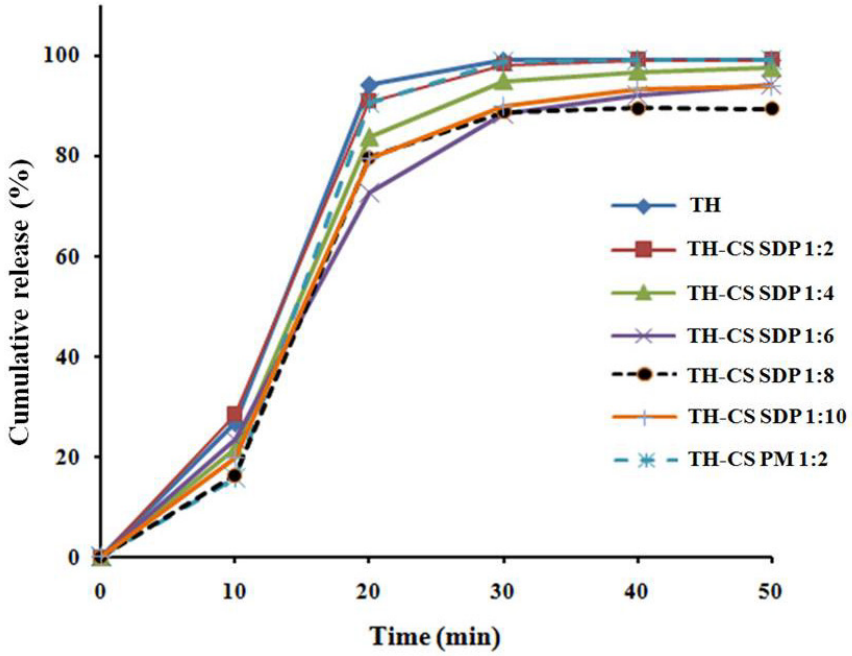

Figure 10 *In vitro* drug release profiles of TH, PM of TH:CS at 1:2 ratio and TH-CS SDPs at various ratios (1:2, 1:4 1:6 1:18 and 1:10).

Conclusions

The spray drying technique altered the thermal behaviors and crystalline properties of the drug-CS SDP system. Acid and alkaline drugs provided different morphologies and sizes of drug-CS SDPs due to their solubility properties. Increasing the portion of CS could not change DS from a crystalline to amorphous state. On the other hand, TH converted to the amorphous state with the spray drying technique using an adequate amount of CS (1:8 and 1:10, TH:CS). Cumulative drug release depended on the CS portion. In case of the insoluble drug, increasing the amount of CS enhanced dispersion of drug, but in a well dispersed system, a high amount of CS formed a gel barrier to retard drug release. For TH, a soluble drug, CS formed gel which retarded drug diffusion and drug release.

Acknowledgements

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