Carbohydrate Polymers 85 (2011) 251–260

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Use of 2-hydroxypropyl-β-cyclodextrin as adjuvant for enhancing encapsulation and release characteristics of asiaticoside within and from cellulose acetate films

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ARTICLE INFO

Article history:
Received 27 August 2010
Received in revised form 13 February 2011
Accepted 14 February 2011
Available online 18 February 2011

Keywords:
Asiaticoside
Centella asiatica
2-Hydroxypropyl-β-cyclodextrin
Host–guest
Cellulose acetate
Drug release

ABSTRACT

The inclusion complex between asiaticoside (AC), an active substance from the medicinal plant *Centella asiatica* L., and 2-hydroxypropyl-β-cyclodextrin (HPβCD) was investigated. The phase solubility profiles of AC in distilled water and 90:10 (v/v) mixture of 80 vol.% acetic acid and *N,N*-dimethylacetamide (DMAc) both in the absence and the presence of HPβCD were classified as A_L-type and indicated a 1:1 molar ratio of the AC/HPβCD complex. The 1:1 stoichiometric molar ratio of the complex was confirmed by both the 1D and the 2D ^{1}H-nuclear magnetic resonance spectrometry. Unlike the highly crystalline nature of the native AC, the stoichiometric AC/HPβCD complex powder was amorphous in nature. The average diameters of the self-assembling aggregates of the AC/HPβCD complex, *a priori* prepared at various HPβCD to AC molar ratios of 0.5, 1, and 2, in the solution state, were about 2.4, 3.3, and 3.8 μm, respectively. The solvent-cast CA films containing these complexes showed strong evidence of the aggregates on their surfaces. The maximal cumulative released amounts of AC, a sparingly water-soluble substance, from the CA films containing a mixture of AC/HPβCD of varying molar ratios (i.e., 1:0.5, 1:1, and 1:2) in the phosphate buffer saline solution (PBS) containing 10% (v/v) methanol (i.e., P/B/M medium) were 2.2, 3.9, and 5.8% (based on the actual weights of the film specimens), respectively, which were clearly an increasing function of the HPβCD content in the films.

1. Introduction

Cellulose acetate (CA) is the acetate ester of cellulose, the primary structural component of cell walls of green plants, and it is one of the most common biopolymers on earth (Anonymous, 2009). CA has been used for a wide variety of applications, including filters (Thostenson, Ren, & Chou, 2001), composite reinforcements (Bergshoef & Vancso, 1999), and topical and/or transdermal delivery of drugs (Wang et al., 2002). In the latter, Taepaiboon, Rungsardthong, and Supaphol (2000) developed electrospun CA fiber mats as carriers for the topical/transdermal delivery of all-trans retinoic acid or vitamin A acid (Retin-A) and α-tocopherol or vitamin E (Vit-E) from CA solutions in 2:1 (v/v) acetone/dimethylacetamide (DMAc) containing Retin-A or Vit-E in the amount of 0.5 or 5 wt.% (based on the weight of CA), respectively. Electrospun CA fiber mats and the corresponding solvent-cast films as carriers for the topical/transdermal delivery of four different non-steroidal anti-inflammatory drugs (NSAIDs), i.e., naproxen (NAP), indomethacin (IND), ibuprofen (IBU), and sulindac (SUL), were reported by Tungprapa, Jangchud, and Supaphol (2007), who used CA solutions in 2:1 (v/v) acetone/DMAc as the base solutions into which NAP, IND, IBU, or SUL in the amount of 20 wt.% (based on the weight of the CA) was added.

Asiaticoside (AC; see Fig. 1 for its chemical structure) is one of four major trisaccharide triterpenoid components (i.e., asiatic acid, asiaticoside, madecassic acid and madecassoside) of the extract from the medicinal plant *Centella asiatica* (L.) Urban which bears a common name in Thai as "Buabok." AC has been regarded as one of the most active compounds associated with the healing of wounds, as evidenced from the observed increase in antioxidant levels at an early stage of recovery of excision-type cutaneous wounds in rats (Shukla, Rasik, & Dhawan, 1999), the observed increase in the proliferation of human dermal fibroblasts and the expression of types I and III pro-collagen mRNA and the protein levels of the cells (Maquart, Bellon, Gillery, Wegrowski, & Borel, 1990; Shim et al., 1996), and the stimulation of extracellular matrix (ECM) accumulation in experimental wounds of rats (Maquart et al., 1999; Suguna, Sivakumar, & Chandrakasan, 1996) in response to the presence of this substance.

Recently, Suwantong, Ruktanonchai, and Supaphol (2008) reported the preparation of electrospun CA fiber mats and the corresponding solvent-cast films containing either *C. asiatica* crude

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doi:10.1016/j.carbpol.2011.02.023

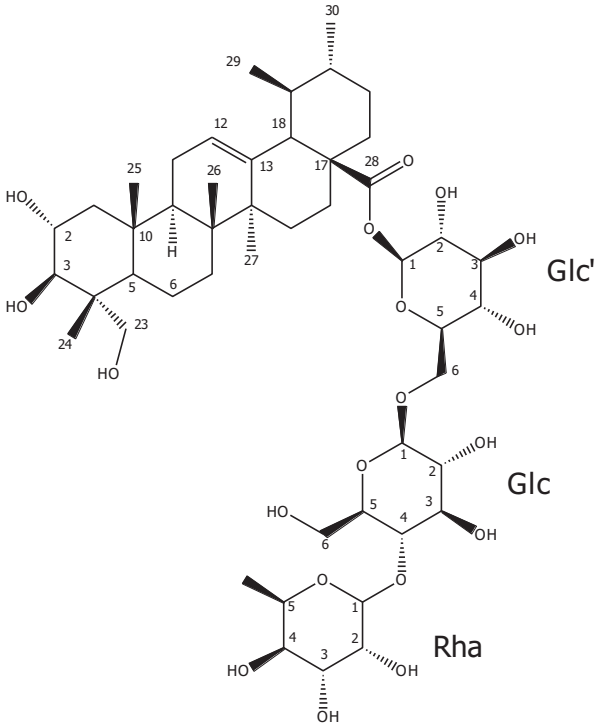


Fig. 1. Chemical structure of asiaticoside (AC).

extract (CACE) or pure AC in the amount of 40 wt.%, based on the weight of the CA powder. The release characteristics of AC from those specimens were tested by the total immersion and the transdermal diffusion through a pig skin methods in either of the acetate or the phosphate buffer solution that contained methanol (i.e., A/B/M or P/B/M medium) at the skin or the physiological temperature of 32 or 37 °C, respectively. However, the release of AC from the CA films containing either CACE or AC was too low (lower than about 2 and 3%, based on the actual weight of the specimens in the A/B/M or P/B/M medium, respectively). This was thought to be a result of the low aqueous solubility of AC (i.e., 0.67 mg mL^{-1}) as well as to the dense structure of the CA films and the inherent insolubility of CA in an aqueous medium. To increase the amount of AC that could be released from the CA matrix, the release should be less dependent on the intrinsic solubility of the active compound in the releasing medium (Sangalli et al., 2001) and/or on the microenvironment through which the drug molecules are to diffuse out (i.e., corresponding to the way in which the drug is incorporated within the matrix) should also be modified (Pose-Vilarnovo et al., 2004).

Cyclodextrins (CDs), complexing agents capable of forming host–guest interactions with a variety of compounds, are capable of interacting with many therapeutic agents, e.g., drugs, by encapsulating them, either wholly or partly, into the hydrophobic cavity of their molecules. Such inclusion complexes have been reported to exhibit several advantages, such as improving the solubility, dissolution rate and bioavailability (Freville, Dollo, Le Corre, Chevanne, & Le Verge, 1996; Loftsson & Brewster, 1996; Pose-Vilarnovo et al., 2001), decreasing toxicity (de Araujoa et al., 2008; Irie & Uekama, 1997; Rajewski & Stella, 1996), and increasing the permeation rate of the encapsulating drug molecules (Loftsson, Jarho, Másson, & Sigurjonsdóttir, 2005; Lopez, Collett, & Bentley, 2000). As a result, they are used in many applications, such as in controlled drug delivery: whether dermally, transdermally, or orally (Challa, Ahuja, Ali, & Khar, 2005). The cyclodextrin complexes have also been reported to form self-assembling entities or micelles (Loftsson, Magnúsdósdottir, Másson, & Sigurjónsdóttir, 2002; Mele, Mendichi, & Selva, 1998). Choi, Ruktanonchai, Min, Chun, and Soottitantawat (2010) prepared either self-assembling aggregates of β-CD and fish oil and studied the release characteristics of fish oil from the obtained β-CD-fish oil complexes at different ratios of the substances.

The aim of the present work was to investigate the complexation of AC within 2-hydroxypropyl-β-cyclodextrin (HPβCD) and the potential for use of the solvent-cast CA films that contained a AC/HPβCD mixture of varying molar ratios as carriers for the topical/transdermal delivery of AC. Various properties (i.e., morphology, size of self-assembling aggregates, water retention and weight loss) of both the AC- and the AC/HPβCD complex-loaded CA films and the release characteristics of AC from these materials in a phosphate buffer saline solution that contained methanol were investigated.

2. Experimental details

2.1. Materials

Cellulose acetate (CA; white powder; $M_w \approx 30{,}000$ Da; acetyl content = 39.7 wt.%; degree of acetyl substitution ≈ 2.4) and 2-hydroxypropyl-β-cyclodextrin (HPβCD; $M_w \approx 1{,}380$ Da; average number of substituent per glucopyranose unit ≈ 0.6) were purchased from Sigma–Aldrich (Switzerland). Asiaticoside (AC; 90% purity) was purchased from Shanghai Angoal Chemical Co., Ltd. (China). *N,N*-Dimethylacetamide (DMAc, Lab-Scan Asia, Thailand), glacial acetic acid (Carlo Erba, Italy), anhydrous disodium hydrogen orthophosphate, sodium dihydrogen orthophosphate, and sodium chloride (Ajax Chemicals, Australia) were used as-received. All chemicals were of analytical reagent grades and used without further purification.

2.2. Stoichiometry of AC/HPβCD complex in solution state

2.2.1. Phase solubility studies

Phase solubility of AC in HPβCD was studied in distilled water. Briefly, excess amounts of AC were put into aqueous HPβCD solutions (1 mL). The amount of HPβCD in the solutions was varied between 0 and 30 mM. The sample solutions were then stirred at room temperature (25 ± 1 °C) for 24 h. It should be noted that the stirring time of 24 h was enough for the solubilization of AC in HPβCD to reach equilibrium. After reaching the equilibrium, unsolubilized AC in the sample solutions was filtered out through a filter membrane (0.45 μm in pore diameter). The filtrates were later quantified for the amounts of the solubilized AC by reversed-phase high performance liquid chromatography (HPLC; see later). The association constant (K_a) was then calculated from the following equation (Higuchi & Connors, 1965):

$$K_a = \frac{m}{s_0(1-m)}, \tag{1}$$

where m is the slope of the plot between the solubility of AC in distilled water as a function of the HPβCD concentration and s_0 is the solubility of AC in distilled water in the absence of HPβCD.

In addition, the complexation efficiency (CE) and the AC:HPβCD molar ratio ([AC]:[CD]) were calculated as follows (Loftsson, Hreindóttir, & Másson, 2007):

$$CE = s_0 K_{1:1} = \frac{[AC]/[CD]}{[CD]} = \frac{m}{1-m}, \tag{2}$$

$$\frac{[AC]}{[CD]} = \frac{1}{1+(1/CE)}, \tag{3}$$

where $K_{1:1}$ is the association constant for the AC:HPβCD molar ratio of 1:1.

The phase solubility of AC in HPβCD was also studied in a 90:10 (v/v) mixture of 80 vol.% acetic acid and DMAc. To ensure the formation of the AC/HPβCD complex in the solvent mixture, the preparation procedure was similar to what had been described above, with an exception to the fact that the mixture of acetic acid and DMAc was used instead of the distilled water.

2.2.2. ^{1}H-NMR studies

A Varian ^{UNITY}*INOVA* ^{1}H-nuclear magnetic resonance spectrometer (^{1}H-NMR), operating at 400 MHz and 25 °C, was used to determine the stoichiometric ratio of the AC/HPβCD complex. Based on the Job's continuous variation method (Job, 1928), the ^{1}H-NMR spectra were recorded from a mixture of AC (10 mM) and HPβCD (10 mM) in 10% (v/v) DMSO-d_6/D_2O mixture at various volumetric ratios between these two solutions at the final concentrations of AC of 1–10 mM, prior to being stirred for 24 h.

2.3. Preparation of AC/HPβCD complex in solid state

The AC/HPβCD complex was prepared according to the method previously described by Higuchi and Connors (1965), with slight modification. Briefly, a weighed amount of HPβCD was first dissolved in distilled water. A weighed amount of AC was then added to the solution and stirred at room temperature (25 ± 1 °C) for 24 h. The stoichiometric AC:HPβCD molar ratio, as obtained from the studies in Section 2.2, was utilized throughout the rest of the studies. The solution/suspension was filtered through a nylon filter (average pore size = 0.45 μm) prior to being frozen at −40 ± 2 °C for 24 h. After that, the obtained mass was lyophilized (Labconco FreeZone® 6-Liter Benchtop Freeze Dry System). A physical blend of AC and HPβCD at the same molar ratio was also prepared for comparison.

2.4. Characterization of solid AC/HPβCD complex

2.4.1. X-ray powder diffractometry

X-ray powder diffractograms of the AC/HPβCD complex and the corresponding physical mixture were obtained on a Bruker Model D8 Advance, equipped with a Cu Kα radiation source, over a scattering (2θ) range of 5 and 60° at room temperature (25 ± 1 °C). The scan rate, the operating voltage, and the filament current were set at 0.2° min^{-1}, 40 kV, and 30 mA, respectively.

2.4.2. Two dimensional ^{1}H-NMR studies

To obtain the 2D ^{1}H-NMR spectrum of the AC/HPβCD complex, the freeze-dried product was dissolved in D_2O at room temperature (25 ± 1 °C) and investigated under the following conditions: acquisition time of 0.205 s, sweep width of 5006.3 Hz, pulse width of 6.3 μs, time domain of 2048, Fourier number of 2048 and temperature of 298 K.

2.5. Preparation of AC- and AC/HPβCD complex-loaded CA films

CA films containing either AC or AC/HPβCD complex were prepared by solvent casting technique. AC or a AC/HPβCD complex that had been prepared at different mixing molar ratios of HPβCD to AC (i.e., 0.5, 1.0, or 2.0) was incorporated at a concentration of 10%, based on the dry weight of CA powder, in a 90:10 (v/v) mixture of 80 vol.% acetic acid and DMAc. The concentration of CA in the final solutions was 4% w/v. The as-prepared solutions were stirred at room temperature (25 ± 1 °C) for 24 h. Prior to the addition of CA powder, each of the sample solutions was characterized for the particle size of the self-assembling aggregates by a Malvern Zetasizer ZS nanosizer [based on the dynamic light scattering (DLS) principle]. CA powder was then added to the sample solution and subsequently stirred at room temperature (25 ± 1 °C) for 3 h until CA was completely dissolved. The solutions were subsequently poured onto glass Petri dishes and dried under a reduced pressure. The films were dried until of a constant weight and stored in a desiccator *in vacuo* for at least 24 h, prior to further use. The thicknesses of the as-cast films were 80 ± 10 μm.

2.6. Characterization of AC- and AC/HPβCD complex-loaded CA films

Morphologies of AC- and AC/HPβCD complex-loaded CA films were studied by a JEOL JSM-6400 scanning electron microscope (SEM). All of the specimens were vacuum-coated with a thin layer of gold using a JEOL JFC-1100E sputtering device. The average diameters of the self-assembling aggregates and the pores that were formed after the films had been immersed in a phosphate buffer saline solution (PBS; see later for its preparation) containing 10% (v/v) methanol (hereafter, P/B/M medium) for 24 h were determined by measuring the diameters of the micro-aggregates and the micro-pores at 100 different points on the SEM images of 7500× magnification with SemAphore 4.0 software. For each sample, the diameters were presented as the average ± standard deviation.

Water retention and weight loss behavior of the AC- and the AC/HPβCD complex-loaded CA films were investigated after their immersion in the P/B/M medium at 37 °C for 24 h. The water retention and the weight loss behavior were calculated as follows:

$$\text{Water retention}\,(\%) = \frac{M - M_d}{M_d} \times 100, \tag{4}$$

and

$$\text{Weight loss}\,(\%) = \frac{M_i - M_d}{M_d} \times 100, \tag{5}$$

where M and M_d are wet and dry weights of the film specimens after immersion in the buffer solution, respectively, and M_i is the initial, dry weight of the specimens. All measurements were carried out in triplicate.

2.7. Release of AC from AC- and AC/HPβCD complex-loaded CA films

2.7.1. Preparation of releasing medium

To prepare 1 L of the PBS solution, 6.177 g of anhydrous disodium hydrogen orthophosphate and 1.1014 g of sodium dihydrogen orthophosphate were dissolved under mechanical stirring in distilled water at room temperature (25 ± 1 °C) until the flakes were completely dissolved. Later, 8.7 g of sodium chloride was added into 20 mL of the solution. The volume was adjusted to the required volume with distilled water. The pH of the final solution was 7.4.

2.7.2. Actual AC content

The actual amounts of AC in the AC- and the AC/HPβCD complex-loaded CA films were quantified by dissolving the samples (circular disc: about 1.5 cm in diameter) in 4 mL of 2:1 (v/v) acetone/DMAc. After that, 0.5 mL of the solutions were pipetted and diluted into 8 mL of the P/B/M medium. Finally, the AC contents in the diluted sample solutions were determined by HPLC (see later) and then back-calculated from the obtained data against a calibration curve for AC.

2.7.3. AC release assay

The release characteristics of AC from the AC- and the AC/HPβCD complex-loaded CA films were determined by the total immersion method. Each of the specimens (circular disc of about 1.5 cm in diameter) was immersed in 25 mL of the P/B/M medium at the physiological temperature of 37 °C. At each time point, ranging between 0 and 24 h (1440 min), 1 mL of the medium was withdrawn

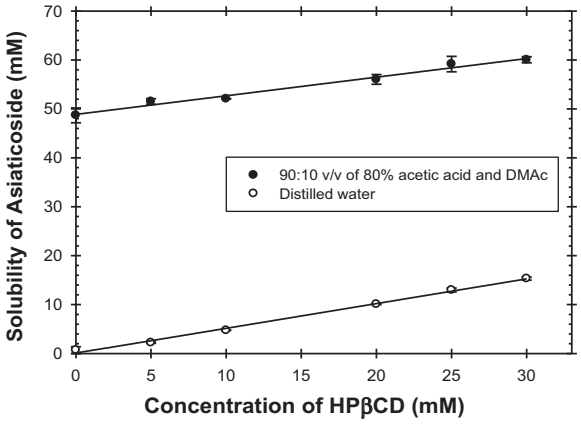


Fig. 2. Phase solubility diagrams of AC in the absence or the presence of 2-hydroxypropyl-β-cyclodextrin (HPβCD) in distilled water and 90:10 (v/v) of 80% acetic acid and DMAc at room temperature.

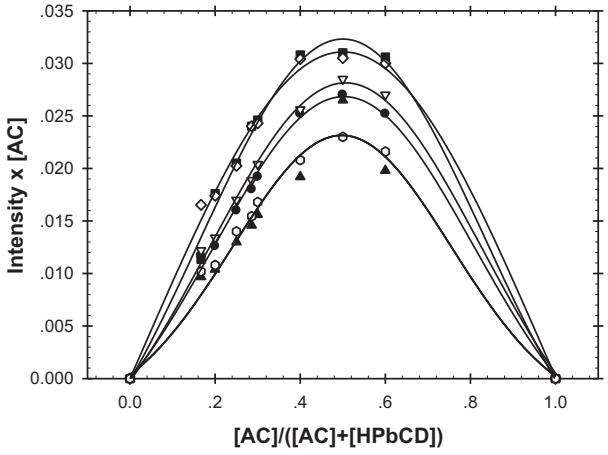


Fig. 3. Job's continuous variation plots of the chemical shifts of the protons, i.e., (▽) H-12, (◊) H-18, (▲) CH_3-26, (●) CH_3-29, (■) Glc-1, and (○) Rha-5, of AC, in the presence of different HPβCD concentrations in the 90:10 (v/v) mixture of D_2O and DMSO-d_6.

(i.e., sample solution) and an equal amount of the fresh medium was refilled. The amounts of AC in the sample solutions were quantified by HPLC (see later) and then back-calculated from the obtained data against the calibration curve for AC.

2.8. Determination of AC content

The amount of AC in a given sample solution was determined by HPLC (Shimadzu LC-10 AD). The sample solution was filtered through a nylon filter (average pore size = 0.45 μm) and then separated in an Inertsil ODS-3 C18 column (particle size = 5 μm; column dimension = 4.6 mm × 250 mm) with an Inertsil ODS-3 guard column (particle size = 5 μm; column dimension = 4.0 mm × 10 mm) at a flow rate of 1 mL min^{-1}. A UV–Visible detector was set at (λ_{max}) 204 nm. The mobile phase for the AC separation consisted of acetonitrile, methanol, and distilled water at 26:24:50 (v/v/v) and the retention time was 7.7 min. The calibration curve for AC was obtained over a concentration range of 0.005–0.200 mg mL^{-1}.

2.9. Statistical analysis

One-way ANOVA was used to analyze the means of different data sets. The significance for all of the tests was accepted at a 0.05 confidence level.

3. Results and discussion

3.1. Stoichiometry of AC/HPβCD complex in solution state

The solubility of AC in the absence or the presence of HPβCD was studied in two types of medium (i.e., distilled water or 90:10 (v/v) of 80% acetic acid and DMAc) and the results are graphically shown in Fig. 2. In the absence of HPβCD, the solubility of AC in any type of the studied medium (i.e., s_0) was low (i.e., 0.7 mM in distilled water and 48.7 mM in 90:10 (v/v) of 80% acetic acid and DMAc). Marked increase in the amounts of soluble AC in either medium was evident in the presence of HPβCD. At the greatest amount of HPβCD investigated (i.e., 30 mM), the amounts of soluble AC increased to 15.3 and 60.0 mM in distilled water and 90:10 (v/v) of 80% acetic acid and DMAc, respectively. Obviously, the amounts of soluble AC in both types of medium exhibited linear relationships with the HPβCD content in the solutions.

The association constants, K_a, characterizing the solubility of AC in distilled water and 90:10 (v/v) of 80% acetic acid and DMAc, can be calculated from the slopes of the plots such as those shown in Fig. 2. The slopes of the least-squared lines drawn through both sets of data were less than one, with the values being 0.505 and 0.380, respectively. According to the definition set forth by Higuchi and Connors (1965), these could be categorized as A_L type and a 1:1 complex between AC and HPβCD could be assumed. Based on the values of the slopes obtained, the K_a values (or the $K_{1:1}$ values, for this particular case) can be calculated, based in Eq. (1), to be 11,726.5 and 12.5 M^{-1}, respectively. According to Eq. (2), the complexation coefficient values, CE, were determined to be 1.020 and 0.613, respectively, while, according to Eq. (3), the AC:HPβCD molar ratios were 1:2 and 1:3, respectively. This means that, despite the stoichiometric 1:1 molar ratio between AC and HPβCD in the as-formed complex, only 1 in every 2 or 3 molecules of HPβCD underwent the complexation with AC.

One dimensional ^{1}H-NMR spectroscopy is useful for studying the inclusion complex of HPβCD with various organic compounds. The variation in the chemical shift of the protons of organic compounds ($\Delta\delta = \delta_c - \delta_0$, where δ_c and δ_0 are the chemical shifts of a specific chemical species in the complex and the free forms, respectively), due to the screening environment by magnetic nuclei within the HPβCD cavity, was recorded. In particular, changes in the chemical shifts of protons of the guest molecule(s) within the CD cavity can be used to quantify the stoichiometry of the complex, simply by plotting them as a function of the mole fraction of the guest molecule(s) or the CD molecule [i.e., Job's continuous variation plots (Job, 1928)]. The mole fraction at the maximum peak(s) is taken as the stoichiometric ratio of the complex. According to Fig. 3, the chemical shifts of CH_3-26, CH_3-29, H-12, H-18, Rha-5, and Glc-1 protons in the AC/HPβCD mixtures, in 90:10 (v/v) mixture of D_2O and DMSO-d_6, exhibited the maximum peaks at [AC]/([AC] + [HPβCD]) = 0.5, corresponding to the AC:HPβCD molar ratio of 1:1. In addition, Table 1 shows the chemical shift signals of protons of AC and HPβCD, both in the free and the complex states, in D_2O at equi-molar ratio between AC and HPβCD, along with their differences.

3.2. Characterization of AC/HPβCD complex in solid state

3.2.1. X-ray powder diffractometry

The freeze-dried product from the equi-molar mixture of AC and HPβCD was white powder. X-ray powder diffractometry was then used to confirm the formation of the AC/HPβCD complex. Fig. 4 shows the X-ray diffractograms of AC, HPβCD, the AC/HPβCD complex, and the physical mixture of AC and HPβCD. Sharp diffraction peaks were evident for the as-received AC, indicating its crys-

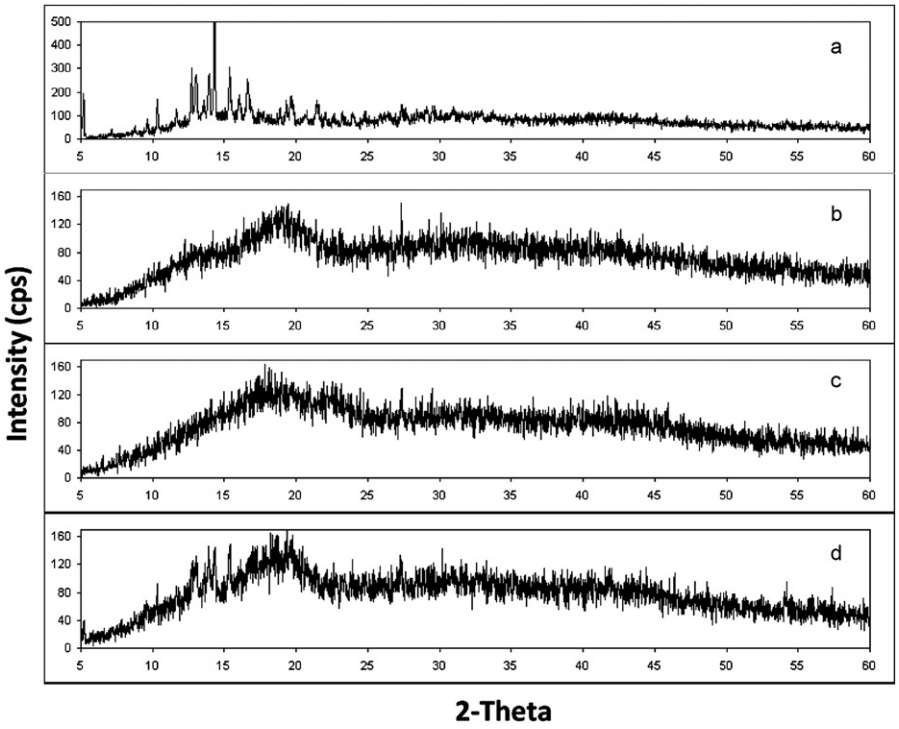


Fig. 4. X-ray diffractograms of (a) AC, (b) HPβCD, (c) AC/HPβCD complex, and (d) physical mixture of AC and HPβCD.

talline nature. On the other hand, only diffused, scattering peaks were observed for the as-received HPβCD, with the scattering center being observed at the scattering angle, 2θ, of about 18°. This indicates the amorphous nature of HPβCD. As for the AC/HPβCD complex, only the scattering peaks similar to those of HPβCD were obtained, indicating the amorphous nature of the material. When AC was physically mixed with HPβCD, however, both the sharp diffraction peaks of AC and the diffused, scattering peaks of HPβCD were evident. These results confirmed the inclusion of AC within the cavity of HPβCD.

3.2.2. Two dimensional ^{1}H-NMR

To investigate the spatial arrangement of AC within the HPβCD cavity, Nuclear Overhauser Effect Spectroscopy (NOESY) was applied on the stoichiometric AC/HPβCD complex (1:1 molar ratio) that had been dissolved in D_2O and the result is shown in Fig. 5a.

Table 1
Chemical shift signals of protons (in ppm) of AC and HPβCD in the free (δ_0) and the complexed (δ_c) states in D_2O at the equi-molar ratio between AC and HPβCD of the inclusion complex, along with their differences.

Proton	δ_0	δ_c	$\Delta\delta$ ($\delta_c - \delta_0$)
AC			
H-12	5.162	5.216	0.054
H-18	2.033	2.087	0.054
CH_3-24	0.644	0.667	0.023
CH_3-26	0.777	0.831	0.054
CH_3-27	1.132	1.134	0.002
CH_3-29	0.844	0.869	0.025
Glc-1	4.385	4.323	−0.062
Rha-CH_3	1.301	1.321	0.002
Rha-5	3.942	3.988	0.046
HPβCD			
H-1	5.090	5.086	−0.004
H-2	3.542	3.546	0.004
H-3	3.817	3.896	0.079
H-4	3.525	3.526	0.001
H-5	3.600	3.620	0.020
H-6	3.682	3.760	0.078

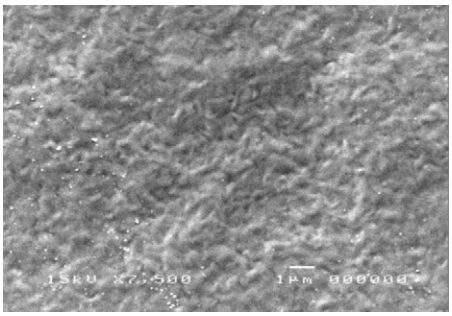
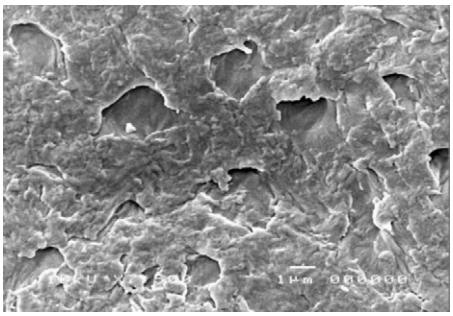
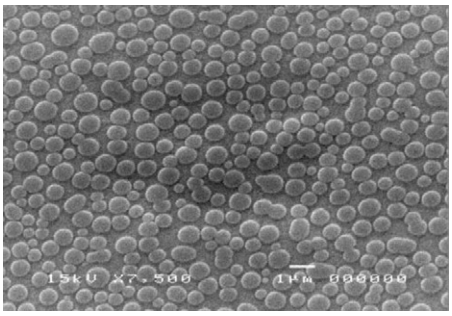
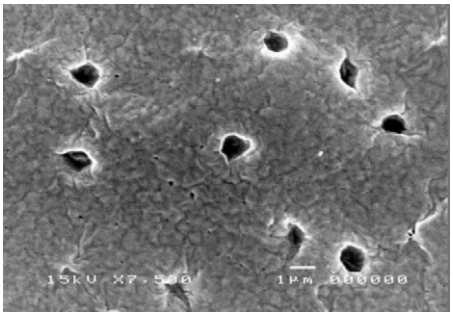
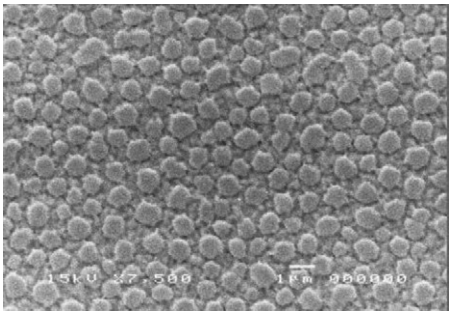
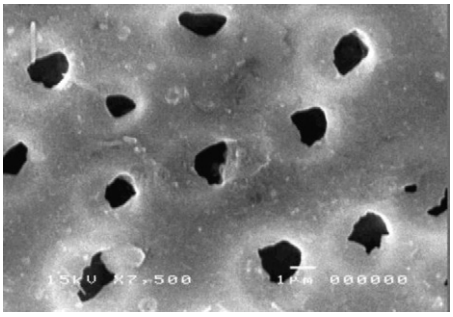
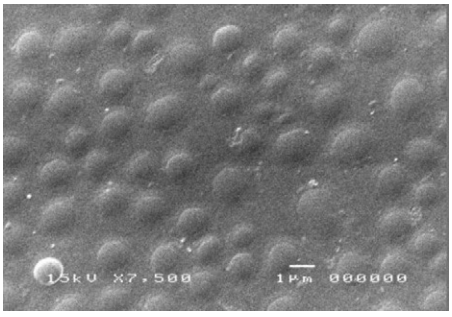
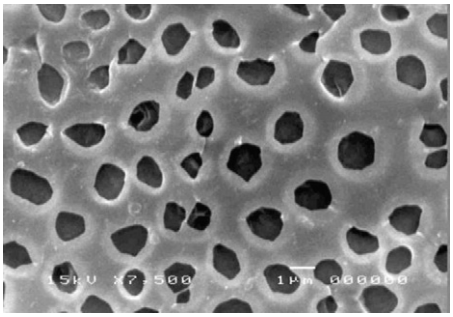
Cross-peaks were observed between the CH_3-29 and CH_3-27 protons of AC and the H-3 inner-cavity proton of HPβCD (located at the wide side of the cavity) and between the CH_3-26 and H-6 protons of AC and the H-5 inner-cavity proton of HPβCD (located at the narrow side of the cavity). This indicates that the cyclohexene ring moiety of AC is captivated within the cavity of HPβCD, as depicted in Fig. 5b, hence confirming the stoichiometric 1:1 molar ratio between AC and HPβCD in the solid complex. Notwithstanding, the specific interactions between H-12, H-18, Glc-1, Rha-5, and Rha-Me protons of AC and the H-2, H-4, and H-6 outer-cavity protons of HPβCD are believed to be the probable cause for the self-aggregation of adjacent AC/HPβCD entities.

3.3. Characterization of AC- and AC/HPβCD complex-loaded CA films

3.3.1. Physical appearance

Prior to the addition of CA powder into the AC/HPβCD solutions, the diametric dimensions of the self-assembling aggregates of the AC/HPβCD complex that had been prepared at different HPβCD to AC molar ratios of 0.5, 1, and 2 were determined by the nanosizer to be 2.38 ± 1.39, 3.34 ± 0.66, and 3.84 ± 0.53 μm, respectively (see Supplementary data). Evidently, the size of the aggregates was an increasing function of the HPβCD proportion in the solution. CDs are known to form into self-assembling aggregates, which is facilitated by their ability to form inter-molecular hydrogen bonding (Coleman & Nicolis, 1992; González-Gaitano et al., 2002; Suzuki, Tsutsui, & Ohmori, 1994). In addition, Mele et al. (1998) reported that β-carotene in its complexation with β and γ-CDs in water existed as large aggregates, as revealed by both light scattering and ^{1}H NMR techniques. Factors that influence the formation of self-assembling aggregates are, for examples, concentration and molecular weight of CDs, pH, and temperature, while high pH, high temperature, and the addition of an electrolyte have been used as means to prevent the aggregation (Bonini et al., 2006; Das, Mallick, Sarkar, & Chattopadhyay, 2008; He, Fu, Shen, & Gao, 2008).

Table 2
Representative SEM images of AC- and AC/HPβCD complex-loaded CA films both before and after their immersion in a phosphate buffer saline solution (PBS) containing 10% (v/v) methanol (i.e., P/B/M medium) for 24 h.

Type of CA film sample	Before immersion in P/B/M	After immersion in P/B/M
Containing 10 wt.% AC		
Containing 10% (w/w) AC/HPβCD at the mixing ratio of 1:0.5		
Containing 10% (w/w) AC/HPβCD at the mixing ratio of 1:1		
Containing 10% (w/w) AC/HPβCD at the mixing ratio of 1:2		

SEM was used to investigate the morphologies of the obtained AC- and AC/HPβCD complex-loaded CA films. Representative images are illustrated in Table 2. Apparently, the surface of the AC-loaded CA film was rough, with no evidence of any specific pattern on it. This should be due to the complete dissolution of AC in the 90:10 (v/v) mixture of 80 vol.% acetic acid and DMAc, prior to the addition of the CA powder, resulting in rather homogeneous distribution of AC throughout the mass of the resulting CA film. On the other hand, specific pattern in the form of spherical aggregates was observed on all of the AC/HPβCD complex-loaded CA films. Specifically, an increase in the HPβCD to AC molar ratio resulted in a monotonous decrease in the sharpness of the boundary of these aggregates. It is believed that these entities were the self-assembling aggregates of the AC/HPβCD complex that had been formed in the AC/HPβCD solutions. The diameters of the aggregates that had been formed at the AC:HPβCD molar ratios of 1:0.5 and 1:1 were 0.73 ± 0.13 and 0.84 ± 0.12 μm, respectively. However, those of the ones that had been formed at the AC:HPβCD molar ratio of 1:2 could not be precisely measured.

Clearly, the dimensions of these self-assembling aggregates of the AC/HPβCD complex were much smaller than those determined by the nanosizer. Such discrepancy could be based on a couple of reasons: (i) the dry or the solvated state of the materials during the measurements by the SEM or the nanosizer, respectively, and (ii) the dependence of diametric projection of the spherical aggregates

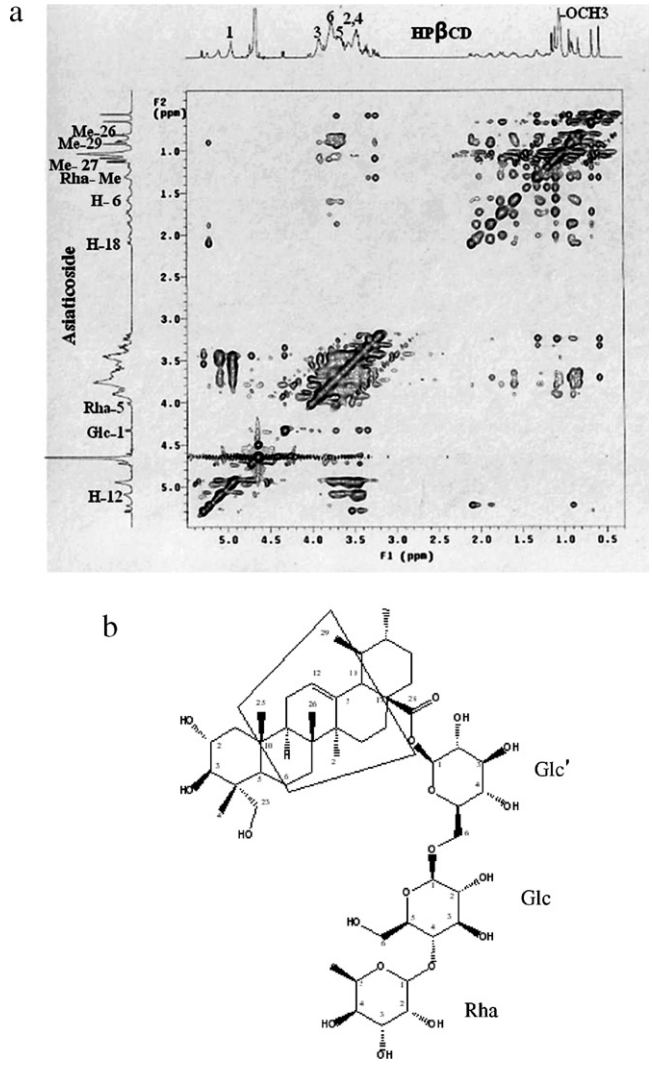


Fig. 5. (a) NOESY diagram of AC/HPβCD complex that had been dissolved in D_2O and (b) schematic arrangement of AC within the cavity of HPβCD.

of the AC/HPβCD complex across the thickness of the AC/HPβCD complex-loaded CA films.

3.3.2. Water retention and weight loss behavior

The CA films containing either AC or the AC/HPβCD complex were further characterized to investigate water retention and weight loss behavior in the P/B/M medium at 37 °C. After the specified immersion time of 24 h had been reached, the weights of the film specimens were recorded and these were calculated to obtain the property values, as reported in Fig. 6. Among the various CA films investigated, the AC-loaded CA films (shown in the figure as the samples with the HPβCD to AC molar ratio of 0) exhibited the lowest water retention (i.e., at about 12%). Such values for the CA films containing either AC or CACE at 40 wt.%, after immersion in the P/B/M medium at 37 °C for 24 h, were reported to be about 39 and 52%, respectively (Suwantong et al., 2008). Compared with the value obtained in the present work, the greater water retention values as reported in the work of Suwantong et al. (2008) could be due to a couple of reasons: (i) the difference in the solvent type used in the fabrication of the films that could affect the physico-chemical properties of the resulting films (Li, Ren, Fane, Li, & Wong, 2006; Romero, Leite, & Goncalves, 2009; Valente, Polishchuk, Burrows, & Lobo, 2005) and (ii) the difference in the as-loaded amounts of the drug within the films that could affect the porosity of the

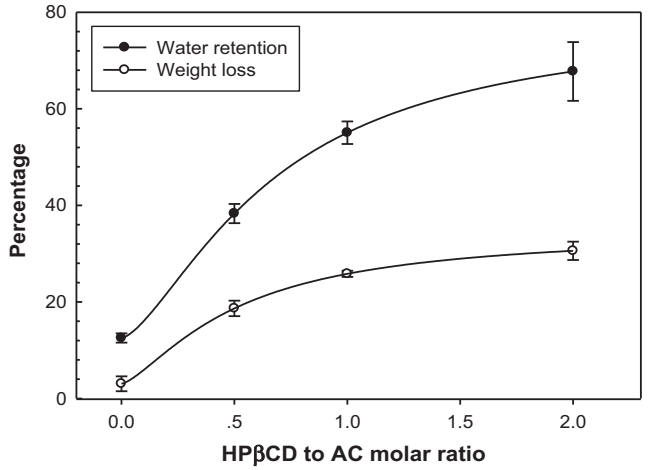


Fig. 6. Water retention and weight loss behavior of the AC/HPβCD complex-loaded CA films at different HPβCD to AC molar ratio after immersion in the P/B/M medium at the physiological temperature of 37 °C for 24 h ($n = 3$).

films after the drug molecules had been released into the medium (Suwantong, Opanasopit, Ruktanonchai, & Supaphol, 2007). On the other hand, the water retention of the AC/HPβCD complex-loaded CA films was much greater than those of the AC-loaded CA films, with the average value increasing from about 38% at the HPβCD to AC molar ratio of 0.5 to about 68% at the HPβCD to AC molar ratio of 2.0.

The AC-loaded CA films also exhibited the lowest weight loss after immersion in the medium (at about 3%). Slightly greater values at about 4 or 7% were reported by Suwantong et al. (2008) for the CA films that contained either AC or CACE at 40 wt.%, respectively. The reasons for the discrepancy in the property values as reported in the present work and those in the work of Suwantong et al. (2008) are similar to those given in the previous paragraph. On the contrary, the loss in the weight of the AC/HPβCD complex-loaded CA films was much greater than those of the AC-loaded CA films, with the average value increasing from about 19% at the HPβCD to AC molar ratio of 0.5 to about 30% at the HPβCD to AC molar ratio of 2.0. The loss in the weight of a drug-loaded material in a medium depends on a number of factors, e.g., the solubility of the drug, the solubility of the carrier material, the diffusion of the drug from the carrier material, and so forth. Due to the low aqueous solubility of both AC and CA in water, the presence of methanol in the medium and of HPβCD in the AC/HPβCD complex-loaded CA films were the obvious reasons for the improvement in the aqueous solubility of both the drug and the matrix materials.

3.4. Release of AC from AC- and AC/HPβCD complex-loaded CA films

The effect of HPβCD on the release characteristics of AC from the CA films at different AC:HPβCD molar ratios was evaluated by the total immersion method in the P/B/M medium. They were reported as the percentages of the ratios of the cumulative amounts of AC released to the amounts of the drug actually loaded within the films or to the actual weights of the films, as shown in Fig. 7. The actual amounts of AC within the AC- and the AC/HPβCD complex-loaded CA films were *a priori* determined to be 70.7, 80.3, 69.2, and 83.0% on average (based on the as-loaded amounts of AC within the casting solutions), for the CA films that had been prepared at the AC:HPβCD molar ratios of 1:0, 1:0.5, 1:1, and 1:2, respectively.

For the AC-loaded CA films (the content of AC initially loaded in the casting solution was 10 wt.%, denoted in Fig. 7 as the samples with the AC:HPβCD molar ratio of 1:0), none of the as-loaded

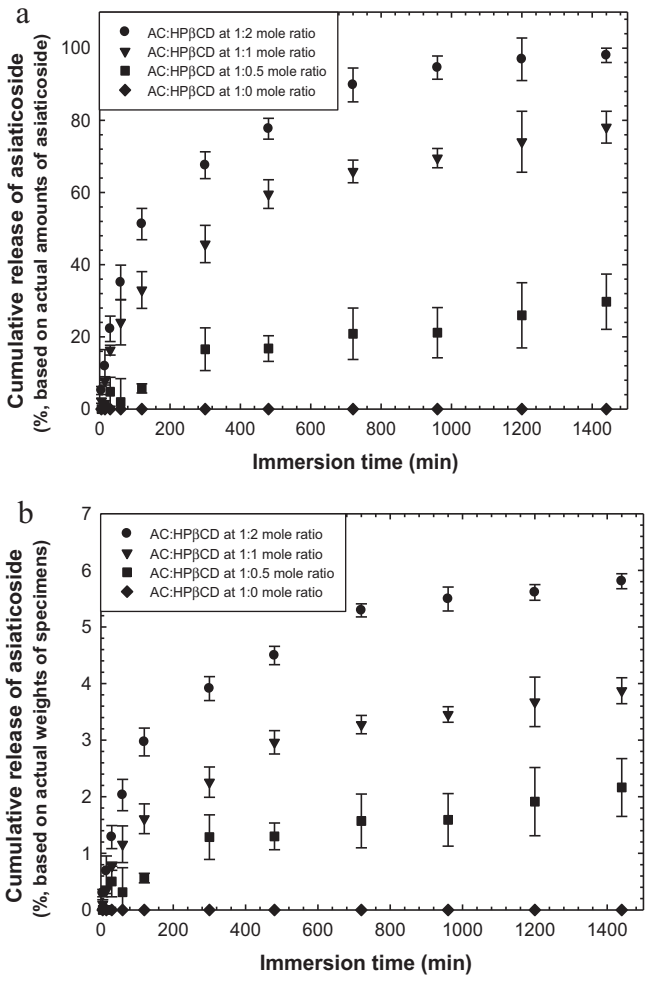


Fig. 7. Cumulative amounts of AC released from AC- and AC/HPβCD complex-loaded CA films upon immersion in the P/B/M medium at 37 °C. The results were reported as the percentages of the ratios of the amounts of the as-released AC to (a) the amounts of AC actually loaded within the films or (b) to the actual weights of the films ($n = 3$).

AC, at any given immersion time point, was able to release into the medium. Suwantong et al. (2008) reported that the maximal amounts of AC released from the CA films that had been prepared from the casting solution containing 40 wt.% of the drug were about 11% on average (based on the AC amounts initially loaded within the films). The discrepancy between the amounts of AC released from the CA films in the present work and those reported in the work of Suwantong et al. (2008) should be influenced by the fact that the amounts of AC initially loaded within the films, as reported in the two studies, were different (i.e., 10 versus 40 wt.%), hence the difference in the driving force for diffusion. The different types of the solvent used in the fabrication of the films [i.e., 90:10 (v/v) mixture of 80 vol.% acetic acid and DMAc as in the present work versus 2:1 (v/v) acetone/DMAc as in the work of Suwantong et al. (2008)], which could render different densities to the obtained films, may also contribute to the discrepancy in the results obtained in the two studies.

In comparison with the AC-loaded CA films, the presence of HPβCD in the AC/HPβCD complex-loaded CA films was clearly responsible for the significantly greater amounts of AC released into the medium. Furthermore, the cumulative amounts of AC released from the films, at any given immersion time point, increased with an increase in the HPβCD content. Specifically, about 25% of the AC actually loaded within the AC/HPβCD complex-loaded CA films, *a priori* prepared at the AC:HPβCD molar ratios of 1:0.5, 1:1, and 1:2, was able to release into the medium within about 1200, 60, and 30 min, respectively. The maximal amounts of AC released from these materials, after having been immersed in the medium for 1440 min, were about 30, 78, and 98% of the amounts of AC actually contained within the CA films, respectively. These values corresponded to about 2.2, 3.9, and 5.8% of the released amounts of AC, when calculated based on the actual weights of the film specimens.

As mentioned, as the HPβCD content in the films increased, significantly more amounts of AC could be released into the medium. The complexation of a poorly water-soluble drug with a native cyclodextrin (CD) as well as its derivatives improves tremendously the aqueous solubility and the dissolution rate of the drug within an aqueous medium. This phenomenon is especially important for a highly hydrophilic derivative of CD, such as HPβCD. Pose-Vilarnovo et al. (2001) reported that the aqueous solubility of sulfamethizole (i.e., a sulfonamide antibiotic drug) was improved significantly upon forming into an inclusion complex with βCD or HPβCD. The difference as observed in the cumulative amounts of AC released from the AC/HPβCD complex-loaded CA films that had been prepared at various AC:HPβCD molar ratios could be explained based on a couple of reasons. Firstly, the ability of AC to form an inclusion complex with HPβCD disfavored the crystallization of the herb, as evidenced from the X-ray results (see Fig. 4). This allows greater opportunity for the solvent molecules to interact with those of the drug. Secondly, the presence of HPβCD increased the hydrophilicity of the films, as evidenced by the increase in the water retention of the films with an increase in the HPβCD content (see Fig. 6). Lastly, due to the highly hydrophilic nature of HPβCD, the dissolution of the unassociated HPβCD as well as the AC/HPβCD inclusion complex into the releasing medium could occur easily, which helped facilitate the solubilization of the drug within the medium. This is evidenced by the observed increases in both the weight loss of the films in and the cumulative amounts of AC released from the films into the medium with an increase in the HPβCD content (see Figs. 6 and 7, respectively).

The latter case was accented by the observation of microholes on the AC/HPβCD complex-loaded CA films after having been immersed in the releasing medium (see Table 2). The diametric dimensions of these holes, as measured directly from the SEM images shown in Table 2, were 0.87 ± 0.14, 1.17 ± 0.28, and $1.20 \pm 0.19\,\mu m$ for the films that had been prepared at the AC:HPβCD molar ratios of 1:0.5, 1:1, and 1:2, respectively. These dimensions were essentially similar to the spherical aggregates that had been observed on the films prior to the immersion (see Table 2 and related texts in Section 3.3.1).

3.5. Release kinetics of AC from AC/HPβCD complex-loaded CA films

The release kinetics of AC from the AC/HPβCD complex-loaded CA films was analyzed by the following equation (Peppas & Khare, 1993; Philip & Peppas, 1987):

$$\frac{M_t}{M_\infty} = kt^n, \quad \text{for} \quad \frac{M_t}{M_\infty} < 0.6, \tag{6}$$

where M_t is the cumulative amount of AC released into the medium at an arbitrary time t, M_∞ is the cumulative amount of AC released into the medium at an infinite time, n is the diffusional exponent used to described the release mechanism, and k is the release rate constant of AC. For the Fickian diffusion, the parameter n is taken as 0.5 and a plot of the fractional cumulative released amounts of AC versus $t^{0.5}$ should give a straight line with a slope of k (Verreck et al., 2003). The values of k (with the values of r^2, signifying the quality of the fit, being given in parentheses) for the AC that had been released from the films, *a priori* prepared at the AC:HPβCD molar ratios of 1:0.5, 1:1, and 1:2, were determined to be $0.0010 s^{-0.5}$

(0.958), $0.0026s^{-0.5}$ (0.951), and $0.0034s^{-0.5}$ (0.899), respectively. Clearly, the rate of AC released from the films increased with an increase in the HPβCD content.

4. Conclusions

Asiaticoside (AC), from the medicinal plant *Centella asiatica* L., readily forms a complex with 2-hydroxypropyl-β-cyclodextrin (HPβCD) at a 1:1 molar ratio both in distilled water and 90:10 (v/v) mixture of 80 vol.% acetic acid and *N,N*-dimethylacetamide (DMAc). The average diametric dimension of the self-assembling aggregates of the AC/HPβCD complex, in the solvated state, increased from about 2.4 μm at the HPβCD to AC molar ratio of 0.5 to about 3.8 μm at the HPβCD to AC molar ratio of 2. In the solid state, the AC/HPβCD complex that had been prepared at the stoichiometric molar ratio of 1:1 did not show the crystalline nature of the as-received AC. Two-dimensional NMR revealed that the cyclohexene ring moiety of AC is captivated well within the HPβCD cavity. The solvent-cast cellulose acetate (CA) films that had been prepared in the presence of a mixture of AC/HPβCD of varying molar ratios (i.e., 1:0.5, 1:1, and 1:2) showed evidence of spherical aggregates on their surfaces. The dimensions of these aggregates were lower than those of the self-assembling aggregates of the AC/HPβCD complex in the solvated state. While AC could not be released from the AC-loaded CA films into the phosphate buffer saline solution (PBS) containing 10% (v/v) methanol (i.e., P/B/M medium), its cumulative amounts that had been released from the AC/HPβCD complex-loaded CA films were much greater, with values increasing with an increase in the HPβCD content. At 24 h of immersion in the medium at 37 °C, the maximal amounts of AC released from these materials were about 2.2, 3.9, and 5.8% (based on the actual weights of the film specimens), respectively.

Acknowledgements

The authors acknowledge the partial support received from (1) The Thailand Research Fund (contact grant number: DBG5280015), (2) The Petroleum and Petrochemical College (PPC), Chulalongkorn University, (3) The Development and Promotion of Science and Technology talents project (DPST).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2011.02.023.

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