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Preparation and characterization of inclusion complexes of naringenin with β-cyclodextrin or its derivative

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ABSTRACT

The inclusion complexation behavior, characterization and binding ability of naringenin with β-cyclodextrin and its derivatives were investigated in both solution and the solid state by means of XRD, DSC, SEM, ^{1}H and 2D NMR and UV–vis spectroscopy. The results showed that the water solubility and thermal stability of naringenin were obviously increased in the inclusion complex with cyclodextrins. This satisfactory water solubility and high thermal stability of the naringenin/CD complexes will be potentially useful for their application as herbal medicines or healthcare products.

1. Introduction

The flavonoid naringenin (5,7,4′-trihydroxyflavanone or 5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one, Fig. 1), a traditional Chinese medicine, is one of the most abundant flavonoids in grapefruits and citrus fruits (El-Mahdy et al., 2008). Daily ingestion of citrus flavonoids has been estimated to be approximately 68 g on an average in the USA. Naringenin is mainly ingested from fruit juices. The concentration of naringenin in the grapefruit juice is found to be 1283 mM (349 mg/L) (Jayachitra & Nalini, 2012). Naringenin plays a key role as an estrogenic substance in humans and as an endogenous regulator in plants, and is known as a safe natural product with various bioactive effects (Fang, Tang, Gao, & Xu, 2010; Kretzschmar et al., 2007). It has been reported to have anticancer, antimutagenic, anti-inflammatory, antiatherogenic, antifibrogenic and free radical scavenging properties (Jayachitra & Nalini, 2012). Naringenin is reported to induce cytotoxicity and apoptosis in various cancer cell line and its treatment at a similar dose showed no toxic effect on normal cells (Jin et al., 2009; Park et al., 2008; Sabarinathan & Vanisree, 2011). In recent years, *in vitro* and *in vivo* studies have shown that naringenin has an insulin-like effect to decrease apoliprotein B (ApoB) secretion in hepatocytes and decreased blood glucose levels in healthy male Wistar rats (Zygmunt, Faubert, MacNeil, & Tsiani, 2010). Furthermore, naringenin is able to traverse the blood–brain barrier and exert a diverse array of neuronal effects through their ability to interact with the Protein Kinase C (PKC) signaling pathways (Sabarinathan & Vanisree, 2011). Additionally, naringenin was shown to reduce the accumulation of collagen fibers by reduced hepatic stellate cell activity in dimethylnitrosamine-induced liver injury rates and to exhibit antifibrogenic effects (Liu et al., 2006).

However, the use of naringenin as a natural herbal medicine is greatly limited by its low water solubility and bioavailability (Hsiu, Huang, Hou, Chin, & Chao, 2002; Yen, Wu, Lin, Cham, & Lin, 2009). Although much effort has been made to improve its water solubility and stability by introducing some nanodispersion or enzymatic condensation techniques (Vila Real, Alfaia, Calado, & Ribeiro, 2007; Yen et al., 2009), it is still not possible to sufficiently dissolve naringenin in water, which prevents its usage for some therapeutic applications. Therefore, the search for an efficient and nontoxic carrier for naringenin has become important in order to further its clinical applications.

It is well known that cyclodextrins (CDs) are truncated-cone polysaccharides mainly composed of six to eight D-glucose

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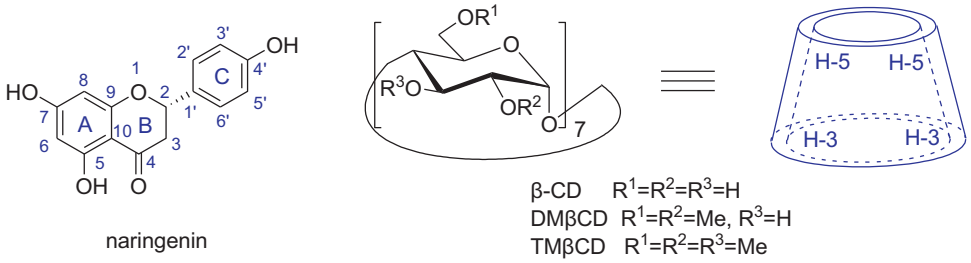


Fig. 1. The structures of naringenin, β-CD and its derivatives.

monomers linked by α-1,4-glucose bonds. They have a hydrophobic central cavity and a hydrophilic outer surface and can encapsulate various inorganic/organic molecules to form host–guest complexes or supramolecular species. This usually enhances drug solubility in aqueous solutions and affects the chemical characteristics of the encapsulated drug in the pharmaceutical industry (Liu & Chen, 2006; Misiuk & Zalewska, 2009; Wu, Liang, Yuan, Wang, & Yan, 2010). This fascinating property enables them to be successfully utilized as drug carriers (Bian et al., 2009; Uekama, Hirayama, & Irie, 1998; Wang & Cai, 2008), separation reagents (Szejtli, 1998), enzyme mimics (Breslow & Dong, 1998), photochemical sensors (Ueno, 1996), etc. Recently, some studies of inclusion complex formation between CDs and flavonoids have been reported (Cannavà et al., 2010; Koontz, Marcy, O'Keefe, & Duncan, 2009; Mourtzinos et al., 2008; Tommasini et al., 2004).

More recently, we reported that the inclusion complexation of CDs with natural medicines such as taxifolin (Yang et al., 2011), crassicauline A (Chen et al., 2011), nimbin (Yang et al., 2010), alpinetin (Ma et al., 2012), and artemether (Yang, Lin, Chen, & Liu, 2009) significantly enhanced the water solubility and bioavailability of the medicines. For example, CDs increased the water solubility of nimbin from 50 μg/mL to 1.3–4.7 mg/mL, and increased the bioavailability of artemether by 1.81-fold (Yang et al., 2009; Yang et al., 2010). As a continuation of our studies on natural medicines/cyclodextrin inclusion complex, an inclusion complex of naringenin with β-CD and its derivatives was investigated.

In this paper, we aim to report the preparation and characterization of some water-soluble inclusion complexes formed by naringenin and β-cyclodextrin and its derivatives: heptakis-(2,6-di-O-methyl)-β-CD (DMβCD) and heptakis(2,3,6-tri-O-methyl)-β-CD (TMβCD) (Fig. 1). We were particularly interested in exploring the solubilization effect of CDs on naringenin and the binding ability of the resulting inclusion complexes, which would provide a useful approach for obtaining novel naringenin-based healthcare products with high water solubility, high bioavailability and low toxicity.

2. Materials and methods

2.1. Materials

Naringenin (FW = 272, PC > 98%) was obtained from the National Institute for Control of Pharmaceutical and Bioproducts (Beijing, PR China); β-CD (average substitution degree = 1135), heptakis(2,6-di-O-methyl)-β-CD (DMβCD, average substitution degree = 1331), and heptakis(2,3,6-tri-O-methyl)-β-CD (TMβCD, average substitution degree = 1429) were purchased from ABCR GmbH & Co. KG and used without further purification. Other reagents and chemicals were of analytical reagent grade. All experiments were carried out using ultrapure water.

2.2. Methods

2.2.1. Preparation of naringenin/β-CD, naringenin/DMβCD and naringenin/TMβCD complexes

Naringenin (0.03 mM, 8.2 mg) and CD (0.01 mM) were completely dissolved in a mixed solution of ethanol and water (ca. 10 mL, v:v = 1:4, given the poor water solubility of naringenin, ethanol was used), and the mixture was stirred for 5 days at room temperature. After evaporating the ethanol from the reaction mixture, the uncomplexed naringenin was removed by filtration. The filtrate was evaporated under reduced pressure to remove the solvent and dried in vacuum to produce the naringenin/CD complexes.

2.2.2. Spectral titration

Absorption spectra measurements were carried out with a Shimadzu UV 2401 (Japan) using a conventional 1 cm path (1 cm × 1 cm × 4 cm) quartz cell in a thermostated compartment, which was kept at 25 °C by a Shimadzu TB-85 Thermo Bath unit. Given the poor water solubility of naringenin, a water/ethanol (v:v = 4:1) solution was used in the spectral measurements. The concentration of naringenin was held constant at 0.03 mM. Then, an appropriate amount of CD was added, and the final concentrations varied from 0 to 2.80–4.00 mM (β-CD: 0, 0.23, 0.47, 0.67, 0.96, 1.37, 1.96, 2.80 mM; DMβCD: 0, 0.23, 0.47, 0.96, 1.37, 2.80, 4.00 mM; TMβCD: 0, 0.33, 0.47, 0.67, 0.96, 1.37, 1.96, 2.80 mM). The absorption spectra measurements were taken after 1 h. The measurements were done in the 220–400 nm spectral range. All experiments were carried out in triplicate.

2.2.3. ^1H and 2D NMR

All NMR experiments were carried out in D_2O. Tetramethylsilane was used as a reference. The samples were dissolved in 99.98% D_2O and filtered before use. ^1H NMR spectra were acquired on a Bruker Avance DRX spectrometer at 500 MHz and 298 K. The one-dimensional spectra of both solutions were run with FID resolution of 0.18 Hz/point. The residual HDO line had a line width at a half-height of 2.59 Hz. Two-dimensional (2D) ROESY spectra were acquired at 298 K with presaturation of the residual water resonance and a mixing (spin-lock) time of 350 ms at a field of ~2 kHz, using the TPPI method, with a 1024 K time domain in F2 (FID resolution 5.87 Hz) and 460 experiments in F1. Processing was carried out with zero-filling to 2 K in both dimensions using sine (F2) and qsine (F1) window functions, respectively.

2.2.4. Powder X-ray diffraction (XRD)

The XRD patterns were obtained using a D/Max-3B diffractometer with Cu Kα radiation (40 kV, 100 mA), at a scanning rate of 5°/min. Powder samples were mounted on a vitreous sample holder and scanned with a step size of $2\theta = 0.02°$ between $2\theta = 3°$ and 50°.

2.2.5. Thermal analyses

Differential scanning calorimetry (DSC) and thermogravimetric (TG) measurements were performed with a 2960 SDT V3.0F

instrument and NETZSCH STA 449F3, respectively, at a heating rate of 10 °C/min from room temperature to 400 °C in a dynamic nitrogen atmosphere (flow rate = 70 mL/min).

2.2.6. Scanning electron microphotographs (SEM)

SEM photographs were determined on a FEI QUANTA 200. The powders were previously fixed on a brass stub using double-sided adhesive tape and then were made electrically conductive by coating, in a vacuum with a thin layer of gold (approximately 300 Å) for 30 s and at 30 W. The pictures were taken at an excitation voltage of 15, 20 or 30 kV and a magnification of 1080×, 1200×, 1400× or 2000×.

2.2.7. Solubilization test

An excess amount of the complex was placed in 2 mL of water (ca. pH 6.0) under nitrogen, sheltered from light, and the mixture was stirred for 1 h at 20 ± 2 °C. The solution was then filtered on a 0.45 μm cellulose acetate membrane. The filtrate was evaporated under reduced pressure to dryness and the residue was dosed by the weighing method.

3. Results and discussion

3.1. Spectral titration

A quantitative investigation of the inclusion complexation behavior of β-CD and its derivatives with naringenin was carried out in a water/ethanol (v:v = 4:1) solution using a spectrophotometric titration method owing to the rather low water solubility of naringenin. As illustrated in Fig. 2, the absorbance intensity of naringenin gradually increased with the stepwise addition of β-CD, DMβCD and TMβCD. The pH of the solution did not change appreciably during any of the experimental procedures. As the size-fit, shape-fit, and charge-fit effects are the dominant controlling factors on the formation of inclusion complexes of CDs (Liu & Chen, 2006), these results indicate that the binding behavior is mainly dependent on the individual structural features of the host and guest. Assuming a 1:1 stoichiometry for the naringenin/CD inclusion complex, the inclusion complexation of naringenin with β-CD could be expressed by Eq. (1), and the stability constant (K_s) could be calculated from Eq. (2), where [naringenin · CD], [naringenin], [CD], $[naringenin]_0$ and $[CD]_0$ refer to the equilibrium concentration of the naringenin/CD inclusion complex, the equilibrium concentration of naringenin, the equilibrium concentration of CD, the original concentration of naringenin, and the original concentration of CD, respectively, and ΔA and $\Delta\varepsilon$ are the differential absorption and molar extinction coefficient of naringenin in the absence and presence of CD. According to Lambert–Beer Law, we can obtain that the concentration of the naringenin/CD complex is equal to $\Delta A/\Delta\varepsilon$ ($[naringenin \cdot CD] = \Delta A/\Delta\varepsilon$). If the naringenin and the CD form complex with a 1:1 stoichiometry, the concentration of the naringenin/CD complex ([naringenin · CD]) is equal to the reduced concentration of the naringenin, and it is also equal to the reduced concentration of CD. So the equilibrium concentration of naringenin is equal to the original concentration of naringenin minus the concentration of the naringenin/CD complex ($[naringenin] = [naringenin]_0 - [naringenin \cdot CD] = [naringenin]_0 - \Delta A/\Delta\varepsilon$), and the equilibrium concentration of CD is equal to the original concentration of CD minus the concentration of the naringenin/CD complex ($[CD] = [CD]_0 - [naringenin$

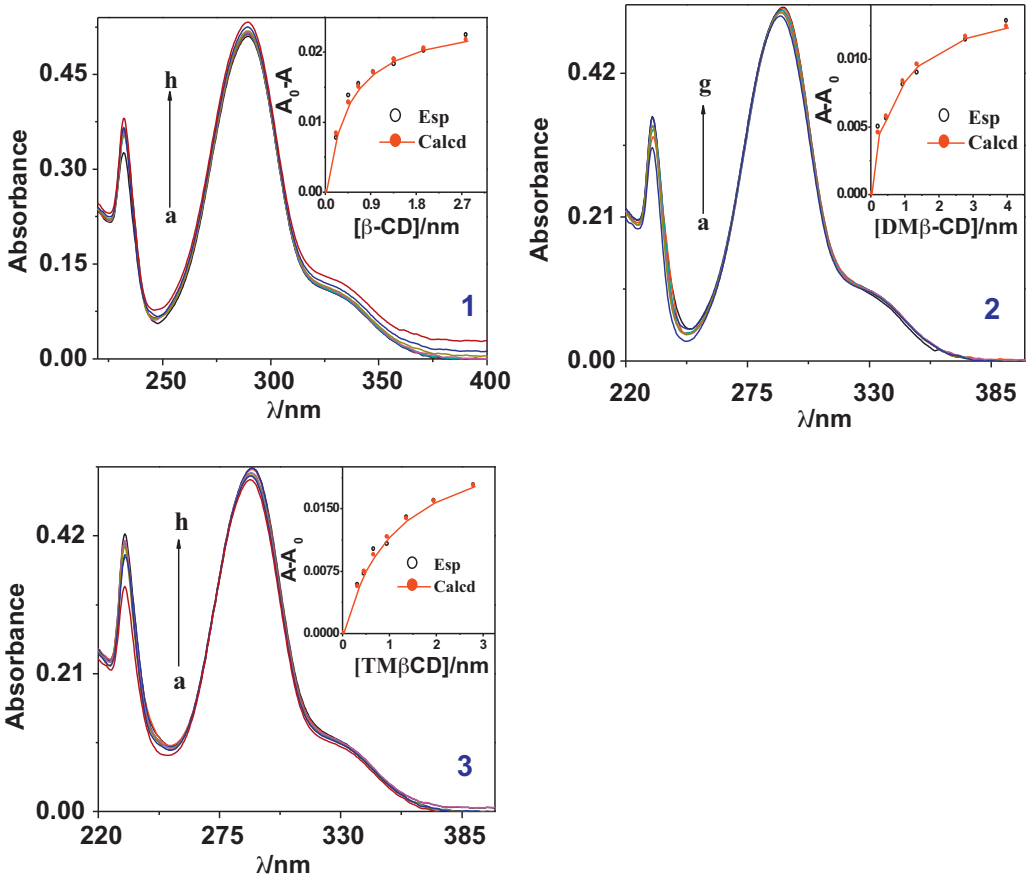


Fig. 2. UV–vis spectral changes in naringenin (0.03 mM) upon addition of β-CD (**1**: 0–2.80 mM, from a to h), DMβCD (**2**: 0–4.00 mM, from a to g) and TMβCD (**3**: 0–2.80 mM, from a to h) in a water/ethanol (v:v = 4:1, ca. pH 3.0) mixed solution, and the nonlinear least-squares analysis (inset) of the differential intensity (ΔA at 289 nm) to calculate the complex stability constant (K_s).

Table 1
The stability constant (K_s and $\log K_s$) and Gibbs free energy change ($-\Delta G^\circ$) for the inclusion complexation of CDs with naringenin guest in a water/alcohol (v/v = 4:1, ca. pH 3.0) mixed solution.

Host	K_s/M^{-1}	$\log K_s$	$-\Delta G^\circ$/kJ mol^{-1}
β-CD	2242	3.35	19.12
DMβCD	1409	3.15	17.97
TMβCD	936	2.97	16.96

$\cdot$ CD] = [CD]$_0$ − $\Delta A/\Delta\varepsilon$). Based on the formula of equilibrium constant, we can obtain K_s = [naringenin$\cdot$ CD]/([naringenin] $\cdot$[CD]) = ($\Delta A/\Delta\varepsilon$)/(([naringenin]$_0$ − $\Delta A/\Delta\varepsilon$)$\cdot$([CD]$_0$ − $\Delta A/\Delta\varepsilon$)) (Eq. (2)). We then derived Eq. (3) from Eq. (2). Finally, the K_s was obtained from the analysis of the sequential changes of absorption (ΔA) at various CD concentrations, with a nonlinear least squares method according to the curve-fitting equation (3).

$$\text{Naringenin} + \text{CD} \overset{K_s}{\rightleftharpoons} \text{Naringenin} \cdot \text{CD} \quad (1)$$

$$K_s = \frac{[\text{Naringenin} \cdot \text{CD}]}{[\text{Naringenin}][\text{CD}]} = \frac{\Delta A/\Delta\varepsilon}{([\text{Naringenin}]_0 - (\Delta A/\Delta\varepsilon))([\text{CD}]_0 - (\Delta A/\Delta\varepsilon))} \quad (2)$$

$$\Delta A = \frac{\Delta\varepsilon([\text{Naringenin}]_0 + [\text{CD}]_0 + 1/K_s) \pm \sqrt{\Delta\varepsilon^2([\text{Naringenin}]_0 + [\text{CD}]_0 + 1/K_s)^2 - 4\Delta\varepsilon^2[\text{Naringenin}]_0[\text{CD}]_0}}{2} \quad (3)$$

Using a nonlinear least squares curve-fitting method (Liu, Li, Wada, & Inoue, 1999), we obtained the complex stability constant for each host–guest combination. Fig. 2 (inset) illustrates a typical curve-fitting plot for the titration of naringenin with β-CD, DMβCD and TMβCD, which shows the excellent fit between the experimental and calculated data and the 1:1 stoichiometry of the naringenin/CD inclusion complexes. In the repeated measurements, the K_s values were reproducible within an error of ±5%. The stability constant (K_s) and Gibbs free energy change ($-\Delta G^\circ$) for the inclusion complexation of CDs with naringenin are listed in Table 1.

3.2. Binding ability

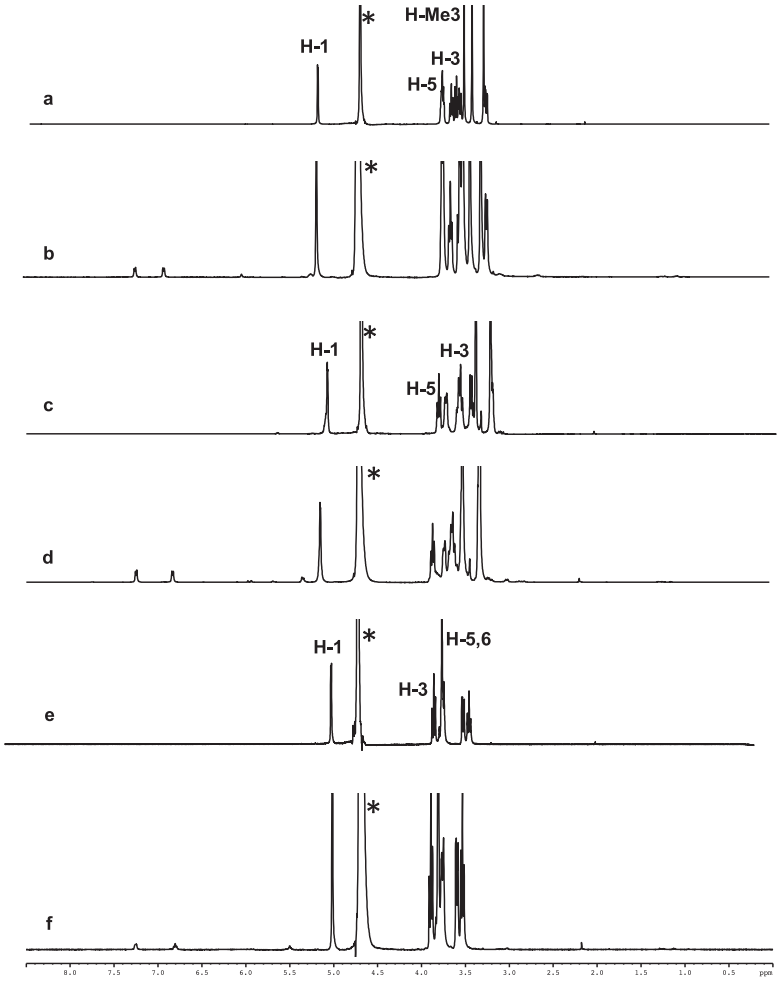
Extensive studies have revealed that the size/shape-fit concept plays a crucial role in the formation of inclusion complexes of host CDs with guest molecules of various structures. On the basis of this concept, several weak intermolecular forces such as ion–dipole, dipole–dipole, van der Waals, electrostatic, hydrogen bond, and hydrophobic interactions are known to cooperatively contribute to inclusion complexation. CDs possess a cyclic truncated cone cavity with a height of 0.79 nm, an inner diameter of 0.62–0.78 nm, and a cavity volume of 0.262 nm^3 for β-CD (Ayala-Zavla, Del-Toro-Sánchez, Alvarez-Parrilla, & González-Aguilar, 2008; Del Valle, 2004; Szejtli, 1998). The host–guest size match may dominate the stability of the complexes formed between CDs and naringenin. From Table 1, we can see that the binding constants for the complexation of naringenin with β-CD, DMβCD and TMβCD were in the following order: β-CD > DMβCD > TMβCD. By comparing the enhancement effect of all kinds of β-CD for naringenin, β-CD gave a stronger K_s value than DMβCD and TMβCD, which demonstrated that β-CD can complex better with the guest naringenin than the methylated CDs. Considering the structural features of the host and guest, we deduced that the hydrogen bond between the hydrogen atoms of β-CD and the oxygen atoms of naringenin may strengthen the host–guest association. In contrast, the methylated CDs (DMβCD and TMβCD) showed a weaker K_s value due to the larger and deeper CD cavity, which caused the intermolecular hydrogen bond to become weaker (Kano, Nishiyabu, Asada, & Kuroda, 2002; Reinhardt, Richter, & Mager, 1996).

3.3. ^{1}H and 2D NMR analysis

In order to explore the possible inclusion mode of the naringenin/CD complex, we compared the ^{1}H NMR spectra of naringenin in the presence of the host CDs (Fig. 3). Owing to its poor water solubility, naringenin is transparent to ^{1}H NMR under most conditions when D_2O is used as a solvent. Assessment of the naringenin complex by ^{1}H NMR clearly demonstrated the presence of the framework protons of the naringenin molecule, consistent with the significant solubilization. As illustrated in Fig. 3, the majority of naringenin protons displayed chemical shifts at δ 5.5–7.5 ppm, which were distinct from the CD protons (usually at δ 3.0–5.0 ppm). By comparing the integration area of these protons with that of the CD's H-1 protons, we calculated the inclusion stoichiometry of the naringenin/CD complexes, that is, 1:1 for the naringenin/β-CD, naringenin/DMβCD and naringenin/TMβCD complexes.

To further explore the inclusion mode, the chemical shifts of β-CD protons in the absence and presence of naringenin were examined (Fig. 3). Inclusion complexation with naringenin had a negligible effect on the δ values of the H-5 and H-6 protons of β-CD (≤0.02 ppm). In contrast, the values of the H-1, H-2, H-3 and H-4 protons exhibited relatively weak but significant changes (0.03–0.04 ppm), which could have been caused by the hydrogen bond between the hydroxyl arms of β-CD and the oxygen atoms of naringenin. It is noteworthy that the H-3 protons shifted ca. 0.03 ppm, but that the H-5 protons shifted ca. 0.01 ppm after inclusion complexation. Because both the H-3 and H-5 protons are located in the interior of the β-CD cavity, and the H-3 protons are near the wide side of the cavity while the H-5 protons are near the narrow side, this phenomenon may indicate that naringenin should penetrate into the β-CD cavity from the wide side. Similarly, all of the TMβCD protons showed appreciable shifts after inclusion complexation with naringenin (0.01–0.08 ppm). By comparing these chemical shifts, we found that the shifts of the H-3 protons (0.08 ppm) were larger than those of the H-5 (0.04 ppm) and H-6 (0.03 ppm) protons, indicating that naringenin may enter the cavity of TMβCD from the wide side as well. It was also revealed that naringenin should penetrate into the DMβCD cavity from the wide side (see Supplementary data).

Two-dimensional (2D) NMR spectroscopy provides important information about the spatial proximity between host and guest atoms via observations of the intermolecular dipolar cross-correlations (Yang et al., 2009). Two protons that are closely located in space can produce a nuclear Overhauser effect (NOE) cross-correlation in NOE spectroscopy (NOESY) or ROESY. The presence of NOE cross-peaks between protons from two species indicates spatial contacts within 0.4 nm (Correia et al., 2002). To gain more conformational information, we obtained 2D ROESY of the inclusion complexes of naringenin with CDs. The ROESY spectrum of the naringenin/β-CD complex (Fig. 4(a)) showed appreciable correlation of the H-2′/H-6′ and H-3′/H-5′ protons of naringenin with the H-3, H-5 and H-6 protons of β-CD (peaks A). These results indicate that the C ring of naringenin was included in



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		δ (ppm)			
		β-CD	naringenin/ β-CD complex	TMβCD	naringenin/ TMβCD complex
H-1	d	4.99	5.02	5.19	5.16
H-2	dd	3.57	3.61	3.25	3.22
H-3	dd	3.88	3.91	3.61	3.53
H-4	dd	3.50	3.54	3.66	3.63
H-5	m	3.80	3.81	3.76	3.72
H-6	dd	3.78	3.76	3.55	3.52
H-Me2	s	–	–	3.42	3.41
H-Me3	s	–	–	3.51	3.49
H-Me6	s	–	–	3.28	3.29

Fig. 3. ^{1}H NMR spectra of naringenin in the absence and presence of β-CD, DMβCD and TMβCD in D_2O at 25 °C, respectively. (a) TMβCD, (b) naringenin/TMβCD complex, (c) DMβCD, (d) naringenin/DMβCD complex, (e) β-CD, and (f) naringenin/β-CD complex (asterisk highlights the water peak); and (g) the chemical shifts (δ) of the β-CD, TMβCD, naringenin/β-CD and naringenin/TMβCD complexes.

the β-CD cavity. The ROESY spectrum of the naringenin/TMβCD complex (Fig. 4(b)) also showed significant correlations between the H-2′/H-6′ and H-3′/H-5′ protons of naringenin and the H-3, H-4, H-5 and H-6 protons of TMβCD (peaks B). These results indicate that the C ring of naringenin was also included in the TMβCD cavity. It was also shown that naringenin should be included in the DMβCD cavity in similar ways (see Supplementary data).

Based on these observations, together with the 1:1 stoichiometry, we deduced the possible inclusion modes of naringenin with CDs as illustrated in Fig. 5.

3.4. XRD analysis

The powder X-ray diffraction (XRD) patterns of naringenin, β-CD and TMβCD as well as their inclusion complexes are

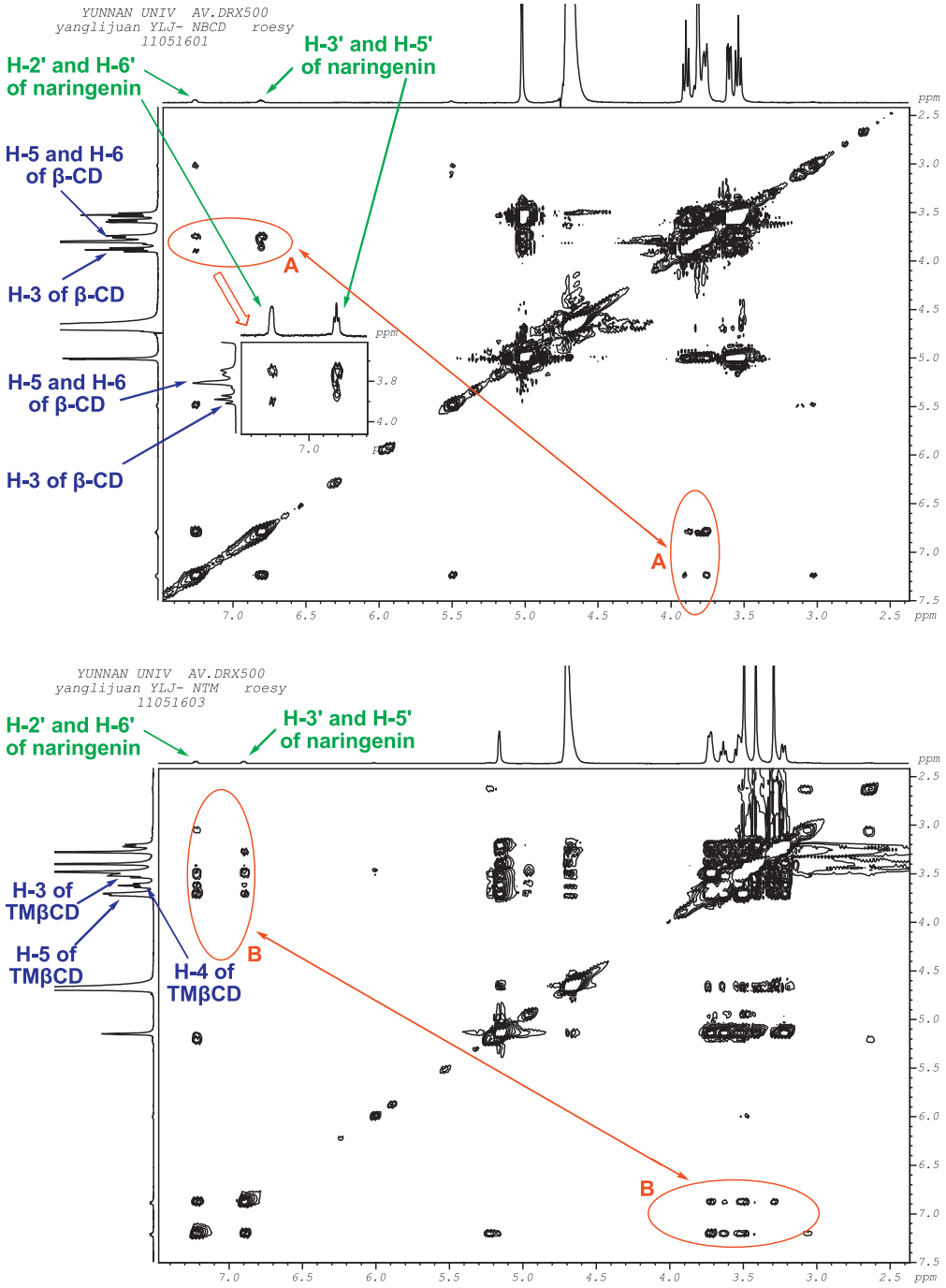

Fig. 4. ROESY spectrum of (a) naringenin/β-CD complex and (b) naringenin/TMβCD in D_2O.

illustrated in Fig. 6A. As indicated in Fig. 6A, naringenin (Fig. 6A(a)), β-CD (Fig. 6A(b)), DMβCD (Fig. 6A(c)) and TMβCD (Fig. 6A(d)) were in a crystalline form. In contrast, the XRD of the naringenin/β-CD, naringenin/DMβCD and naringenin/TMβCD complexes (Fig. 6A(e)–(g)) are amorphous and show halo patterns, which are quite different from the superimposition of crystalline naringenin in β-CD, DMβCD and TMβCD, indicating the formation of the inclusion complex between β-CD (or α-CD) and naringenin. In addition, most of the crystalline diffraction peaks of β-CD, DMβCD and TMβCD disappeared after complexation with naringenin, indicating that the complexation of naringenin reoriented the CD molecules to some extent.

3.5. DSC analysis

The thermal properties of the naringenin/β-CD, naringenin/DMβCD and naringenin/TMβCD complexes were investigated by thermogravimetric (TG) methods (see Supplementary data). A systemic analysis of the TG curves showed that naringenin decomposes at ca. 210 °C, β-CD at ca. 200 °C, DMβCD at ca. 205 °C and TMβCD at ca. 170 °C. However, the thermal stability of their inclusion complexes differed; that is, the decomposition temperatures were ca. 225, 240 and 245 °C for the naringenin/β-CD, naringenin/DMβCD and naringenin/TMβCD complexes, respectively. These results indicate that naringenin's

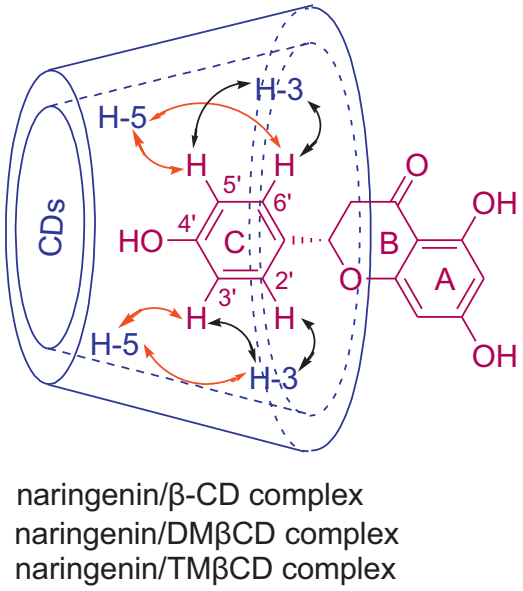

Fig. 5. Possible inclusion mode and significant NOESY (↔) correlations of the naringenin/β-CD, naringenin/DMβCD and naringenin/TMβCD inclusion complexes.

usual thermal properties were altered after inclusion complexation, and that the naringenin/CD complexes possessed high decomposition temperatures.

The differential scanning calorimetry (DSC) thermogram gave further information about the thermal properties of the naringenin/β-CD, naringenin/DMβCD and naringenin/TMβCD complexes. As shown in Fig. 6B, the DSC curve of naringenin contained an endothermic peak at 255 °C (Fig. 6B(a)). In contrast, the DSC curves of pristine β-CD, DMβCD and TMβCD had an endothermic peak at 118, 68 and 182 °C (Fig. 6B(b)–(d)), respectively. However, in the DSC curves of the naringenin/CD complexes, the endothermic peaks at about 255 °C corresponding to the free naringenin disappeared, coinciding with the appearance of a new exothermic peak at 89, 103, and 105 °C in the case of the naringenin/β-CD, naringenin/DMβCD and naringenin/TMβCD system (Fig. 6B(e)–(g)), respectively. This suggested that the naringenin/TMβCD and naringenin/DMβCD complexes are more stable than the naringenin/β-CD complex.

These results not only further confirm the formation of naringenin/CDs complexes, but they also indicate that the naringenin/CD complexes started to decompose only at a temperature above 225 °C, which means that these complexes are fairly stable from a thermal viewpoint.

3.6. SEM analysis

Scanning electron microscopy (SEM) is a qualitative method used to study the structural aspects of raw materials, i.e., CDs and drugs or the products obtained by different methods of preparation, such as physical mixing, solution complexation, coevaporation and others (de Araujo et al., 2008; Duchêne, 1987). The SEM photographs of DMβCD, naringenin, their inclusion complexes and their physical mixtures are shown in Fig. 7. Typical crystals of DMβCD and naringenin are found in many different sizes. Pure naringenin crystallizes in a cubic columnar form with medium dimensions (Fig. 7(a)) and DMβCD appear as irregularly shaped crystal particles (Fig. 7(b)). The physical mixture of naringenin/DMβCD revealed some similarities with the crystals of the free molecules and showed both crystalline components (Fig. 7(c)). However, the naringenin/DMβCD inclusion complex appeared as a compact and homogeneous plate-like structure crystal and was quite different from the sizes and shapes of β-CD and naringenin (Fig. 7(d)), which confirms the formation of the naringenin/β-CD inclusion complex.

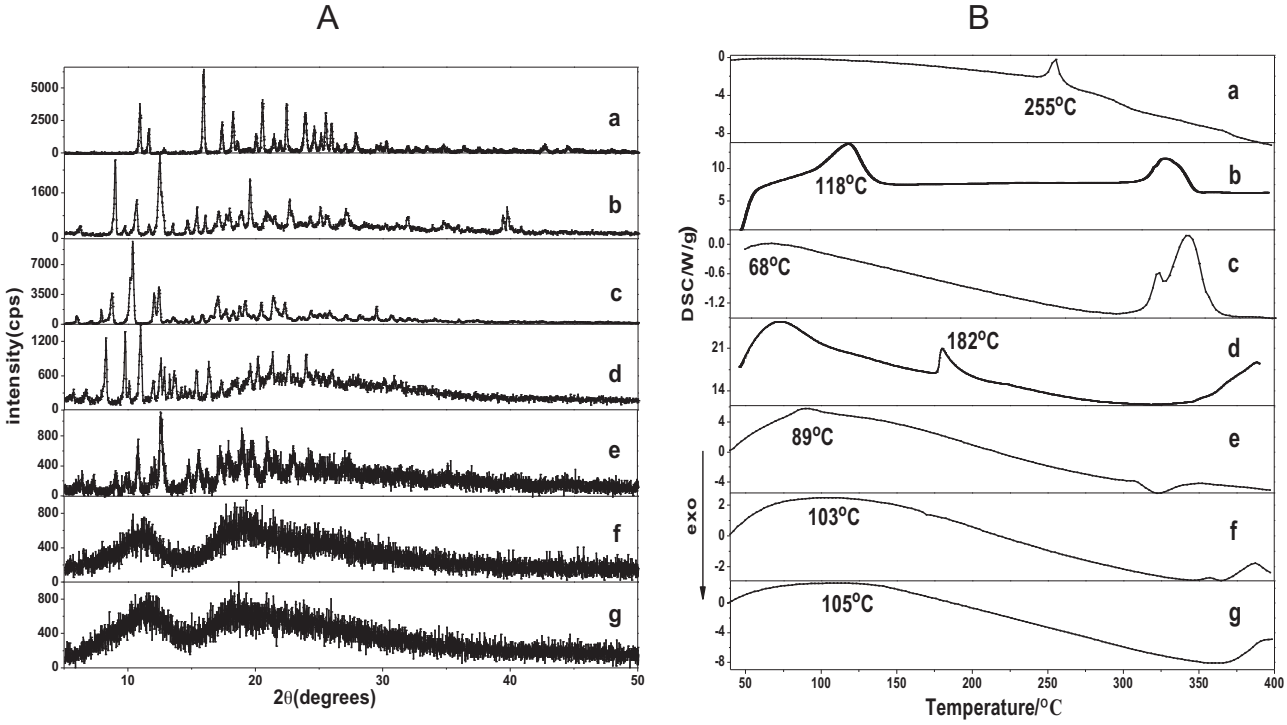

Fig. 6. (A) XRD patterns: (a) naringenin, (b) β-CD, (c) DMβCD, (d) TMβCD, (e) naringenin/β-CD inclusion complex, (f) naringenin/DMβCD inclusion complex and (g) naringenin/TMβCD inclusion complex. (B) DSC thermograms: (a) naringenin, (b) β-CD, (c) DMβCD, (d) TMβCD, (e) naringenin/β-CD inclusion complex, (f) naringenin/DMβCD inclusion complex and (g) naringenin/TMβCD inclusion complex.

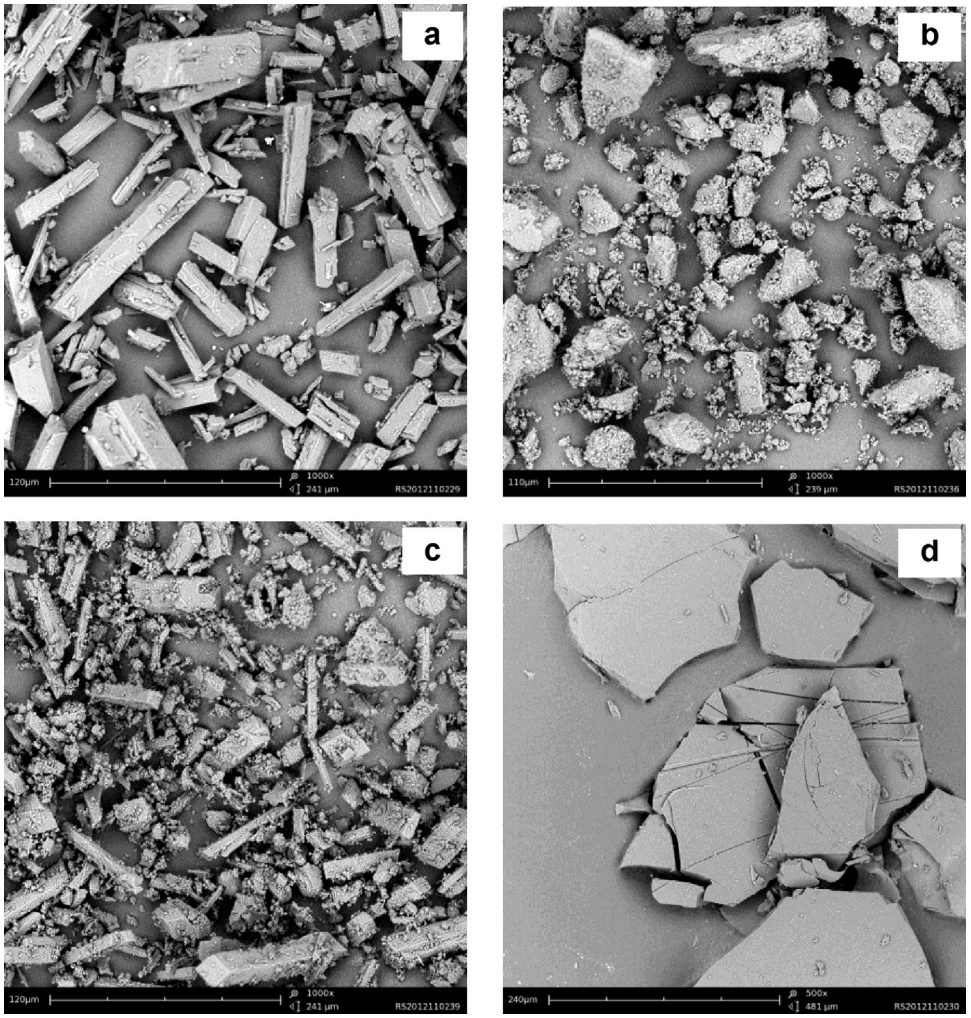

Fig. 7. Scanning electron microphotographs: (a) naringenin, (b) DMβCD, (c) naringenin and DMβCD physical mixture (1:1 molar ratio) and (d) naringenin/DMβCD inclusion complex.

3.7. Solubilization

The water solubility of the naringenin/CD complex was assessed by the preparation of its saturated solution (Montassier, Duchêne, & Poelman, 1997). An excess amount of the complex was placed in 2 mL of water (ca. pH 6.0) and the mixture was stirred for 1 h. After removing the insoluble substance by filtration, the filtrate was evaporated under reduced pressure to dryness and the residue was dosed by the weighing method. The results show that the water solubility of this naringenin, compared to that of native naringenin (ca. 4.38 μg/mL), was remarkably increased to approximately 1.34, 1.60 and 1.52 mg/mL by the solubilizing effects of β-CD, DMβCD and TMβCD, respectively. In the control experiment, a clear solution was obtained after dissolving the naringenin/β-CD (6.9 mg), naringenin/DMβCD (9.4 mg) and naringenin/TMβCD (9.5 mg) complexes, which was equivalent to 1.34, 1.60 and 1.52 mg of naringenin, respectively, in 1 mL of water at room temperature. This confirmed the reliability of the obtained satisfactory water solubility of the naringenin/CD complex, which will be beneficial for the medical utilization of this compound.

4. Conclusions

The inclusion complexation behavior, characterization and binding ability of naringenin with β-CD and its derivatives DMβCD and TMβCD were investigated. The results showed that CDs and its derivatives can enhance the water solubility and thermal stability of naringenin. Given the shortage of applications for naringenin and the easy and environmentally friendly preparation of the naringenin/CD complexes, this inclusion complexation should be regarded as an important step in the design of a novel formulation of naringenin for herbal medicine or healthcare products.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.07.010.

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