Carbohydrate Polymers 85 (2011) 629–637

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Host–guest system of taxifolin and native cyclodextrin or its derivative: Preparation, characterization, inclusion mode, and solubilization

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ARTICLE INFO

Article history:
Received 6 February 2011
Received in revised form 2 March 2011
Accepted 11 March 2011
Available online 24 March 2011

Keywords:
Taxifolin
Cyclodextrin
Inclusion complex
Characterization
Inclusion mode

ABSTRACT

The inclusion complexation behavior, characterization and binding ability of taxifolin with native cyclodextrin (α-, β-, or γ-CD) and its derivative hydroxypropyl-β-cyclodextrin (HPβCD) 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 taxifolin were obviously increased in the inclusion complex with cyclodextrins. This satisfactory water solubility and high thermal stability of the taxifolin/CD complexes will be potentially useful for their application as herbal medicines or healthcare products.

1. Introduction

Flavonoids are a large group of phenolic compounds and constitute one of the largest groups of secondary metabolites in plants (Hollman & Katan, 1997; Lin, Mukhopadhyay, Robbins, & Harnly, 2007; Mateus & Costa, 2007; Soares, Mateus, & Freitas, 2007; Xiao et al., 2008). These substances, with variable polyphenolic structures, are found in numerous food products, such as fruit, vegetables, nuts and beverages (coffee, tea, red wine), as well as in different parts of herbs (Nijeveldt et al., 2001; Shiko et al., 2009). They are known to possess the ability to scavenge free radicals, and have antimicrobial, antithrombotic, antimutagenic and anticarcinogenic activities (Belinha et al., 2007; Makris, Boskou, & Andrikopoulos, 2007; Tu, Lian, Yen, Chen, & Wu, 2007; Wei, Chen, Jiang, Ma, & Xiao, 2009).

The flavonoid taxifolin (3,5,7,3′,4′-pentahydroxyflavanone or dihydroquercetin, Fig. 1) is widely distributed in barks of the genus *Pinus* or *Larix* and in seeds of the genus *Silybum* (Daniel, Renaud, Patrick, & Fabrice, 2006). Several of the plants described as possessing taxifolin are used in traditional and clinical medicine; some are ingested in the human diet; and others are being studied for their potential use in drug development (Vega-Villa et al., 2009). Taxifolin significantly dilates blood vessels, improves microcirculation, increases cerebral blood flow, and inhibits platelet aggregation activity. It has been widely used in the treatment of cerebral infarction and sequela, cerebral thrombus, coronary heart disease and angina pectoris (Landolfi, Mower, & Steiner, 1984; Shiko et al., 2009; Tzen, Ko, Ko, & Teng, 1991). In recent years, taxifolin has become a common antioxidant additive in the food industry (Dapkevicius et al., 2002), and it has also been described as having antimicrobial activity (Chatzopoulou et al., 2010), anti-inflammatory and analgesic properties (Delporte et al., 2005), anti-adipogenic capacity (Theriault et al., 2000), radical scavenger activity (Willfor et al., 2003), and a protective role in plants against pathogens (Bais, Walker, Kennan, Stermitz, & Vivanco, 2003; Brignolas et al., 1995). Taxifolin also affects the gastrointestinal tract by possessing antiulcer, antispasmodic, and antidiarrheal activities (Di Carlo, Mascolo, Izzo, & Capasso, 1999). Furthermore, taxifolin has been described as possessing a tyrosinase inhibitory capacity and thus it is used in depigmentation drugs and whitening cosmetics, as well as a food additive and an insect control agent (Miyazawa & Tamura, 2007; Vega-Villa et al., 2009). Additionally, taxifolin alters the expression of several genes including those coding for detoxification enzymes, cell cycle regulatory proteins, growth factors, and DNA repair proteins (Lee, Cha, Selenge, Solongo, & Nho, 2007). Taxifolin is reported to upregulate the phase II detoxification enzymes, NQO1 and GSTM1, and to downregulate the phase I detoxification enzyme, CYP2E (Makena, Pierce, Chung, & Sinclair, 2009). However, the use of taxifolin as a natural antioxidant or herbal medicine is greatly limited by its low water solubility and bioavailability (Daniel et al., 2006; Shiko et al., 2009). Although much effort has been made to improve its water solubility and stability by introduc-

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doi:10.1016/j.carbpol.2011.03.029

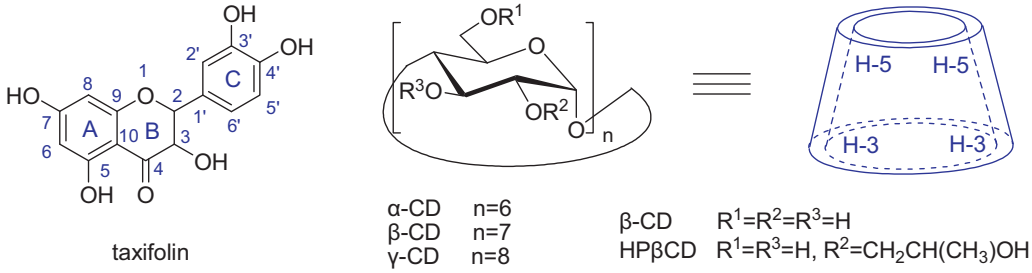


Fig. 1. The structures of taxifolin, α-CD, β-CD, γ-CD and HPβCD.

ing some nanodispersion or enzymatic condensation techniques (Daniel, Renaud, Patrick, & Fabrice, 2009; Shiko et al., 2009), it is still not possible to sufficiently dissolve taxifolin in water, which prevents its usage for some therapeutic and cosmetic applications. Therefore, the search for an efficient and nontoxic carrier for taxifolin 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 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), and 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). However, to the best of our knowledge, no scientific study on the inclusion behavior of taxifolin/CD complexes has hitherto been reported.

More recently, we reported that the inclusion complexation of CDs with natural medicines such as nimbin (Yang et al., 2010), artemether (Yang, Lin, Chen, & Liu, 2009a), scutellarin (Yang, Yang, Lin, Chen, & Liu, 2009b), azadirachtin B (Yang, Chen, Lin, & Liu, 2008; Yang & Lin, 2009) and crassicauline A (Chen et al., 2011), 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, Lin, et al., 2009a; Yang, Yang, et al., 2009b; Yang et al., 2010). As a continuation of

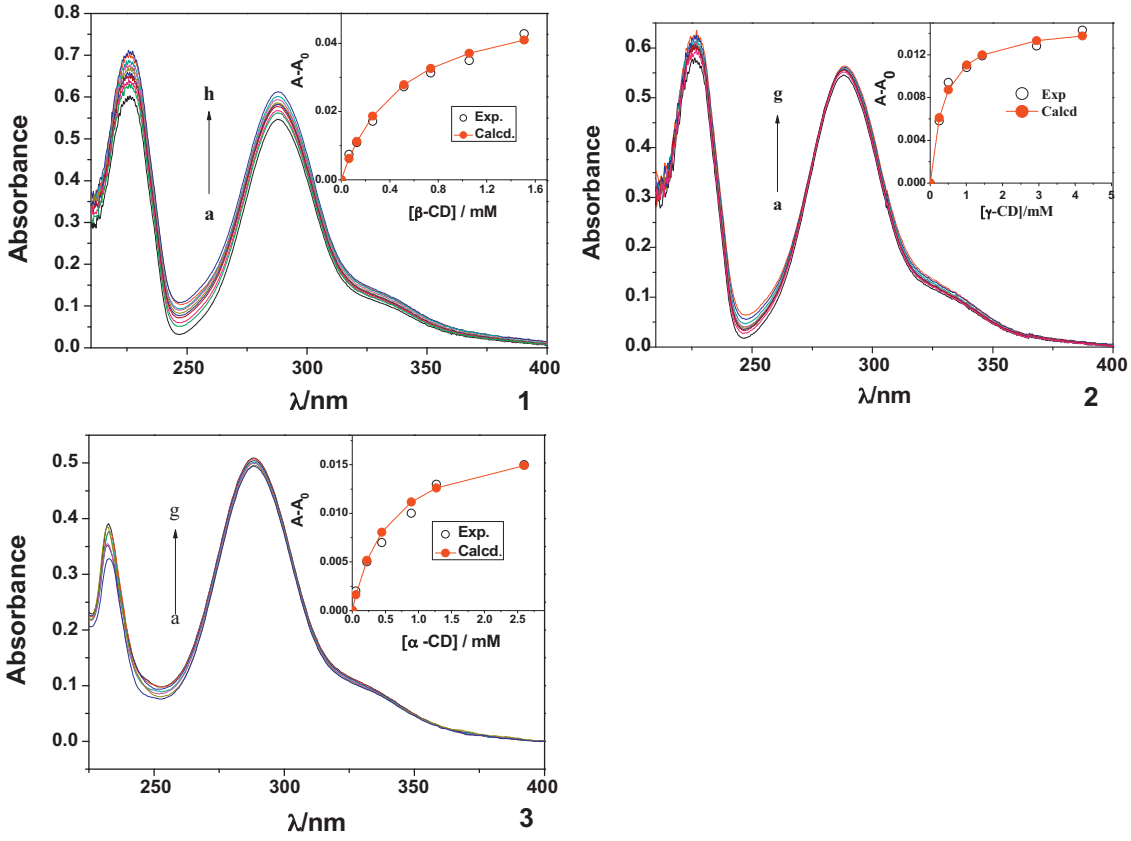
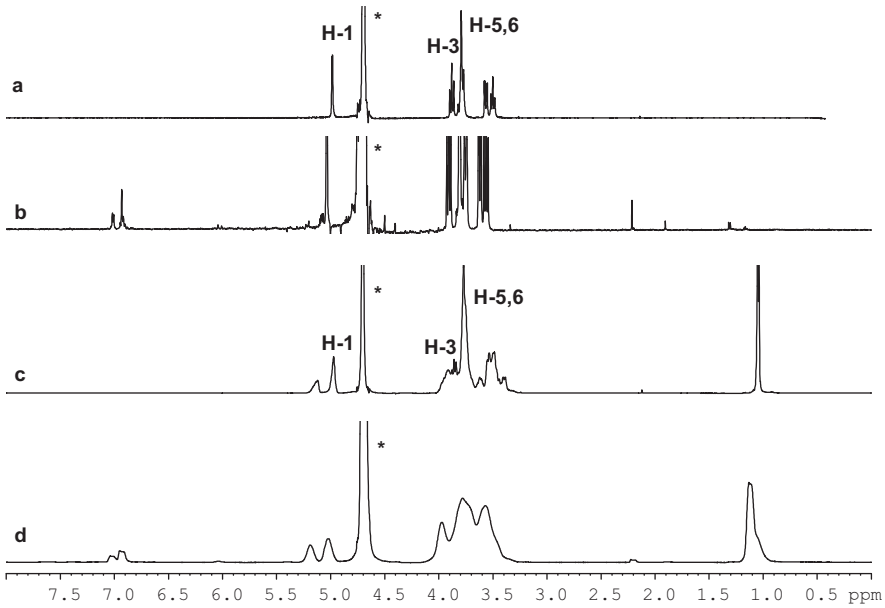


Fig. 2. UV–vis spectral changes in taxifolin (0.03 mM) upon addition of β-CD (**1**: 0–1.50 mM, from a to h), γ-CD (**2**: 0–4.20 mM, from a to g) and α-CD (**3**: 0–2.60 mM, from a to g) in a water/ethanol (V:V = 4:1, ca. pH 3.0) mixed solution, and the nonlinear least-squares analysis (inset) of the differential intensity (ΔA at 287 nm) to calculate the complex stability constant (K_s).



e		δ (ppm)			
		β-CD	taxifolin/β-CD complex	HPβCD	taxifolin/HPβCD complex
H-1	d	4.99	5.04	4.99	5.02
H-2	dd	3.57	3.62	3.54	3.57
H-3	dd	3.88	3.91	3.87	3.97
H-4	dd	3.50	3.56	3.49	3.45
H-5	m	3.80	3.81	3.77	3.78
H-6	dd	3.78	3.76	3.77	3.75
H-Me	s			1.03	1.13

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

our studies on natural medicines/cyclodextrin inclusion complex, an inclusion complex of CLA with β-CD was investigated.

In this paper, we aim to report the preparation and characterization of some water-soluble inclusion complexes formed by taxifolin and native cyclodextrin (α-, β-, or γ-CD) and its derivative: 2-hydroxypropyl-β-cyclodextrin (HPβCD) (Fig. 1). We were particularly interested in exploring the solubilization effect of CDs on taxifolin and the binding ability of the resulting inclusion complexes, which would provide a useful approach for obtaining novel taxifolin-based healthcare products with high water solubility, high bioavailability and low toxicity.

2. Materials and methods

2.1. Materials

Taxifolin (FW = 304, PC > 98%) was obtained from Nanjing Zelang Medical Technology Co., Ltd. (Jiangu, PR China); α-CD (average substitution degree = 972), β-CD (average substitution degree = 1135), γ-CD (average substitution degree = 1297), and 2-hydroxypropyl-β-cyclodextrin (HPβCD, average substitution degree = 1380) were purchased from ABCR GmbH and Co. KG and used without further purification. Other reagents and chemicals were of analytical reagent grade. All experiments were carried out using ultrapure water.

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 taxifolin 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}
α-CD	1872	3.27	18.68
β-CD	2145	3.33	19.02
γ-CD	2908	3.46	19.77

2.2. Methods

2.2.1. Preparation of taxifolin/β-CD, taxifolin/HPβCD, taxifolin/α-CD and taxifolin/γ-CD complexes

Taxifolin (0.03 mM, 9.1 mg) and CD (0.01 mM) were completely dissolved in a mixed solution of ethanol and water (ca. 7 mL, V:V = 1:5, given the poor water solubility of taxifolin, ethanol was used), and the mixture was stirred for 5 days at room temperature. After evaporating the ethanol from the reaction mixture, the uncomplexed taxifolin was removed by filtration. The filtrate was evaporated under reduced pressure to remove the solvent and dried in vacuum to produce the taxifolin/CDs complexes. Taxifolin/β-CD complex (yield 92%): ^{1}H NMR (500 MHz, D_2O, TMS): δ 6.85–7.05 (m, 3H, H-2′, 5′, 6′ of C ring protons for taxifolin), 3.45–3.95 (m, 42H, H-2–6 of β-CD), 4.97–4.99 (s, 7H, H-1 of β-CD). Taxifolin/HPβCD complex (yield 91%): ^{1}H NMR (500 MHz, D_2O, TMS): δ 6.85–7.05 (m, 3H, H-2′, 5′, 6′ of C ring protons for taxifolin), 3.30–4.15 (m, 100H, H-2–6 and CH_2- and CH_3-2, 3, 6 of HPβCD), 4.95–5.10 (s, 7H, H-1 of HPβCD). Taxifolin/α-CD complex (yield 80%): ^{1}H NMR (500 MHz, D_2O, TMS): δ 6.85–7.05 (m, 3H, H-2′, 5′, 6′ of C ring protons for taxifolin), 3.45–4.00 (m, 36H, H-2–6 of α-

CD), 4.90–5.05 (s, 7H, H-1 of α-CD). Taxifolin/γ-CD complex (yield 82%): ^{1}H NMR (500 MHz, D_2O, TMS): δ 6.85–7.05 (m, 3H, H-2′, 5′, 6′ of C ring protons for taxifolin), 3.40–3.85 (m, 56H, H-2–6 of γ-CD), 4.94–5.00 (s, 7H, H-1 of γ-CD).

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 taxifolin, a water/ethanol (V:V = 4:1) solution was used in the spectral measurements. The concentration of taxifolin was held constant at 0.05 mM. Then, an appropriate amount of CD was added, and the final concentrations varied from 0 to 1.50–4.20 mM (β-CD: 0, 0.06, 0.13, 0.26, 0.52, 0.74, 1.06, 1.50 mM; γ-CD: 0, 0.25, 0.50, 1.01, 1.44, 2.97, 4.20 mM; α-CD: 0, 0.06, 0.22, 0.45, 0.89, 1.27, 2.60 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. ^{1}H 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. ^{1}H 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 the 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 α-, β-, γ-CDs and HPβCD with taxifolin 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 taxifolin. As illustrated in Fig. 2, the absorbance intensity of taxifolin gradually increased with the stepwise addition of β-CD, γ-CD and α-CD. 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 taxifolin/CD inclusion complex, the inclusion complexation of taxifolin with β-CD could be expressed by Eq. (1), and the stability constant (K_s) could be calculated from Eq. (2), where [taxifolin/CD], [taxifolin], [CD], $[taxifolin]_0$ and $[CD]_0$ refer to the equilibrium concentration of the taxifolin/CD inclusion complex, the equilibrium concentration of taxifolin, the equilibrium concentration of CD, the original concentration of taxifolin, and the original concentration of CD, respectively, and $\Delta\varepsilon$ is the differential molar extinction coefficient of taxifolin in the absence and presence of CD. According to Lambert–Beer Law, it was found that the concentration of the taxifolin/CD complex was equal to $\Delta A/\Delta\varepsilon$ 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 Eq. (3).

$$\text{Taxifolin} + \text{CD} \overset{K_s}{\rightleftharpoons} \text{Taxifolin} \cdot \text{CD} \tag{1}$$

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

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

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 taxifolin with β-CD, γ-CD and α-CD, which shows the excellent fit between the experimental and calculated data and the 1:1 stoichiometry of the taxifolin/CDs 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 ($-\Delta G°$) for the inclusion complexation of CDs with taxifolin 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,

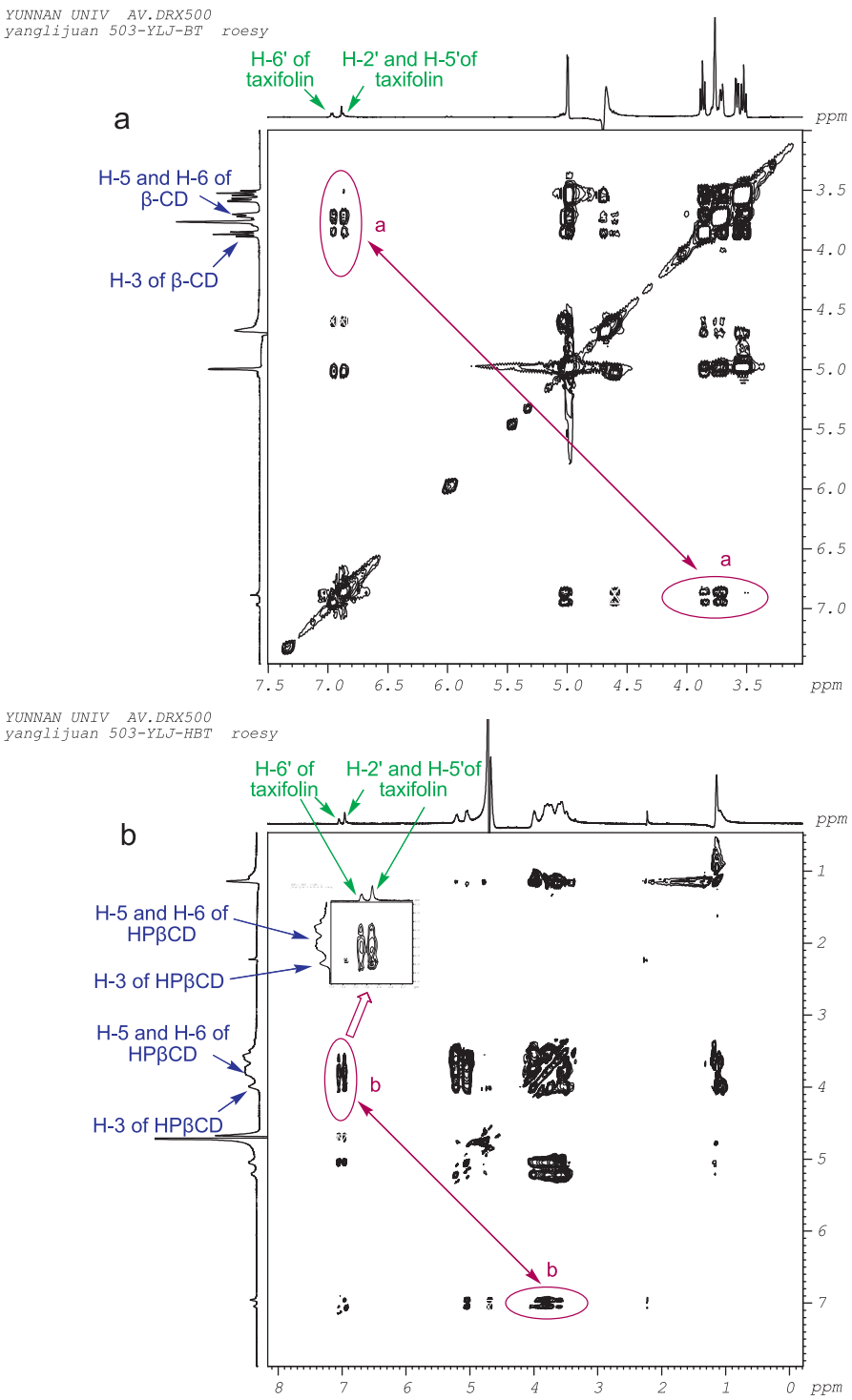


Fig. 4. ROESY spectrum of (a) taxifolin/β-CD complex and (b) taxifolin/HPβCD complex in D_2O.

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 0.47–0.53, 0.62–0.78 and 0.75–0.83 nm, and a cavity volume of 0.174, 0.262 and 0.427 nm^3 for the α-, β- and γ-CDs, respectively (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 taxifolin.

From Table 1, we can see that the binding constants for the complexation of taxifolin with α-, β- and γ-CDs were in the following order: γ-CD > β-CD > α-CD. By comparing the enhancement effect of all kinds of native CDs for taxifolin, γ-CD and β-CD gave a stronger K_s value than α-CD, which showed that γ-CD and β-CD, which possessed a larger cavity size, can complex better with the guest taxifolin than α-CD. It was showed that the size-fit effect was the dominant controlling factor on the formation of inclusion complexes of taxifolin/CDs.

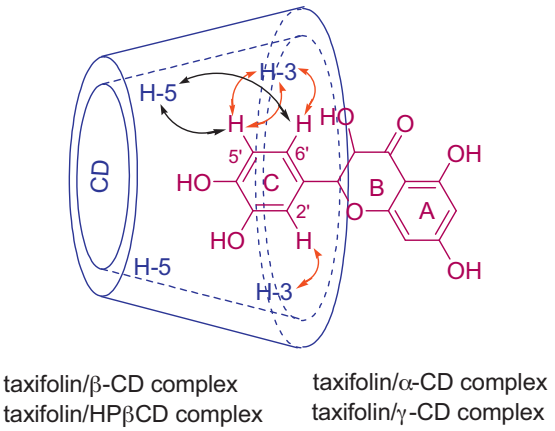


Fig. 5. Possible inclusion mode and significant NOESY (↔) correlations of the taxifolin/β-CD, taxifolin/HPβCD, taxifolin/α-CD and taxifolin/γ-CD inclusion complexes.

3.3. ^1H and 2D NMR analysis

In order to explore the possible inclusion mode of the taxifolin/CD complex, we compared the ^1H NMR spectra of taxifolin in the presence of the host CDs (Fig. 3). Owing to its poor water solubility, taxifolin is transparent to ^1H NMR under most conditions when D_2O is used as a solvent. Assessment of the taxifolin complex by ^1H NMR clearly demonstrated the presence of the framework protons of the taxifolin molecule, consistent with the significant solubilization. As illustrated in Fig. 3, the majority of taxifolin protons displayed chemical shifts at δ 5.0–7.5 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 CDs H-1 protons, we calculated the inclusion stoichiometry of the taxifolin/CD complexes, that is, 1:1 for the taxifolin/β-CD and taxifolin/HPβCD complexes. This 1:1 inclusion stoichiometry was also observed in the taxifolin/α-CD and taxifolin/γ-CD complexes.

To further explore the inclusion mode, the chemical shifts of β-CD protons in the absence and presence of taxifolin were examined (Fig. 3). Inclusion complexation with taxifolin had a negligible effect on the δ values of the H-5 and H-6 protons of β-CD ($\leq$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 (0.03–0.06 ppm), which could have been caused by the hydrogen bond between the hydroxyl arms of β-CD and the oxygen atoms of taxifolin. 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 taxifolin should penetrate into the β-CD cavity from the wide side. Similarly, all of the HPβCD protons showed appreciable shifts after inclusion complexation with taxifolin (0.01–0.10 ppm). By comparing these chemical shifts, we found that the shifts of the H-3 protons (0.10 ppm) were larger than those of the H-5 (0.01 ppm) and H-6 (0.02 ppm) protons, indicating that taxifolin may enter the cavity of HPβCD from the wide side as well. It was also revealed that taxifolin should penetrate into the α-CD and γ-CD cavities 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, Lin, et al., 2009a; Yang, Yang, et al., 2009b). 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 taxifolin with CDs. The ROESY spectrum of the taxifolin/CD complex (Fig. 4a) showed appreciable correlation of the H-2′, H-5′ and H-6′ protons of taxifolin with the H-3, H-5 and H-6 protons of β-CD (peak a). These results indicate that the C ring of taxifolin was included in the β-CD cavity. The ROESY spectrum of the taxifolin/HPβCD complex

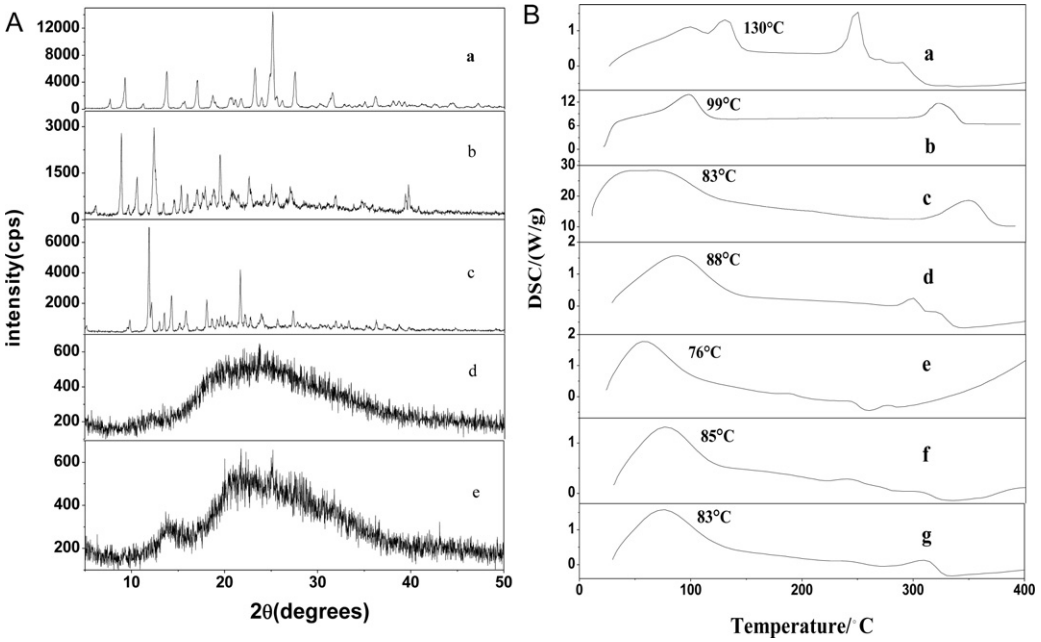


Fig. 6. (A) XRD patterns: (a) taxifolin, (b) β-CD, (c) α-CD, (d) taxifolin/β-CD inclusion complex, (e) taxifolin/α-CD inclusion complex; (B) DSC thermograms: (a) taxifolin, (b) β-CD, (c) HPβCD, (d) γ-CD, (e) taxifolin/β-CD inclusion complex, (f) taxifolin/HPβCD inclusion complex, (g) taxifolin/γ-CD inclusion complex.

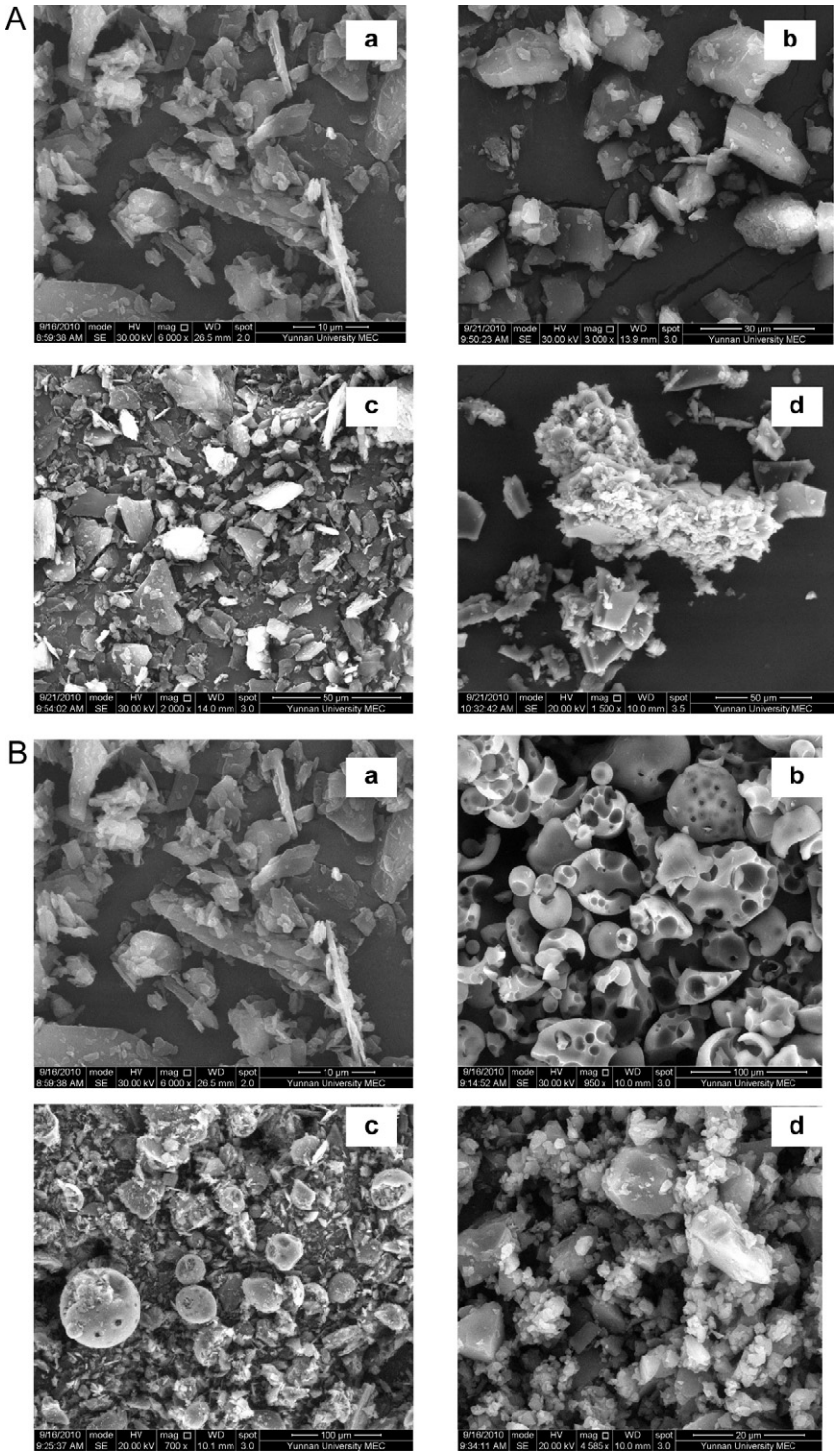


Fig. 7. (A) Scanning electron microphotographs: (a) taxifolin, (b) β-CD, (c) taxifolin β-CD physical mixture (1:1 molar ratio), (d) taxifolin/β-CD inclusion complex; (B) Scanning electron microphotographs: (a) taxifolin, (b) HPβCD, (c) taxifolin and HPβCD physical mixture (1:1 molar ratio), (d) taxifolin/HPβCD inclusion complex.

(Fig. 4b) also showed significant correlations between the H-2′, H-5′ and H-6′ protons of taxifolin and the H-3, H-5 and H-6 protons of HPβCD (peaks b). These results indicate that the C ring of taxifolin was also included in the HPβCD cavity. It was also shown that taxifolin should be included in the α-CD and γ-CD cavities in similar ways (see Supplementary data).

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

3.4. XRD analysis

The powder X-ray diffraction (XRD) patterns of taxifolin, β-CD and α-CD as well as their inclusion complexes are illustrated in Fig. 6A (the XRD of HPβCD and γ-CD and their inclusion complexes with taxifolin can be seen in the Supplementary data). As indicated in Fig. 6A, taxifolin (Fig. 6A(a)), β-CD (Fig. 6A(b)) and α-CD (Fig. 6A(c)) were in a crystalline form. In contrast, the XRD of the taxifolin/β-CD and taxifolin/α-CD complexes (Fig. 6A(d) and

(e)) are amorphous and show halo patterns, which are quite different from the superimposition of crystalline taxifolin in β-CD and α-CD, indicating the formation of the inclusion complex between β-CD (or α-CD) and taxifolin. A similar phenomenon for HPβCD and γ-CD and their inclusion complexes was found (see Supplementary data). In addition, most of the crystalline diffraction peaks of β-CD, α-CD, HPβCD and γ-CD disappeared after complexation with taxifolin, indicating that the complexation of taxifolin reoriented the CD molecules to some extent.

3.5. DSC analysis

The thermal properties of the taxifolin/β-CD, taxifolin/HPβCD and taxifolin/γ-CD complexes were investigated by thermogravimetric (TG) methods (see Supplementary data). A systemic analysis of the TG curves showed that taxifolin decomposes at ca. 190 °C, β-CD at ca. 200 °C, γ-CD at ca. 208 °C and HPβCD at ca. 210 °C. However, the thermal stability of their inclusion complexes differed; that is, the decomposition temperatures were ca. 230, 220 and 250 °C for the taxifolin/β-CD, taxifolin/γ-CD and taxifolin/HPβCD complexes, respectively. These results indicate that taxifolin's usual thermal properties were altered after inclusion complexation, and that the taxifolin/CD complexes possessed high decomposition temperatures.

The differential scanning calorimetry (DSC) thermogram gave further information about the thermal properties of the taxifolin/β-CD, taxifolin/HPβCD and taxifolin/γ-CD complexes. As shown in Fig. 6B, the DSC curve of taxifolin contained an endothermic peak at 130 °C (Fig. 6B(a)). In contrast, the DSC curves of pristine β-CD, HPβCD and γ-CD had an endothermic peak at 99, 83 and 88 °C (Fig. 6B(b)–(d)), respectively. However, in the DSC curves of the taxifolin/CD complexes, the endothermic peaks at about 130 °C corresponding to the free taxifolin disappeared, coinciding with the appearance of a new exothermic peak at 76, 85 and 83 °C in the case of the taxifolin/β-CD, taxifolin/HPβCD and taxifolin/γ-CD system (Fig. 6B(e)–(g)), respectively. This suggested that the taxifolin/HPβCD complex was more stable than the taxifolin/β-CD or taxifolin/γ-CD complexes.

These results not only further confirm the formation of taxifolin/CDs complexes, but they also indicate that the taxifolin/CD complexes started to decompose only at a temperature above 220 °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 β-CD, taxifolin, their inclusion complexes and their physical mixtures are shown in Fig. 7A. Typical crystals of β-CD and CLA are found in many different sizes. Pure taxifolin appear as irregularly shaped crystal particles (Fig. 7A(a)) and β-CD crystallizes in a polyhedral form with large dimensions (Fig. 7A(b)). The physical mixture of taxifolin/β-CD revealed some similarities with the crystals of the free molecules and showed both crystalline components (Fig. 7A(c)). However, the taxifolin/β-CD inclusion complex appeared as a compact and homogeneous plate-like structure with crystal particles and was quite different from the sizes and shapes of β-CD and taxifolin (Fig. 7A(d)), which confirms the formation of the taxifolin/β-CD inclusion complex. In similar tests, HPβCD appeared as a spherical crystal with cavity structures (Fig. 7B(b)). However, the taxifolin/HPβCD inclusion complex appeared to be quite different from the sizes and shapes of HPβCD and taxifolin (Fig. 7B(d)), which confirms the formation of the taxifolin/HPβCD inclusion complex.

3.7. Solubilization

The water solubility of the taxifolin/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 taxifolin, compared to that of native taxifolin (ca. 1.0 mg/mL), was remarkably increased to approximately 12.1, 6.8, 8.5, and 7.0 mg/mL by the solubilizing effects of β-CD, HPβCD, α-CD, and γ-CD, respectively. In the control experiment, a clear solution was obtained after dissolving the taxifolin/β-CD (23.8 mg), taxifolin/HPβCD (18.0 mg), taxifolin/α-CD (22.0 mg), and taxifolin/γ-CD (20.0 mg) complexes, which was equivalent to 12.1, 6.8, 8.5, and 7.0 mg of taxifolin, respectively, in 1 mL of water at room temperature. This confirmed the reliability of the obtained satisfactory water solubility of the taxifolin/CD complex, which will be beneficial for the medical utilization of this compound.

4. Conclusions

The inclusion complexation behavior, characterization and binding ability of taxifolin with native cyclodextrin (α-, β-, or γ-CD) and its derivative hydroxypropyl-β-cyclodextrin (HPβCD) were investigated. The results showed that CDs and its derivatives can enhance the water solubility and thermal stability of taxifolin. Given the shortage of applications for taxifolin and the easy and environmentally friendly preparation of the taxifolin/CD complex, this inclusion complexation should be regarded as an important step in the design of a novel formulation of taxifolin for herbal medicine or healthcare products.

Acknowledgements

This work was supported by NSFC (30860342), Natural Science Foundation of Yunnan Province (2010CD090, 2009cc017), the Opening Foundation of the State Key Laboratory of Elemento-Organic Chemistry of Nankai University (0704 and 0815), and Natural Science Foundation of Yunnan Education Department (2010Y429).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2011.03.029.

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