Spectrochimica Acta Part A 71 (2009) 1891–1895

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Preparation and spectral investigation of inclusion complex of caffeic acid with hydroxypropyl-β-cyclodextrin

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ARTICLE INFO

Article history:
Received 13 May 2008
Received in revised form 3 July 2008
Accepted 5 July 2008

Keywords:
CA
HP-β-CD
Fluorescence
^{1}H NMR
2D NMR

ABSTRACT

The inclusion complexation behavior of caffeic acid (CA) with hydroxypropyl-β-cyclodextrin (HP-β-CD) was studied by UV–vis, fluorescence spectroscopy and nuclear magnetic resonance spectroscopy (NMR). Experimental conditions including the concentration of HP-β-CD and media acidity were investigated in detail. The result suggested HP-β-CD was more suitable for including CA in acidity solution. The binding contants (K) of the inclusion complexes were determined by linear regression analysis and the inclusion ratio was found to be 1:1. The water solubility of CA was increased by inclusion with HP-β-CD according to the phase-solubility diagram. The spatial configuration of complex has been proposed based on ^{1}H NMR and two-dimensional (2D) NMR, the result suggested that CA was entrapped inside the hydrophobic core of HP-β-CD with the lipophilic aromatic ring and the portion of ethylene.

1. Introduction

Cyclodextrins (CDs) are non-toxic cyclic oligosaccharides, consisting of (α-1, 4)-linked α-D-glucopyranose units, with a hydrophilic outer surface and hollow hydrophobic interior. The most abundant natural cyclodextrins are α-cyclodextrin (α-CD), β-cyclodextrin (β-CD), γ-cyclodextrin (γ-CD) containing six, seven and eight glucopyranose units, respectively [1–4].

Investigations of molecular recognition have attracted much attention in supramolecular chemistry involving natural and artificial host-guest systems [4,5]. Cyclodextrin complex has been successfully used to improve the solubility, chemical stability and bioavailability of a number of poorly soluble compounds [4,6,7]. Recently, various hydrophilic, hydrophobic and ionic cyclodextrin derivatives have been utilized to extend the physicochemical properties and inclusion capacity of natural cyclodextrin [8,9]. HP-β-CD is a water-soluble derivative of β-CD, which has been widely studied as a complexion agent for many pharmaceuticals. The ability of CDs to form inclusion complexes is highly affected by size, shape, hydrophobicity and the form of the guest molecular.

Phenolic compounds are secondary plant metabolites and naturally present in almost all plant materials, including food products of plant origin. These compounds are thought to be an integral part of both human and animal diet [10]. Phenolic acids are simple phenols because of their structure. Hydroxycinnamic acid is the major subgroup of phenolic compounds [11]. Hydroxycinnamates are phenylpropanoid metabolites and occur widely in plants [12] and plant products [13]. Hydroxycinnamates and their derivates are bioactiveplant food ingredients. They exhibit in vitro antioxidant activity, which might have beneficial health impact in vivo [14].

CA (3,4-dihydroxycinnamic acid) is one of the hydroxycinnamates derivatives, has been shown to be a α-tocopherol protectant in low-density lipoprotein (LDL) [15]. Also, its conjugates such as chlorogenic and caftaric acids were demonstrated to be more powerful antioxidants in a number of different systems [16]. CA and its derivatives are good may undergo oxidation in plant tissues or products of plant origin [17].

CA exists in four molecular forms in aqueous solution, three charged forms and neutral form. It is an easily available drug, and has extensive bacteriostasic activity [18]. So it is undoubtedly that the interaction between CA and HP-β-CD depends on pH values of the aqueous medium. According to our knowledge, there is not any research on inclusion interaction between the CA and cyclodextrins, but we have only found some research on biologic effect of CA derivatives [19]. So we are interesting to investigate the inclusion mechanism of CA with HP-β-CD.

2. Experimental

2.1. Apparatus

UV-757CRT spectrophotometer (Shanghai precise and Scientific Instrument Co. Ltd.); Fluorescence measurements were performed

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doi:10.1016/j.saa.2008.07.014

by F-2500 FL spectrofluoremeter (Hitachi) using 1 cm quartz cell and both the slits were set at 10 nm with the excitation wavelength at 330 nm and the emission at 350 nm. All the NMR data was obtained on Bruker Avance DRX 300 MHZ NMR spectrometer.

2.2. Reagents

The stock solution of 1.0×10^{-3} mol/L CA was prepared by dissolving it in water and diluted by water. Phosphate buffer solution (0.2 mol/L) was used to control the pH-value of the media. All other reagents were of analytical-reagent grade and were used without purification. Doubly distilled water was used throughout. All experiments were carried out at room temperature.

2.3. Procedure

A 0.1 ml aliquot of the stock solution (1.0×10^{-3} mol/L) of CA was transferred into a 10 mL volumetric flask, then appropriate amount of HP-β-CD (1.0×10^{-2} mol/L) solution was added. The pH was controlled by 0.2 mol/L phosphate buffer solution. The mixed solution was diluted to final volume with distilled water and shaken thoroughly, following ultrasonic for 30 min at $20 \pm 1\,^{\circ}$C. All the measurements of absorption, fluorescence were made against the blank solution treated in the same way without HP-β-CD by using 1.0 cm quartz cell.

2.4. Phase-solubility study

Solubility measurements were based on the phase-solubility technique established by Higuchi and Connors [20]. For that purpose, aqueous solutions of CDs with different increasing concentrations were prepared, excess amount of CA were added to each solutions of CDs, the solutions were reacted completely by ultrasonic for 1 h, then equilibrated for 24 h, then centrifuged and filtered. Their absorption was measured by UV spectrophotometer (315 nm). The phase-solubility profile was therefore obtained by plotting the solubility of CA versus the concentration of CDs.

2.5. NMR measurements

NMR measurements were taken by Bruker Avance DRX 300 MHZ superconducting NMR spectrometer. All the concentrations of CA and HP-β-CD solution were 1.0×10^{-4} mol/L and CA solution is diluted with HP-β-CD solutions at the volume ratio of 1:1. ^{1}H NMR of CA solution as well as its inclusion complexes solutions were also performed to get further evidence.

3. Results and discussion

3.1. UV spectroscopy

The formation of complex of CA with HP-β-CD in aqueous solution was characterized by UV spectroscopy. Fig. 1 shows the absorption spectra of CA in the absence and presence of HP-β-CD. The absorption peaks of CA itself were near 283 and 309 nm. With increasing concentration of HP-β-CD, increasing absorption depended on the concentration of HP-β-CD was observed at 283 nm. Simultaneously, the absorption wavelengths of 283 nm appeared blue shifts, and the absorption peaks at 309 nm appeared the same trend, but the shape of the peaks appeared blur. All of these suggested the formation of inclusion complex between CA and HP-β-CD.

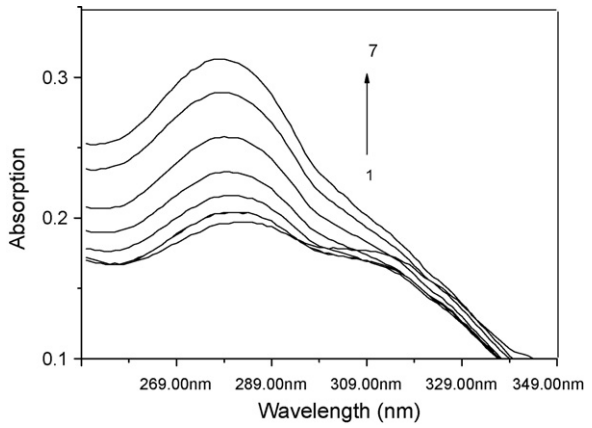

Fig. 1. The absorption spectra of 1.0×10^{-5} mol/L CA in the presence of HP-β-CD. The concentration of HP-β-CD (M) 0–6.0×10^{-3}.

3.2. Fluorescence study

Fig. 2 shows fluorescence spectra of CA in the absence and presence of HP-β-CD. The maximum excitation and emission wavelengths were 330 and 413 nm, respectively. From figure, the fluorescence intensity was enhanced and the emission wavelengths have blue shifts with increasing concentration of HP-β-CD. These data suggested that stable complexes were formed between HP-β-CD and CA. The HP-β-CD cavity provided an apolar environment for CA molecule and thus increased the quantum yield of the fluorescence of CA.

The formation constant (K) and the ratio of the complex were calculated from these data by use of the modified Benesi–Hildebrand equation:

$$\frac{1}{F - F_0} = \frac{1}{(Kk[P]_0[\mathrm{CD}]_0)} + \frac{1}{(KQ[P]_0)}$$

where F and F_0 represent the fluorescence intensity of CA in the presence and absence of HP-β-CD, respectively; K is a forming constant; α is a constant. Fig. 3 shows the double reciprocal plots of $1/(F - F_0)$ versus $1/[\mathrm{CD}]$. They exhibit good linearity. These implied that the inclusion complexes have a stoichiometry of 1:1. The value of K is 112 M^{-1}.

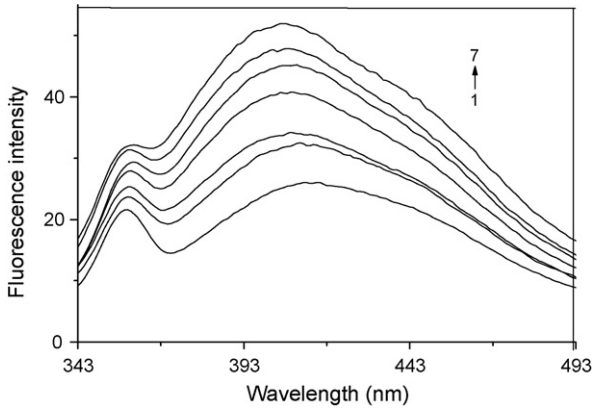

Fig. 2. Fluorescence emission spectra of 1.0×10^{-5} mol/L CA in the presence of HP-β-CD. The concentration of HP-β-CD (M): 0–6.0×10^{-3}.

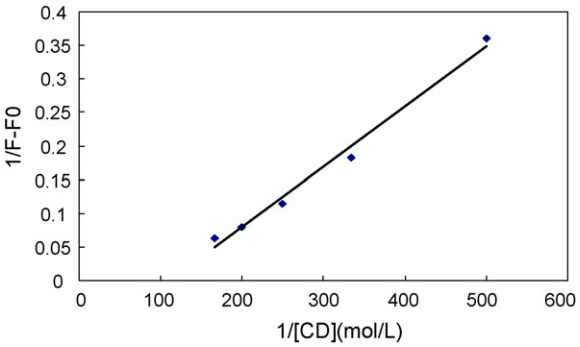

Fig. 3. Double reciprocal plot for CA in the presence of HP-β-CD at 293 K.

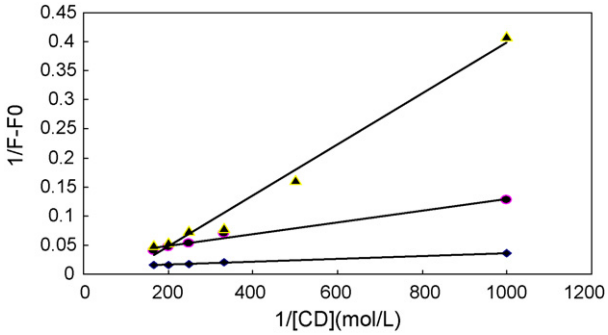

Fig. 4. Double reciprocal plots for CA in the presence of HP-β-CD at different pH values: (♦) pH 3.0, (●) pH 6.5 and (▲) pH 10.5.

Table 1
The binding constants (*K*) of CA/HP-B-CD at different pH values

CA/HP-B-CD	*K*
pH 3	580 ± 56
pH 6.5	279 ± 11
pH 10.5	104 ± 5

3.3. Influence of pH

Fig. 4 shows the double reciprocal plots $1/(F-F_0)$ versus $1/[CD]_0$ for CA to HP-β-CD at different values of pH. The plots exhibit good linearity. This implies that the formation of inclusion complexes with a stoichiometric ratio of 1:1 (HP-β-CD: CA).

It is noted that the formation constants are very sensitive to the change of pH values. The inclusion complexation interaction of HP-β-CD with CA is the order: $K_{pH\,3} > K_{pH\,6.5} > K_{pH\,10.5}$. The formation constants are listed in Table 1.

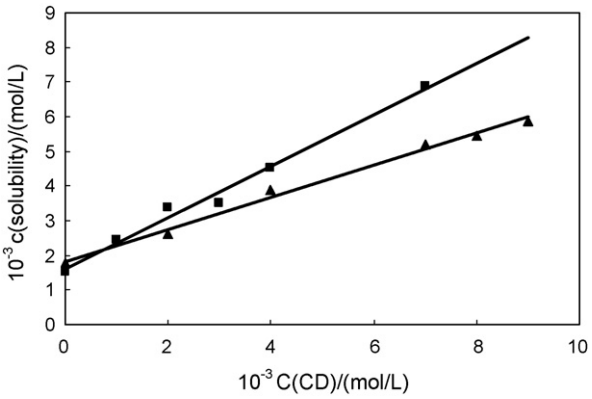

Fig. 6. Phase-solubility profile for CA in β-CD (♦) and HP-β-CD (▲).

One of the major factors affecting the inclusion interaction is the hydrophobic degree of the guest, which is related to the form of CA. CA has four forms: three charged forms and one neural. There existed following equilibrium in aqueous solution (Fig. 5) In pH 2.0–3.5, the neutral form of CA is predominant; while pH 5.5–7.4, the charged form of H_2CAF^- is predominant; while pH > 8.5, the form of $HCAF^{2-}$ is predominant gradually, and pH > 12.5, the form of CAF^{3-} is predominant [21].

The normal HP-β-CD are not charged (2 < pH < 11) and the major inclusion interactions are hydrophobic interactions between the guest and the cyclodextrin cavity and hydrogen bonding of the guest to –OH groups or other introduced groups on the CD rings. In acidic media, the neutral (uncharged) form of CA is predominant, which is more hydrophobic than other forms, so it is more easily to form the inclusion with HP-β-CD.

3.4. Phase-solubility studies

The phase-solubility diagrams of CA in β-CD and HP-β-CD solutions are shown in Fig. 6. It could be observed that the solubility of CA increased as the concentrations of β-CD and HP-β-CD increased, and the increasing was more obvious when complexed with HP-β-CD as opposed to β-CD. When $c\,(CD) = 1 \times 10^{-2}$ mol/L, the solubility of CA/HP-β-CD enhanced nearly four times and the solubility of CA/β-CD enhanced nearly three times. The phase-solubility diagrams of CA with β-CD and HP-β-CD resulted both in A_L-types [22]. Higuchi phase-solubility diagram ($r = 0.9943$ and 0.9987) similar to that reported by Higuchi and Connors [20]. This may again indicate that the formation of 1:1 stoichiometric ratios of CA/β-CD and CA/HP-β-CD complexes.

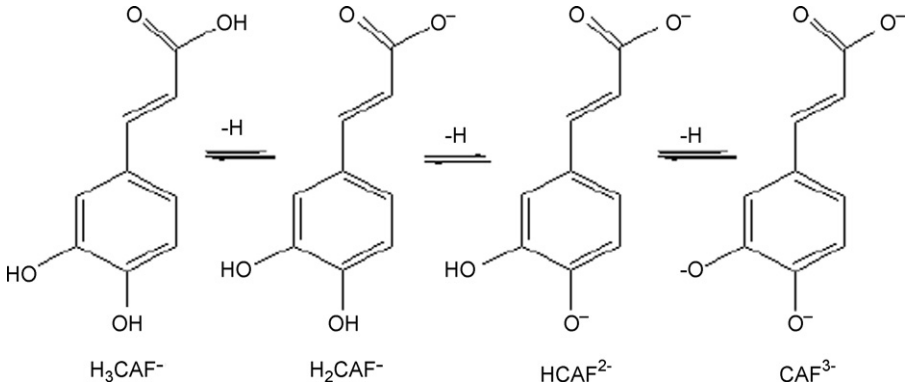

Fig. 5. The equilibrium of CA in aqueous solution.

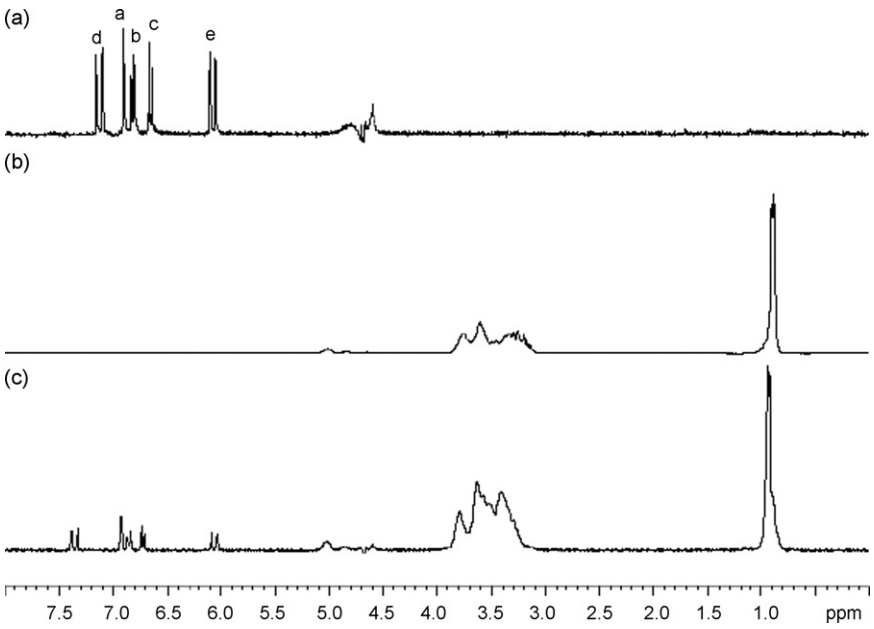

Fig. 7. ^{1}H NMR spectra of (a) CA, (b) HP-β-CD and (c) CA complexed with HP-β-CD.

3.5. ^{1}H NMR studies

^{1}H NMR spectroscopy affords direct evidence for the inclusion of a guest molecule inside the CD cavity. This is based on the expectation that, if the inclusion occurs, the physical or chemical environment affected will be felt by hydrogens of the internal surface of the cavity (H_3 and H_5 from any glucopyranose unit of CD), but not by that of the external surface (H_1, H_2 and H_4). It seems that the addition of the ligand makes variable levels of changes on the chemical shift ($\Delta\delta$) of the hydrogen both of the ligand or CD. These values are critical functions of the position of the molecule and of the size of the CD cavity. The magnitude of the shift of H_3 and H_5 hydrogens and their relation ($\Delta\delta H_3/\Delta\delta H_5$) an be used, respectively, as quantitative measurements of the complex stability and of the deepness of inclusion of the ligand inside the cavity [23].

The CA resonances are affected by the inclusion: we can observed that in the NMR spectrum a modification of the chemical shift values of the anisotropically shielded atoms in Fig. 7. The ^{1}H chemical shift values of free CA and those of the complex are reported in Table 2.

CA has five types of hydrogen: a-H, b-H, c-H, d-H and e-H. When CA complexed with HP-β-CD, we observed that high delta values ($\Delta\delta$) for an aromatic proton b ($\Delta\delta = 0.033$), c ($\Delta\delta = 0.066$) and d ($\Delta\delta = 0.227$) belonging to the adjacent –CH=CH– group. So it is reasonable to speculate that part of the molecule, which is highly hydrophobic, corresponding to the benzene ring and portion of ethylene, must be deeply inside the lipophilic core of HP-β-CD. However, the most polar groups of the molecule, –COOH and the hydroxyl groups, exposed outside the cavity.

Table 2
Changes of chemical shifts of CA before and after forming inclusion complex

^{1}H assignment	Δ CA free (ppm)	δ CA complexed (ppm)	$\Delta\delta$ (CA complexed – CA free)
H-a	6.907	6.807	0.019
H-b	6.807	6.840	0.033
H-c	6.674	6.740	0.066
H-d	7.100	7.327	0.227
H-e	6.100	6.083	−0.017

3.6. 2D NMR studies

^{1}H NMR spectroscopy is an effective method for studying spatial conformations of cyclodextrin inclusions. Two-dimensional (2D) NMR is a powerful tool for investigating inter- and intra-molecular interaction. The presence of NOE cross-peaks between protons from two species indicates spatial contacts within 0.4 nm. To gain more conformational information, we used 2D ROESY to study the inclusion complexes.

Fig. 8 shows a contour plot of a section of the ROESY spectrum of CA/HP-β-CD complex. In order to assign unambiguously H-3, H-5 and H-6 of HP-β-CD region, an HSQC spectrum of HP-β-CD system was obtained in the conditions used for the ROESY spectrum (data not shown). The ROESY spectrum of the HP-β-CD complex shows correlation between H-a,b,c and e of CA with H-3 and H-2 of the cyclodextrin, indicating that phenyl ring of the guest molecular

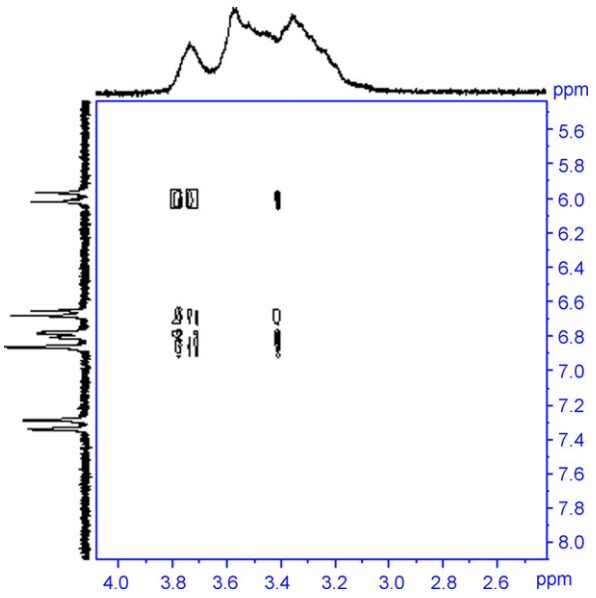

Fig. 8. The ROESY spectrum of CA in the presence of HP-β-CD.

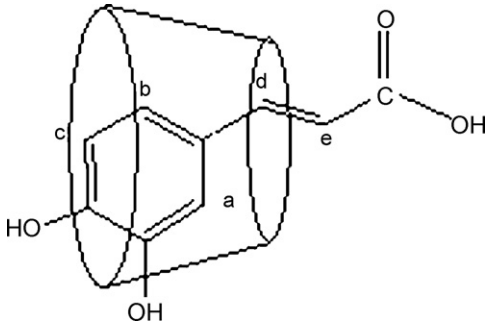

Fig. 9. Proposed model for CA with HP-β-CD.

is included in the HP-β-CD cavity and the more polar group, the –COOH group, is exposed outside the HP-β-CD cavity and towards the secondary hydroxyl group, as shown in Fig. 9.

4. Conclusion

In our work, UV and fluorescence spectroscopy give originally support for the formation of complexes of CA/HP-β-CD, while NMR analyses unequivocally demonstrate that CA is embedded inside the cavity of HP-β-CD with the aromatic and portion of ethylene, and the more polar groups exposed outside the HP-β-CD cavity. In addition, HP-β-CD was more suitable for including CA in acidity solution. In summary, major factors affecting molecular recognition is size matching between CD and guest and the hydrophobic degree of the guest molecule. The water solubility of CA was increased by inclusion with HP-β-CD according to the phase-solubility diagram. ^{1}H NMR and 2D NMR provides good description regarding structural information of the inclusion phenomena, and results evidence that HP-β-CD void pocket is viable for CA and orientations of the guest molecule are considerably restricted. The result suggested that HP-β-CD is powerful pharmaceutical tools for the encapsulation or releases of this potent antitoxiant.

Acknowledgements

The work was supported by the Foundation of Shanxi Province (No. 2006011017) and High-tech Research and development projects (No. 2006BAI06A20-10).

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