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Investigation on the Inclusion Behavior of Caffeic Acid with Cyclodextrin

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The formation of the inclusion complex of caffeic acid with cyclodextrin (β-CD) was studied by fluorescence, absorption spectroscopy, nuclear magnetic resonance (NMR) and the binding constant (*K*) of the inclusion complexes was obtained by steady-state fluorescence measurements. Experimental conditions including concentrations of β-CD and media acidity were investigated for the inclusion formation in detail. The results suggested that caffeic acid exists in four molecular forms in aqueous solution, (charged forms and neutral form) and β-CD were most suitable for inclusion of one of the charged forms of caffeic acid and could cause enhanced fluorescence emission and absorption of caffeic acid. Moreover, the spatial information of complexes have been investigated by NMR. The related mechanism is proposed to explain the inclusion process.

Keywords: β-Cyclodextrin; Caffeic acid; Fluorescence; Absorption; Nuclear magnetic resonance

INTRODUCTION

Cyclodextrins (CDs) are polysaccharides of six to eight D-glucose monomers connected at the 1 and 4 carbon atoms, and they can include guest molecules of appropriate polarity and dimension due to their hydrophobic cavity and hydrophilic exterior surface [1–5], which give them applications in many fields [6–8]. CDs can be used as models to probe proteins and enzymes [9], or as important additives to enhance solubility, chemical stability and bioavailability of poorly soluble drugs, reduce toxicity, or control the release rate, etc. [10–12]. Nuclear magnetic resonance spectroscopy (NMR) plays a vital role in the analysis of CD complexes [9], mostly because it can provide detailed spatial information on the interaction between guest molecules and CDs in solution. Two-dimensional nuclear Overhauser effect spectroscopy proves to be a powerful method in studying intermolecular interaction and supermolecule conformation. Moreover, it is feasible to investigate fluorescence properties when inclusion occurs, since many guest compounds are fluorescent and the non-radiative decay process often significantly attenuate as fluorescence increases [13–15]. The complexation phenomenon often results in remarkable variations in photophysical and photochemical properties of guest molecules because of the microenvironmental difference between the CD interior and aqueous medium. The study of supramolecules has been increasing in many applications. It has been demonstrated that several weak forces, including van der Waals, hydrophobic, electrostatic, dipole–dipole, and hydrogen-bonding interactions, cooperatively govern the inclusion complexation behavior of cyclodextrin host [15,16]. The ability of CDs to form inclusion complexes is highly affected by the size, shape, and hydrophobicity of guest molecules. We have investigated some drug-CD systems [17–21].

Caffeic acid exists in four molecular forms in aqueous solution, three charged forms and a neutral form. It is an easily available drug, and has extensive bacteriostasic activity [22]. As we have known that caffeic acid exists mostly as neutral form under the pH range of 3.0–10.0 [23]. So the interaction between caffeic acid and β-CD depends on the pH values of the aqueous medium. According to our knowledge, there is not any research on inclusion interaction between caffeic acid and cyclodextrin, we have only found some research on the biologic effect of caffeic

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acid derivatives [24]. So, it is interesting to investigate the mechanism of caffeic acid CD complex. The formation of the interaction complexation of CD with guest molecule can enhance solubility, chemical stability and bioavailability of poorly soluble drugs, reduce toxicity, or control the release rate, etc.

In this paper, the interaction of caffeic acid with β-CD derivatives was studied in detail based on fluorescence, absorption measurement and NMR. The factors affecting the inclusion process were discussed, especially the pH value which is related to the form of caffeic acid in solution. Enhanced fluorescence and absorption characteristics served as an aid for better understanding the inclusion mechanism, including the size/shape-fit, hydrophobicity. Especially, detailed spatial information in solution has been studied by 1H NMR and 2D NMR.

RESULTS AND DISCUSSION

Formation of Inclusion Complex of Caffeic Acid with β-CD

Figure 1 shows the absorption spectra of caffeic acid in the absence and presence of β-CD at pH = 12.5. The maximal absorption wavelength of caffeic acid itself exhibited at 260 nm, which resulted from the absorption of charged forms of caffeic acid [23]. With the increasing concentration of β-CD, an interesting independence of absorption wavelength on the concentration of β-CD was observed, so does the absorption at 280 nm. With the increasing concentration of β-CD, the absorption at 260 nm for charged form gradually increased, so does the absorption at 280 nm increased. At the same time, we can draw such conclusion: an absorption wavelength depending on the value of pH was also observed.

The formation of β-CD-caffeic acid inclusion complexes at pH = 3.05, 7.5, 10.53, 12.5 were studied by fluorescence spectra, respectively. Figure 2 showed fluorescence spectra of caffeic acid in the absence and presence of β-CD at pH = 12.5. With the increasing concentration of β-CD, the fluorescence spectra for all forms of caffeic acid gradually increased. At 4.5, the maximum wavelength of excitation and emission were at 285 nm and 420 nm; at 7.5, the maximum wavelength of excitation and emission were at 310 nm and 425 nm; at 10.53, the maximum wavelength of excitation and emission were at 330 nm and 465 nm; at 12.5, the maximum wavelength of excitation and emission were at 260 nm and 460 nm, respectively. Addition of β-CD at different pH caused a noticeable increase of caffeic acid fluorescence intensity. These remarkable changes were due to the interaction between CDs and caffeic

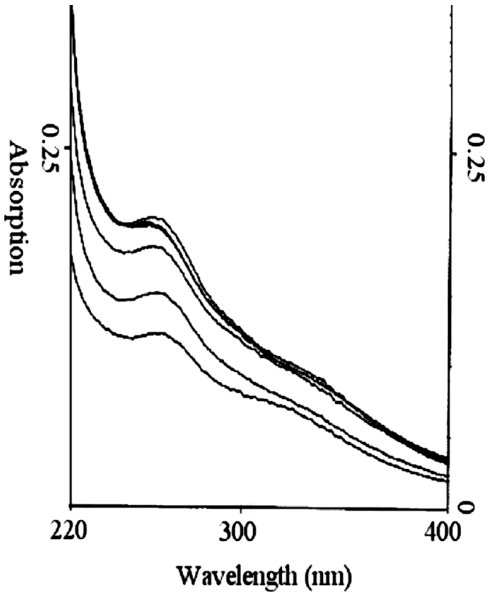


FIGURE 1 The absorption spectra of 4.0×10^{-5} M caffeic acid in the presence of β-CD at pH = 6.5. The concentration of β-CD (M): (1) 0; (2) 1.0×10^{-3}; (3) 2.0×10^{-3}; (4) 3.0×10^{-3}; (5) 4.0×10^{-3}; (6) 5×10^{-3}.

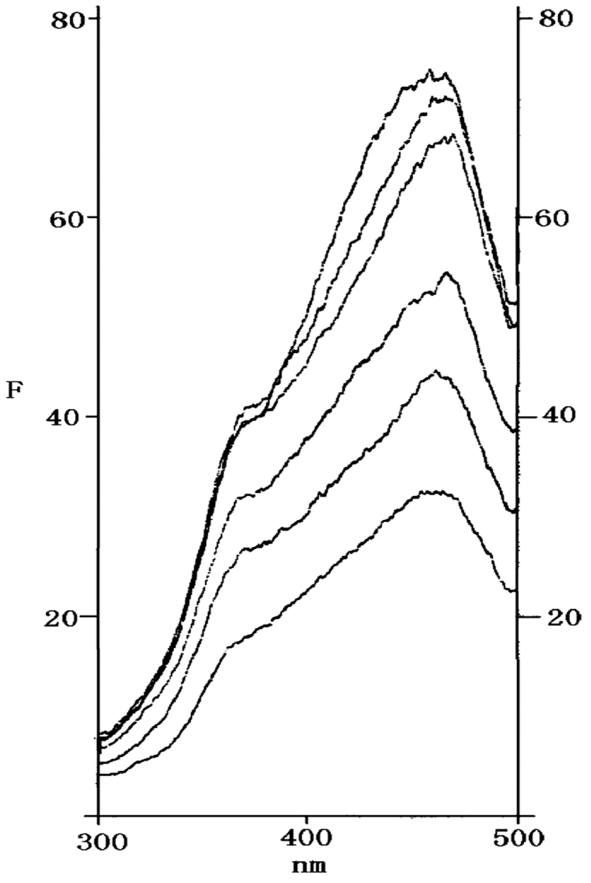


FIGURE 2 Fluorescence emission spectra of 1.0×10^{-5} M caffeic acid at pH = 12.5 in β-CD, β-CD concentration (M): (1) 0; (2) 1.0×10^{-3}; (3) 2.0×10^{-3}; (4) 3.0×10^{-3}; (5) 4×10^{-3}; (6) 5.0×10^{-3}.

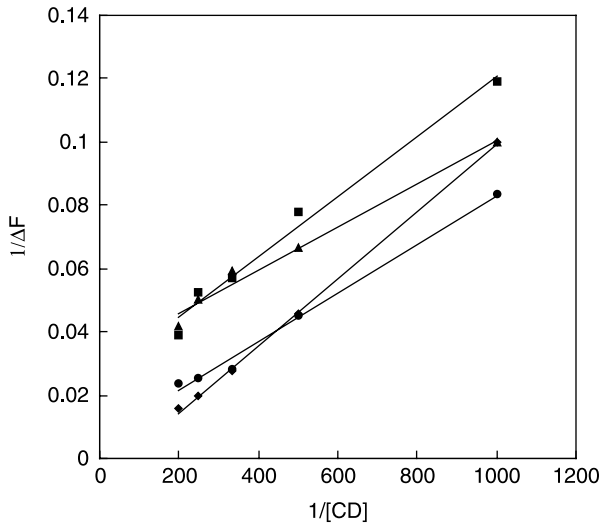


FIGURE 3 Double reciprocal plots for caffeic acid complexed to β-CD at different pH values. (•) pH = 3.05; (■) pH = 7.5; (▲) pH = 10.53; (◆) pH = 12.50.

TABLE I Formation constants K (M^{-1}) for CDs–CA complexes at different pH values

	pH = 3.05	pH = 7.5	pH = 10.53	pH = 12.5
Formation constants	268	253	475	73
Excitation wavelength (nm)	285	310	330	260
Emission wavelength (nm)	420	425	465	460

acid, implying the formation of CDs- caffeic acid inclusion complexes.

Formation Constants of CA–β-CD Complexes

Inclusion formation constant (K) was a measure for complexing capacity of β-CD. The formation constants of caffeic acid with β-CD were evaluated at different pH values assuming a 1:1 (β-CD: caffeic acid) inclusion model. The inclusion equilibrium is as follows:

$$\text{CD} + \text{CAFFEIC ACID} \rightleftharpoons \text{CD–CAFFEIC ACID}$$

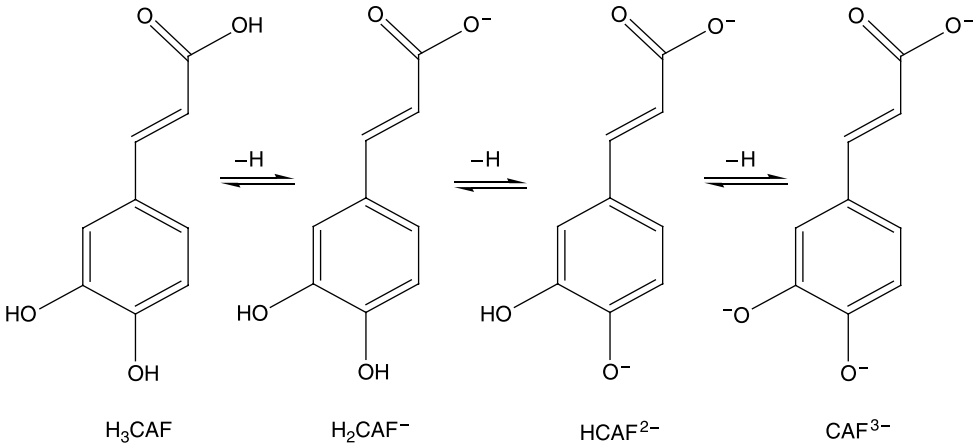
Where the symbols CD, caffeic acid, and CD-caffeic acid stand for β-CD, caffeic acid and the inclusion complex, respectively. The formation constant can be obtained from fluorescence data by the modified Benesi–Hildebrand equation (double the reciprocal plot).

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

Where F and F_0 represent the fluorescence signals of caffeic acid in the presence and absence of β-CD; $[P]_0$ and $[CD]_0$ represent the initial concentration of caffeic acid and cyclodextrin; k is an instrumental constant; K is the formation constant of the complex; Q is the quantum yield for the complex. Figure 3 shows the double reciprocal plots $1/(F - F_0)$ vs. $1/[CD]_0$ for caffeic acid to β-CDs at different value of pH. The plots exhibit good linearity. This implies that the formation of inclusion complexes with a stoichiometry of 1:1 (β-CD: CAFFEIC ACID).

It is noted that the formation constants are very sensitive to the change of pH values. The inclusion complexation interaction of β-CD with caffeic acid is the order: $K_{pH10.53} > K_{pH7.5} > K_{pH12.5} > K_{pH3.05}$. The formation constants are listed in the Table I. One of the major factors affecting the inclusion interaction is the hydrophobicity of the guest, which is related to the form of caffeic acid. Caffeic acid has four forms: three charged forms and a neutral one. There exists the following equilibrium in aqueous solution (Scheme 1).

In pH = 2.0–3.5, the neutral form of caffeic acid is predominant; while pH = 5.5–7.4, the charged form of H_2CAF^- is predominant; while pH > 8.5,



SCHEME 1 The equilibrium of Caffeic acid in aqueous solution.

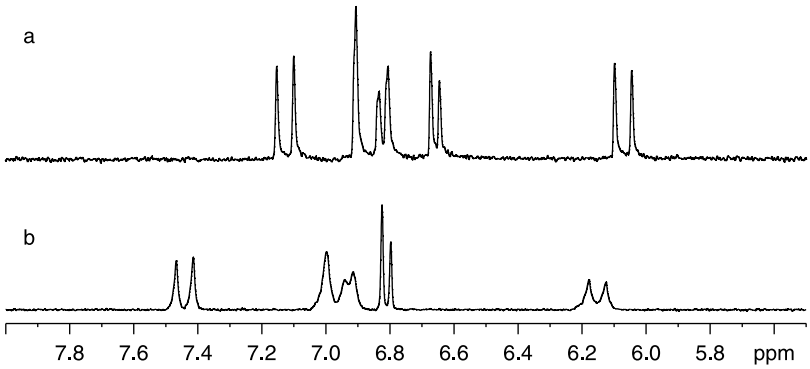


FIGURE 4 1H NMR spectra of: **a**. CA; **b**. CA complexed in β-CD.

the form of $HCAF^{2-}$ is predominant gradually, and pH > 12.5, the form of CAF^{3-} is predominant [25]. Implying that the carboxyl charged group is out of the face of β-CD, and it is proved further in the experiment below.

Nuclear Magnetic Resonance (1H-NMR)

The formation of inclusion complexes can be proved from the changes of chemical shifts in 1H NMR spectra. Figure 4 illustrates that most of the hydrogen atoms of CA are influenced owing to the presence of β-CD. Table II lists the detailed variation of proton chemical shifts of CA before and after forming inclusion complexes with β-CD, CA has five types of hydrogen: a-H, b-H, c-H, d-H and e-H.

Figure 5 illustrates that when the molar ratio between CA and β-CD is 1:1, there is always a turning point which leads to a consistent conclusion with the aforementioned fluorescence method.

Figure 6 illustrates that most of the hydrogen atoms of CD are influenced owing to the presence of CA. Table III lists the detailed variation of proton chemical shifts of β-CD before and after forming inclusion complexes with CA, 3-H, 5-H and 6-H experience greater up field shifts because of interaction caused by the CA penetration into β-CD cavity. However, 1-H, 2-H, and 4-H outside of β-CD cavities experience minor changes in chemical shift.

Nuclear Magnetic Resonance (2D-NMR)

Figure 7 shows a contour plot of a section of the ROESY spectrum of CA and β-CD complexes. The dimensional spectrum shows several intermolecular cross-peaks between d-H, e-H of CA and H-5, H-6 of β-CD, with additional relation between a-H, b-H, c-H of CA and H-3, H-5 of β-CD, but no sign between d-H, e-H of CA and H-3 of β-CD.

This clearly suggests that the vinyl piece of CA is on secondary face of the β-CD, and the carboxyl group is out of the secondary face of β-CD, and phenyl ring of the guest molecular is on the primary face or at the edge of it, as shown in Fig. 8.

So it can be concluded that CA is deeply included into the inner cavity of β-CD to form a supermolecular system, where van der Waals force and pole–pole interaction are the main forces.

TABLE II Variation of 1-H chemical shifts of CA before and after inclusion in β-CD

	a	b	c	d	e
Caffic acid	6.910	6.654	6.821	7.122	6.069
Complex	7.005	6.814	6.934	7.443	6.153
Δδ	−0.095	−0.16	−0.113	−0.331	-0.084

The Related Inclusion Mechanism

The inclusion interactions are based on the cooperation of several weak forces working between receptor (CDs) and substrates (caffeic acid), including dipole–dipole, electrostatic, van der Waals, hydrogen bonding, and hydrophobic interactions [15,16]. The regular cyclodextrins are not charged and the major inclusion interactions are hydrophobic interactions between the guest and cyclodextrin cavity and hydrogen bonding of the guest to —OH groups or other introduced groups on the CD ring.

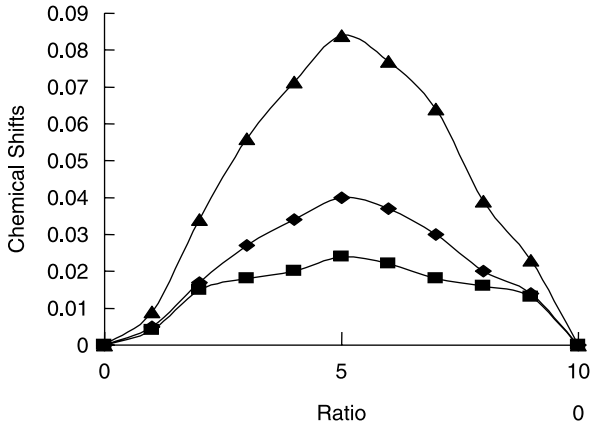


FIGURE 5 Chemical shifts changes of β-CD in the case of CA. (◆) H-3; (▲) H-5; (■) H-6.

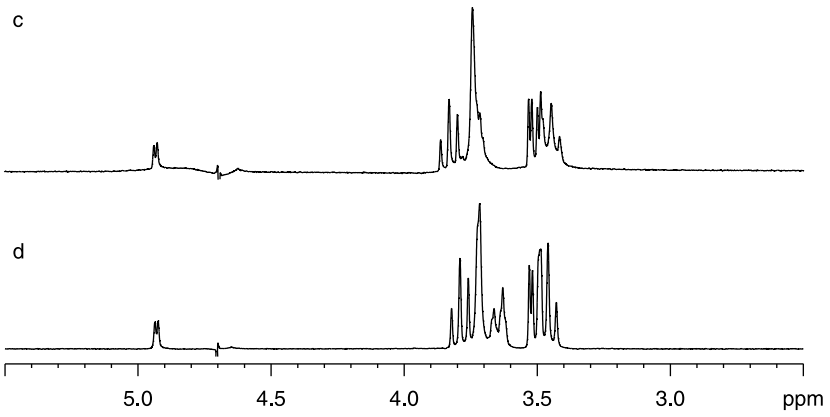


FIGURE 6 1H NMR spectra of: **c**. β-CD without CA; **d**. β-CD with CA.

EXPERIMENTAL

Apparatus

The absorption and fluorescence measurements were performed with a UV-265 spectrophotomter (Shimadzu, Japan), and a RF-540 spectrofluorometer (Shimadzu, Japan), respectively. Excitation and emission bandwidths were both set at 10 nm. The pH meter (pHs-2 meter) was made in the 2nd instrument factory of Shanghai in China. nuclear magnetic resonance (DRX300, Bruker, Switzerland). All experiments were carried out at 20 ± 1°C.

Reagents

The stock solution of 1.0×10^{-4} mol/L caffeic acid, caffeic acid was isolated based on the literature method [23]. β-CD (95%, Yunnan Gourmet Factory) was recrystallized twice from doubly distilled water before use. Phosphate buffer solution was used to control the pH-value of the media. All other reagents were of analytical-reagent without further purification. Doubly distilled water was used throughout.

Procedure

A 1 ml aliquot of the stock solution (1.0×10^{-4} mol/L) of caffeic acid was transferred into a 10 ml volumetric flask, then appropriate amount of 1.0×10^{-3} mol/L β-CD solution was added. The pH was controlled by 0.5 mol/L phosphate buffer solution. The mixed solution was diluted to final volume with distilled water and shaken thoroughly, following equilibration for 30 minutes at 20 ± 1°C. All the measurements of absorption, fluorescence were made against the blank solution treated in the same way without CDs by using 1.0 cm quartz cell. For NMR data, all the concentrations of caffeic acid and CDs solutions are 1.0 mM, and caffeic acid solution is diluted with β-CDs solutions, respectively at the volume ratio of 1:1. ^{1}H NMR and 2D NMR of caffeic acid CD solution are performed. To get further evidence, changes of β-CD in the case of caffeic acid has also been performed.

TABLE III Chemical shifts changes of β-CD in the presence of CA

	H-3	H-6	H-5
β-CD	3.831	3.746	3.714
Complex	3.791	3.722	3.630
Δδ	−0.04	−0.024	−0.084

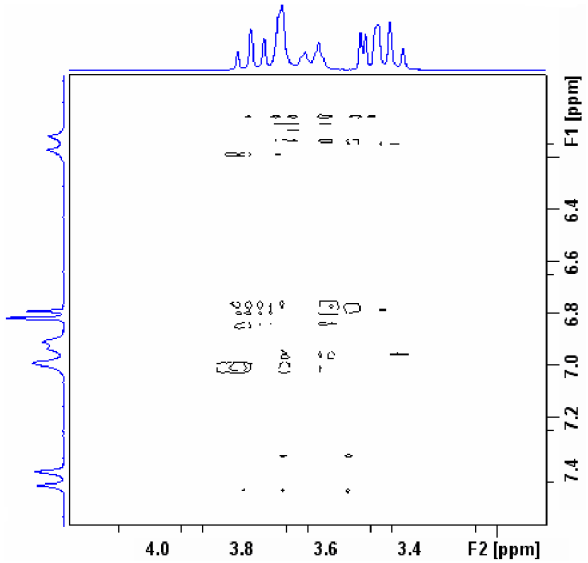


FIGURE 7 2-D NMR spectra of CA with β-CD.

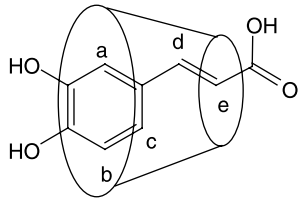


FIGURE 8 Proposed model for CA with β-CD.

CONCLUSION

Absorption and fluorescence investigation has demonstrated the inclusion complexation interaction between caffeic acid and the β-CD. In summary, major factors affecting molecular recognition is size matching between CD and guest and the hydrophobicity of the guest molecule. β-CD were suitable for including of $HCAF^{2-}$, which is due to the effect of hydrophobicity and hydrogen bonding.

Acknowledgements

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References

[1] Lehn, J. M. *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 1304.
[2] Hamilton, A. D. Molecular Recognition (Tetrahedron Symposia No. 56). *Tetrahedron* **1995**, *51*, 343.
[3] Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry*; Springer: New York, 1997.
[4] Shuang, S. M.; Yang, Y.; Pan, J. H. *Anal. Chim. Acta.* **2002**, *458*, 307.
[5] Szejtli, J. *Cyclodextrins and their Inclusion Complexes*; Akademiai Kiado: Budapest, Hungary, 1982.
[6] Scyrinski, S.; Cline Love, L. J. *Anal. Chem.* **1984**, *46*, 331.
[7] Ndou, T. T.; Zung, J. B.; Greene, K. L.; Live, D. H.; Warner, I. M. *J. Am. Chem. Soc.* **1991**, *113*, 1572.
[8] Munoz de la Pena, A.; Duyan-Meras, I.; Salinas, F.; Warner, I. M.; Ndou, T. T. *Anal. Chim. Acta.* **1991**, *370*, 351.
[9] Escandar, G. M.; Munoz de la Pena, A. *Anal. Chim. Acta.* **1998**, *370*, 199.
[10] Shuang, S. M.; Guo, S. Y.; Li, L.; Cai, M. Y. *Anal. Lett.* **1998**, *31*, 1357.
[11] Liu, Y.; Li, B.; Zhang, Y. *Chin. Sci. Bull. (in China)* **1995**, *40*, 1858.
[12] Ueno, A.; Suzuki, K.; Osa, T. *J. Am. Chem. Soc. (in China)* **1989**, *111*, 6391.
[13] Breslow, R.; Chung, S. *J. Am. Chem. Soc.* **1990**, *112*, 9659.
[14] Zhang, B.; Breslow, R. *J. Am. Chem. Soc.* **1991**, *115*, 9353.
[15] Inoue, Y.; Hakuhsi, T.; Liu, Y.; Tong, L. J.; Shen, B. J.; Jin, D. S. *J. Am. Chem. Soc.* **1993**, *115*, 475.
[16] Inoue, Y.; Liu, Y.; Tong, L. H.; Shen, B. J.; Jin, D. S. *J. Am. Chem. Soc.* **1993**, *115*, 10637.
[17] Chao, J. B.; Chen, L.; Xu, H.; Meng, D. P. *Spectrochim. Acta Part A* **2002**, *58*, 2809.
[18] Chao, J. B.; Li, J. S.; Meng, D. P.; Huang, S. P. *Spectrochim. Acta Part A* **2003**, *59*, 705.
[19] Chao, J. B.; Tong, H. B.; Huang, S. P.; Liu, D. S. *Spectrochim. Acta Part A* **2004**, *60*, 161.
[20] Chao, J. B.; Meng, D. P.; Li, J. S.; Xiu, H.; Huang, S. P. *Spectrochim. Acta Part A* **2004**, *60*, 729.
[21] Chao, J. B.; Tong, H. B.; Liu, D. S.; Huang, S.P. *Spectrochim. Acta Part A* **2006**, *64*, 166.
[22] Kocsis, I.; Hagymási, K. *Acta Biol. Szeged.* **2003**, *47*, 143.
[23] Zhang, L. W.; Yuan, C. X.; Yang, P. *Spectrosc. Spec. Anal.* **2003**, *23*, 127.
[24] Natarajan, K.; Singh, S.; Burke, T. R.; Grunbergert, D.; Aggarwal, B. B. *Proc. Natl. Acad. Sci.* **1996**, *93*, 9090.
[25] Giacomelli, C.; Ckless, K.; Galato, D. *J. Braz. Chem. Soc.* **2002**, *13*, 332.