Spectrochimica Acta Part A 85 (2012) 298–302

Contents lists available at SciVerse ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Inclusion complexes of phosphorylated daidzein derivatives with β-cyclodextrin: Preparation and inclusion behavior study

Yongmei Xiao*, Liangru Yang, Pu Mao, Jinwei Yuan, Yuxia Deng, Lingbo Qu

College of Chemistry & Chemical Engineering, Henan University of Technology, Zhengzhou 450001, People's Republic of China

ARTICLE INFO

Article history:
Received 11 August 2011
Received in revised form
29 September 2011
Accepted 6 October 2011

Keywords:
Phosphorylated daidzein
β-Cyclodextrin
Inclusion complex

ABSTRACT

In the present work the feasibility of β-cyclodextrin in complexation was explored, as a tool for improving the solubility and biological ability of daidzein derivatives. A series of phosphorylated daidzein derivatives featuring different chain lengths were synthesized through a modified Atherton–Todd reaction and their inclusion complexes with βCD were prepared by coprecipitation method. The inclusion complexation behavior was studied by fluorescence, UV, FT-IR, MS and ^{1}H NMR. The results showed that only phosphorylated daidzein derivative carrying small substituent group ($(C_2H_5O)_2P{=}O$) entered the cavity of βCD and formed 1:1 inclusion complex. The formation constant was 175 $(mol/L)^{-1}$.

1. Introduction

Cyclodextrins (CDs) are truncated, cone-shaped cyclic oligosaccharides, composed of 6 (in α-), 7 (in β-), 8 (in γ-) or more α-1, 4 linked glucoses and the most abundant natural cyclodextrin is β-cyclodextrin (βCD). Due to its special molecular structure/hydrophobic internal cavity and hydrophilic external surface, βCD can form inclusion complexes with various guest molecules featuring suitable polarity and dimension [1]. Moreover, formation of βCD inclusion complex generates aqueous drug solutions without the use of organic co-solvents, surfactants, or lipids, as formulation adjuncts [2]. Recently, βCD inclusion complexes have been used in pharmaceutical industry to increase the solubility, stability and bioavailability of the guest molecules [3]. Many poorly soluble drugs have been converted to βCD inclusions and their physicochemical properties could be significantly improved [4,5].

Due to the impressive biological activities of flavonoids, they have attracted extensive interests, while research on the interaction between flavonoids and βCD are quite rare [6,7]. Daidzein (7,4′-dihydroxyisoflavone) is one of the most important isoflavonoids, which has been pharmacologically shown featuring the bioactivities of anti-oxidant [8], anti-allergic, anti-inflammatory [9], anti-microbial [10], anti-cancer [11–13], and playing important role in female hormone [14]. However, the bioavailability of daidzein by oral absorption is quite low because of its poor solubility, which limits its clinical application. Introducing of phosphate ester to daidzein could change its physicochemical properties and increase the biological ability through the formation of non-covalent complexes with the proteins, while the change of the solubility is quite limited [15,16]. To increase the solubility and biological ability of daidzein, we synthesized a series of phosphorylated daidzein derivatives and studied their inclusion with βCD using fluorescence, IR, MS and NMR techniques.

2. Experimental

2.1. Materials

βCD (98%) was purchased from Kermel (China) and recrystallized from doubly distilled water before using. Daidzein was purchased from Shanxi Huike Botanical Development Co. (China). Dialkyl phosphates were synthesized in our lab. The water used was doubly distilled, deionized and filtered through 0.22 μm Millipore filters. All other materials were of analytical reagent grade purity.

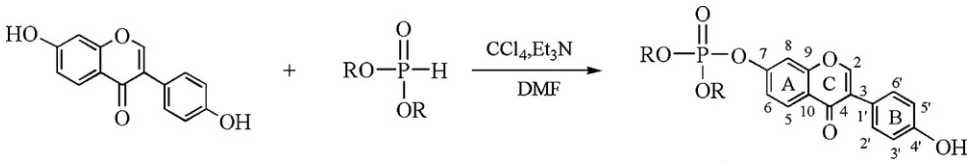
2.2. Apparatus

UV–vis absorption spectra were recorded on a Spekol 2000 UV–vis double beam spectrophotometer using 1 cm quartz cell, with a wavelength ranging between 200 and 400 nm. The fluorescence spectra were recorded on a Cary Eclipse fluorescence spectrometer using 1 cm quartz cell, 5 nm slit width, excitation at 257 nm and fluorescence emission obtained at 417 nm. Each measurement was carried out in triplicate at 23 °C.

IR spectra were recorded using an IR-200 Fourier transform spectrometer in KBr pellets. ^{1}H NMR experiments were carried out

* Corresponding author. Tel.: +86 371 67756712; fax: +86 371 67756718.
E-mail address: henangongda@yahoo.com (Y. Xiao).

1386-1425/$ – see front matter.
doi:10.1016/j.saa.2011.10.014



1 2 3

R = -CH_2CH_3 (**a**), -$CH_2CH_2CH_3$ (**b**), -$CH(CH_3)_2$ (**c**), -$CH_2CH_2CH_2CH_3$ (**d**), -$CH(CH_3)CH_2CH_3$ (**e**), -$CH_2CH_2CH_2CH_2CH_3$ (**f**), -$CH(CH_3)CH_2CH_2CH_3$ (**g**)

Scheme 1. Synthesis of phosphorylated daidzein derivatives.

using a Bruker Avance 400 MHz instrument in DMSO-d_6-D_2O (1:1, v/v) mixture, using TMS as internal standard. Mass spectra were analyzed on a Bruker Esquire 3000 mass spectrometer fitted with an ion spray source working in positive ion mode, using methanol as solvent.

Sonication was performed using a Jiangsu Kunshan KQ-200VDE ultrasonic cleaner.

2.3. Procedure

2.3.1. Phase-solubility measures

Phase-solubility measurements were carried out according to the method of Higuchi and Connors [17]. A fixed amount of phosphorylated daidzein derivative, exceeding its solubility, was added to 10 mL aqueous solutions of βCD with concentrations of 0, 1.0×10^{-3}, 2.0×10^{-3}, 2.5×10^{-3}, 3.5×10^{-3}, 4.0×10^{-3} mol/L in capped tubes. The mixture was sonicated in ultrasonic cleaner for 20 min and then magnetically stirred for 3 days to reach equilibrium in a thermostated bath at 23 °C in darkness. The suspension was filtered through Sartorius Minisart®-SRP 15 PTFE 0.45 μm filters, respectively. 1 mL filtrate was withdrawn from each vial and then measured by UV–vis absorption spectroscopy (260 nm). Each experiment was carried out in triplicate.

2.3.2. Fluorescence spectra measurements

In the fluorescence spectra measurements of compound **3a**, different volumes of βCD (0, 1, 1.5, 2.5, 3, 4 mL) with concentration of 0.01 mol/L was added to 1 mL compound **3a** solution (1×10^{-3} mol/L) and diluted to 10 mL with distilled water. In the fluorescence spectra measurements of compound **3b**, different volumes of βCD (0, 1, 2, 2.5, 3, 4, 5 mL) with concentration of 0.01 mol/L was added to 1 mL compound **3b** solution (1×10^{-3} mol/L) and diluted to 10 mL with distilled water.

2.4. Preparation of samples

2.4.1. Synthesis of phosphorylated daidzein derivatives *3a–3g*

Synthesis of phosphorylated daidzein derivatives was shown in Scheme 1. Daidzein (0.6 g, 2.36 mmol) was added to a solution of NEt_3 (0.8 mL) in DMF (6 mL) in a three-neck flask and the mixture was stirred until all the daidzein was dissolved. The flask was put into an ice-water bath and then a solution of dialkyl phosphate (2.36 mmol) in CCl_4 (6 mL) was added dropwise with vigorous stirring. After addition, the ice-water bath was removed and the reaction proceeded at room temperature for 24 h. The mixture was filtered and the filtrate was evaporated *in vacuo*. The residue was then dispersed in 30 mL water, filtered and further purified by column chromatography using chloroform–methanol (30:1, v/v) as eluent.

2.4.2. Preparation of solid inclusion complexes

The inclusion complexes of compounds **3a–3g** with βCD were prepared by the coprecipitation method. Molar equivalent compounds **3a–3g** in distilled water and βCD in ethanol were combined and stirred at 25 °C for 1 h. The mixture was concentrated and cooled to 4 °C for 24 h. Collection of the precipitate gave the inclusion complexes.

2.4.3. Preparation of physical mixtures

Molar equivalent compounds **3a–3g** and βCD were combined and ground in an agate mortar until the mixture was homogeneous.

3. Results and discussion

3.1. Fluorescence study

As a highly sensitive and selective technology, fluorescence method has been extensively used to study the interaction between host and guest molecules [18,19]. We synthesized a series of phosphorylated daidzein derivatives **3a–3g** through a modified Atherton–Todd reaction, and studied their interaction with βCD using fluorescence method (Figs. 1 and 2).

Fig. 1 showed that the fluorescence intensity of complex **3a** enhanced with concentration increasing of βCD. These data suggested that a stable inclusion complex was formed between βCD and compound **3a**. Since the CD cavity provided an apolar environment for compound **3a** and confined the motion of the molecule, protecting the excited states of **3a** molecule from nonradiative and quenching processes that normally readily occurred in bulk aqueous solution [20], the quantum yield of the excited fluorophore of compound **3a** was increased in combination with βCD [21,22].

Fig. 2 showed that the fluorescence intensity of complex **3b** decreased with concentration increasing of βCD. The phenomena maybe explained that the increased bulkiness of the phosphate group in compound **3b** made it difficult to enter the cavity of βCD to form inclusion complex. Instead, there may exist the formation

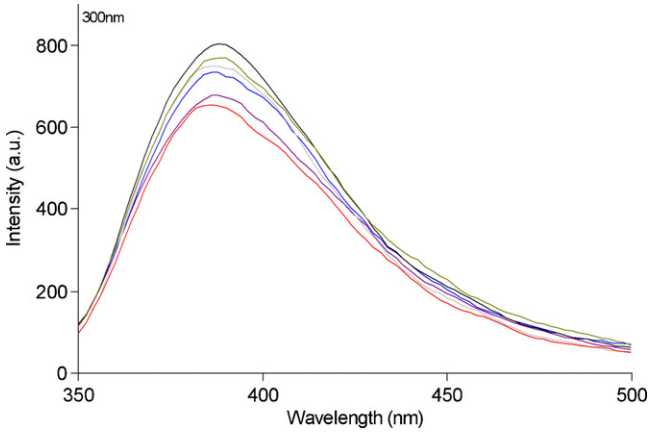


Fig. 1. The fluorescence spectra of compound **3a** combined with different concentrations of βCD, 0, 1×10^{-3}, 1.5×10^{-3}, 2.5×10^{-3}, 3×10^{-3}, 4×10^{-3} mol/L (from bottom to top).

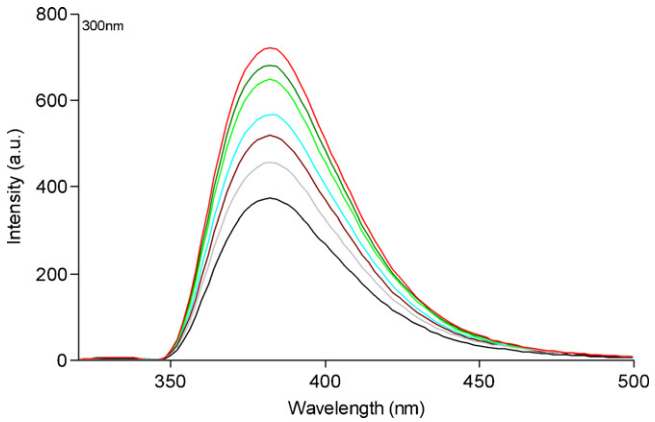


Fig. 2. The fluorescence spectra of compound **3b** combined with different concentrations of βCD, 0, 1×10^{-3}, 2×10^{-3}, 2.5×10^{-3}, 3×10^{-3}, 4×10^{-3}, 5×10^{-3} mol/L (from top to bottom).

of complexes between βCD, solvent molecules and compound **3b**, which led to the enhanced quenching processes with the increasing of βCD. The fluorescence spectra of complexes **3c**–**3g** combined with different concentrations of βCD showed the same trend with those of complex **3b**.

The formation constant can be obtained from fluorescence data in Fig. 1 by the modified Benesi–Hildebrand equation [23,24].

$$\frac{1}{F - F_0} = \frac{1}{K\,[F_\infty - F_0]\,[\beta\mathrm{CD}]^n} + \frac{1}{F_\infty - F_0}$$

Here F is the observed fluorescence intensity of compound **3a** solution at each βCD concentration; F_0 presents fluorescence intensity of compound **3a** solution in the absence of βCD. K is formation constant of the complex; n represents the stoichiometry of the complex formed. The plot curve of $1/(F - F_0)$ against $1/[\beta\mathrm{CD}]^2$ deviated from linearity (data not shown), while the plot curve of $1/(F - F_0)$ against $1/[\beta\mathrm{CD}]$ (Fig. 3) exhibited excellent linearity, which indicated that compound **3a** formed a 1:1 stoichiometry inclusion complex with βCD. The calculated formation constant K was 1.75×10^2 $(\mathrm{mol/L})^{-1}$.

3.2. Solubility studies

It has been extensively reported in the literatures that βCD molecules are able to increase the guest molecule solubility [25,26].

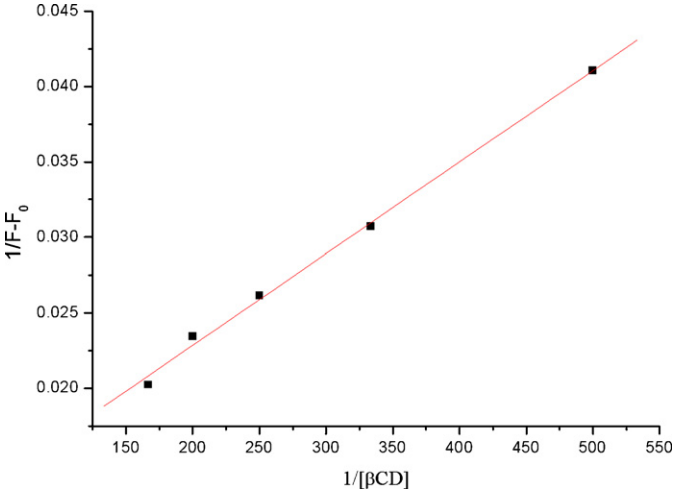


Fig. 3. Benesi–Hildebrand plots for 1:1 inclusion of compound **3a** with βCD.

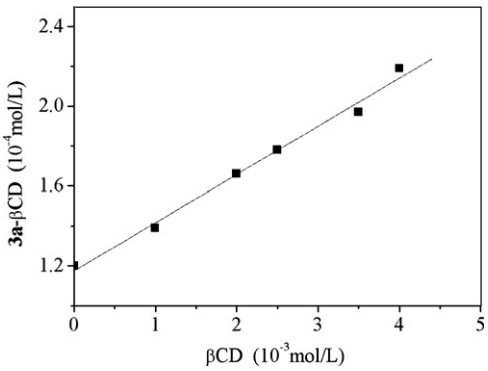


Fig. 4. Phase-solubility diagrams of compound **3a** in aqueous solutions of βCD.

Fig. 4 showed the phase solubility diagrams of inclusion complex **3a**-βCD. It could be observed that the solubility of compound **3a** increased linearly as the concentrations of βCD increased. According to the Higuchi and Connors classification, the diagrams obtained were of A_L type, where the stoichiometry of the inclusion complex was assumed to be 1:1, in consistent with the result from fluorescence study. Compound **3a** had a solubility of 1.19×10^{-3} mol/L in water, while the solubility of **3a** in a 1:1 inclusion complex was increased to 2.2×10^{-3} mol/L. This phenomenon could be attributed to the interaction between host/guest molecules, during the supramolecular complex formation.

3.3. FT-IR spectra studies

The FT-IR spectroscopic analysis confirmed the interaction and inclusion complex formation between compound **3a** and βCD [27,28]. Fig. 5 showed the FT-IR spectra of βCD (a), compound **3a** (b), **3a**/βCD physical mixture (c) and inclusion complex (d). Comparison of these spectra indicated the significant change in the characteristic bands of the pure substances. In the inclusion complex, absorbances at 1650–1700 cm^{-1} and 1250 cm^{-1}, characteristic bands attributed to the carbonyl and P=O groups, disappeared respectively. Meanwhile, the characteristic bands of daidzein at

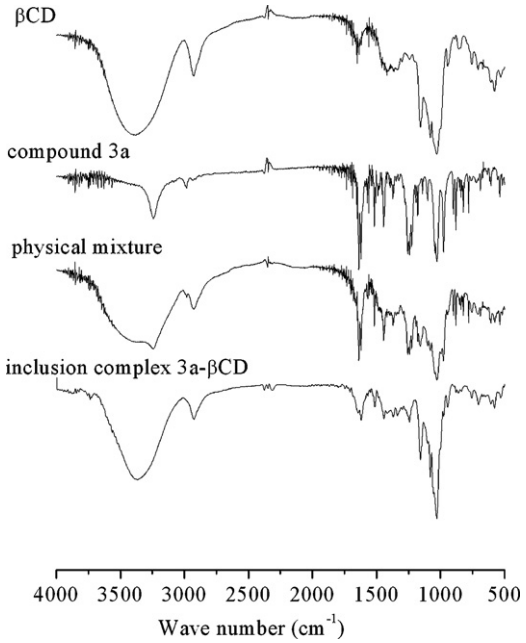


Fig. 5. FT-IR spectra of (a) βCD, (b) compound **3a**, (c) physical mixture of **3a** and βCD and (d) inclusion complex **3a**-βCD.

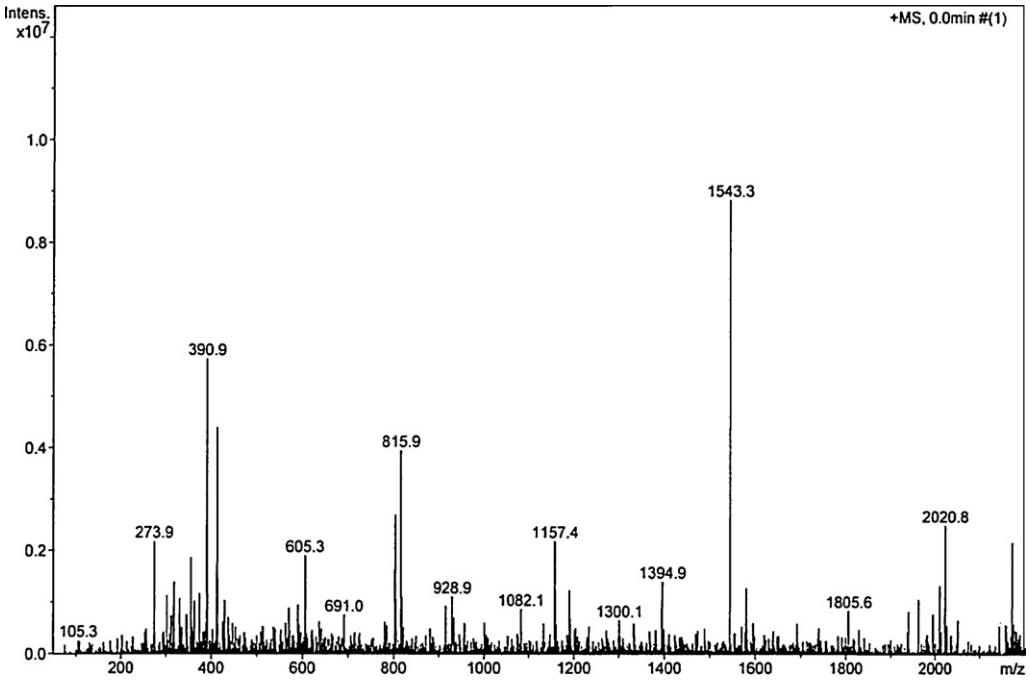


Fig. 6. Mass spectrum of **3a**-βCD.

1480–1600 cm^{-1} also disappeared in the FT-IR spectrum of the inclusion complex. Additionally, in the inclusion complex, the intensity and shape of these bands changed obviously compared to those of compound **3a** and physical mixture. The dramatic differences indicated that the bending and vibrating of the structure of compound **3a** were restricted, resulting from the interaction of compound **3a** with the cavity of βCD.

3.4. MS studies

Mass method is already known as a powerful tool to detect the formation of complex. Fig. 6 showed the mass spectrum of the inclusion complex **3a**-βCD. Ion peaks at *m*/*z* 1543.3 and 390.9 corresponded to [**3a**-βCD+H_2O+H]$^+$ and compound **3a**, respectively.

3.5. ^1H NMR studies

Further support for the inclusion complex formation can be obtained using ^{1}H NMR spectroscopy, which has proved to be useful in the study of βCD inclusion complex [29]. Here the formation of inclusion complex could be proved from the comparison of the chemical shifts of **3a** before and after interaction with βCD, which was defined as $\Delta\delta$. The broadening of the proton signals of **3a** in the presence of βCD suggested that **3a** entered the cavity of βCD and the motions of these protons were restricted [30]. Table 1 listed the characteristic chemical shifts of **3a**, **3a**-βCD and $\Delta\delta$ in ^{1}H NMR spectra. Downfield shifts were observed for H-2, H-6 and H-8 of the aromatic protons of the A- and C-rings, indicating that A- and C-rings probably entered the inner cavity of βCD.

Table 1
Comparison of the characteristic ^{1}H NMR of **3a** and **3a**-βCD.

H-number	**3a** (δ, ppm)	**3a**-βCD (δ, ppm)	$\Delta\delta$
H-2	8.452	8.455	0.003
H-5	8.183	8.177	−0.006
H-6	7.372	7.387	0.015
H-8	7.518	7.519	0.001
H-2′,6′	7.415	7.403	−0.012
H-3′,5′	6.845	6.825	−0.020

4. Conclusions

In this study, we synthesized a series of phosphorylated daidzein derivatives through a modified Atherton–Todd reaction, and studied their interaction with βCD. The data obtained from fluorescence, UV–vis, FT-IR, MS and NMR studies showed that phosphorylated daidzein derivatives carrying small substituent (**3a**) entered the cavity of βCD and formed 1:1 inclusion complex with the formation constant being 175 $(mol/L)^{-1}$, while phosphorylated daidzein derivatives carrying bulky substituent (**3b**–**3g**) failed to form inclusion complexes with βCD.

Acknowledgements

We thank the Science and Technology hall of Henan Province (Nos. 072300420070 and 82102330014), China, for financial support to this work.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.saa.2011.10.014.

References

[1] A. MunozPen, T.T. Ndou, J.B. Zung, K.L. Greene, D.H. Live, I.M. Warner, J. Am. Chem. Soc. 113 (1991) 1572–1577.
[2] N.E. Polyakov, T.V. Leshina, T.A. Konovalova, E.O. Hand, L.D. Kispert, Free Radic. Biol. Med. 36 (2004) 872–880.
[3] F. Hirayama, K. Uekama, Adv. Drug Deliv. Rev. 36 (1999) 125–141.
[4] K. Uekama, F. Hirayama, T. Irie, Chem. Rev. 98 (1998) 2045–2076.
[5] M.E. Cortés, R.D. Sinisterra, M.J. Avila-Campos, N. Tortamano, R.G. Rocha, J. Incl. Phenom. Macrocyclic Chem. 40 (2001) 297–302.
[6] M.L. Calabro, S. Tommasini, P. Donato, R. Stancanelli, D. Raneri, S. Catania, C. Costa, V. Villari, P. Ficarra, R. Ficarra, J. Pharm. Biomed. Anal. 36 (2005) 1019–1027.
[7] S. Tommasini, M.L. Calabro, R. Stancanelli, P. Donato, C. Costa, S. Catania, V. Villari, P. Ficarra, R. Ficarra, J. Pharm. Biomed. Anal. 39 (2005) 572–580.

[8] M.J. Tlkkanen, K. Wähälä, S. Ojala, V. Vihtna, H. Adletcteutr, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 3106–3110.
[9] S.V. Bhat, B.A. Nagasampagi, M. Sivakumar, Chemistry of Natural Products, Springer, 2005, p. 585.
[10] T.P. Cushnie, A.J. Lamb, Int. J. Antimicrob. Agents 38 (2011) 99–107.
[11] Y.K. Jing, R. Han, Chin. J. Pharmacol. Toxicol. 6 (1992) 278–280.
[12] N. Sarhyyamoonhy, T.T.Y. Wang, Eur. J. Cancer 33 (1997) 2384–2389.
[13] Y. Jing, K. Nakaya, R. Han, Anticancer Res. 13 (1993) 1049–1054.
[14] R.J. Miksicek, Mol. Pharm. 44 (1993) 37–43.
[15] X.L. Chen, X.N. Shi, L.B. Qu, J.W. Yuan, J.S. Lu, Y.F. Zhao, Chin. J. Chem. 25 (2007) 1008–1013.
[16] X.L. Chen, X.N. Shi, L.B. Qu, J.W. Yuan, J.S. Lu, Y.F. Zhao, Chin. J. Magn. Reson. 24 (2007) 85–90.
[17] T. Higuchi, A.K. Connors, in: C.N. Reilley (Ed.), Advances in Analytical Chemistry and Instrumentation, Interscience, New York, 1965, pp. 117–212.
[18] L.A. Blyshak, I.M. Warner, G. Patonay, Anal. Chim. Acta 232 (1990) 239–243.
[19] A. Örstan, J.B.A. Ross, J. Phys. Chem. 91 (1987) 2739–2745.
[20] T.T. Ndou, I.M. Warner, Chem. Rev. 91 (1991) 493–507.
[21] J. Li, M. Zhang, J. Chao, S. Shuang, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 73 (2009) 752–756.
[22] C. Jullian, C. Cifuentes, M. Alfaro, S. Miranda, G. Barriga, C. Olea-Azar, Bioorg. Med. Chem. 18 (2010) 5025–5031.
[23] F.V. Bright, T.L. Keiming, L.B. McGown, Anal. Chim. Acta 175 (1985) 189–201.
[24] G.C. Ctana, F.V. Bright, Anal. Chem. 61 (1989) 905–909.
[25] J. Szejtli, Chem. Rev. 98 (1998) 1743–1754.
[26] T. Loftsson, D. Duchêne, Int. J. Pharm. 329 (2007) 1–11.
[27] D. Bonenfant, P. Niquette, M. Mimeault, A. Furtos-Matei, R. Hausler, Water Res. 43 (2009) 3575–3581.
[28] B. Cappello, C.D. Maio, M. Iervolino, J. Incl. Phenom. Macrocyclic Chem. 43 (2002) 251–257.
[29] X.H. Wen, F. Tan, Z.J. Jing, Z.Y. Liu, J. Pharm. Biomed. Anal. 34 (2004) 517–523.
[30] J. Chao, J.S. Li, D.P. Meng, S. Huang, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 59 (2003) 705–711.