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Study on the Supramolecular Interaction of Curcumin and β-cyclodextrin by Spectrophotometry and Its Analytical Application

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The supramolecular interaction of curcumin and β-cyclodextrin (β-CD) has been studied by spectrophotometry. The mechanism of the inclusion was studied and discussed based on the variations of pK_a, absorption intensity, and infrared spectrograms. The results show that β-CD reacts with curcumin to form a 2:1 host−guest complex with an apparent formation constant of 5.53×10^5 mol^{-2} L^2. Based on the enhancement of the absorbance of curcumin produced through complex formation, a spectrophotometric method for the determination of curcumin in bulk aqueous solution in the presence of β-CD was developed. The linear relationship between the absorbance and curcumin concentration was obtained in the range of 0−15 μg/mL, with a correlation coefficient (r) of 0.9991. The detection limit was 0.076 μg/mL. The proposed method was used to determine the curcumin in curry and mustard with satisfactory results.

KEYWORDS: ***Curcumin*; β-cyclodextrin; spectrophotometry; supramolecular complex**

INTRODUCTION

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], the main constituent of the rhizomes of the plant *Curcuma longa*, is a common ingredient used in spices, cosmetics, and traditional Chinese medicine. It has been reported that curcumin has many pharmacological functions, such as antioxygenation, antibiosis, and antitumor (*1*, *2*). As a powerful antioxidant in both enzymic and nonenzymic systems, the antioxidant mechanism of curcumin has been extensively investigated (*3*). Methods such as thin-layer chromatography (*4*−*6*), high performance liquid chromatography (*7*−*11*), electrochemical method (*12*, *13*), spectrofluorometry (*14*−*16*), and UV−vis spectrophotometry (*11*, *17*−*21*) have been employed to determine curcumin in a variety of matrixes such as *Curcuma longa*, foodstuffs, and biological materials. Curcumin has extensive absorption around 420 nm in organic solvent, but its absorbance strongly decreased in aqueous solution. This fact precludes the determination of trace amount of curcumin in aqueous solution. Cyclodextrins, the cyclic oligosaccharides consisting of six or more D-(+)-glucopyranose units, are well-known to have the property of forming inclusion complexes with guest molecules which possess suitable polarity and dimension. This ability has been widely used in the studies of general inclusion phenomena and enzyme−substrate interactions, applied in food, cosmetics, and pharmaceutical industries, and has also been used for analytical purpose (*22*). In the present work, a spectrophotometric study of the supramolecular interaction between curcumin and β-cyclodextrin is carried out. All the data on the complexation of curcumin refer to the fact that curcumin is a good complex forming guest in terms of its chemical structure, polarity, and molecular dimensions. The stoichiometry of the complex and the apparent formation constant have been estimated. The thermodynamic parameters ($\Delta H°$, $\Delta S°$, $\Delta G°$) for the formation of complexes were obtained from the van't Hoff equation. Based on the significant enhancement in the absorbance of curcumin produced through complexation with β-CD, a spectrophometric method having the improved sensitivity for the determination of curcumin in the bulk aqueous solution was developed.

MATERIALS AND METHODS

Apparatus. All absorbance measurements were carried out on a shimadzu UV-265 spectrophotometer equipped with 1.0 cm quartz cells. The sample chamber accommodated a thermostated cuvette holder, controlled to 20 ± 1 °C via a constant temperature circulator. Infrared spectrograms were obtained from a PE-983G IR-spectrophotometer (Perkin-Elmer). The fluorescence measurements were made on a Perkin-Elmer LS-5 spectrofluorimeter, equipped with a xenon lamp, 1.0 cm quartz cells. All pH measurements were made with a pHs-3C digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass-calomel electrode.

Reagents. Curcumin (purchased from China Medicine (Group) Shanghai Chemical Reagent Corporation) was of analytical reagent grade. Its stock solution (1.0×10^{-2} mol/L) was prepared in acetonitrile and stored in the dark in amber bottles

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at 4 °C. β-Cyclodextrin (obtained from China Medicine (Group) Shanghai Chemical Reagent Corporation) was purified by twice recrystallization in double-distilled water, followed by vacuum-drying at ca. 60 °C for 12 h and used with a concentration of 1.0×10^{-2} mol/L aqueous solution. Other chemicals used were of analytical reagent grade. Doubly distilled water was used throughout.

Influence of pH. The changes in the absorbance of both curcumin and curcumin−β-CD complex as a function of pH were carried out. The initial concentrations of curcumin and β-CD in the experimental solutions were 1.0×10^{-5} mol/L and 6.0×10^{-3} mol/L, respectively. The ionic strength and temperature were maintained at 0.10 mol/L and 20 ± 1 °C, respectively. The absorbance of curcumin (or curcumin−β-CD complex) was measured at 470 nm against a reagent blank prepared with the same reagent concentration but without curcumin. The measurements were performed in duplicate.

Determination of the Apparent Formation Constant. The curcumin concentration was held constant at 1.0×10^{-5} mol/L while the β-CD concentration was varied. The pH of the system was adjusted to 2.4 by the addition of 2 mL of tri-sodium citrate-HCl buffer solution (pH = 2.4). The mixed solution was diluted to 10 mL with water, shaken thoroughly and allowed to equilibrate at 20 ± 1 °C for 15 min. The absorbance at 431 nm was read against a reagent blank which was prepared with the same reagent concentration but without curcumin. All measurements were made in duplicate. The apparent formation constant of the inclusion complex was obtained from the double reciprocal plot.

Spectrophotometric Determination of Curcumin. An aliquot of the curcumin stock solution containing $(0.0-4.0) \times 10^{-7}$ mol of curcumin, 7.5 mL of 1.0×10^{-2} mol/L β-CD, and 2.0 mL of trisodium citrate-HCl buffer solution (pH = 2.4) was sequentially added into a 10-mL colorimetric tube. The mixture was diluted to the mark with water, shaken thoroughly, and equilibrated at 20 ± 1 °C for 15 min. The absorbance of the solution was measured at 431 nm against a reagent blank which was prepared with the same reagent concentration but without curcumin. This procedure was replicated in order to obtain three or more absorbance values for each of the curcumin concentrations studied.

The procedures for the spectrophotometrtic determination of curcumin in the absence of β-CD were similar to those described above except that β-CD was not added.

Preparation of CD−Curcumin Inclusion Complex. The β-CD−curcumin inclusion complex was prepared for the purpose of an infrared spectrogram. The calculated amount of curcumin to be complexed was dissolved in a minimum volume of methanol at 60 °C and then added dropwise into the 2.5 equiv. of β-CD aqueous solution at 60 °C with continuous, intensive stirring. The mixture solution was refluxed with vigorous agitation at 70 °C for about 4 h. Then the reflux equipment was taken down and the solution was stirred for an additional hour at 70 °C to remove methanol. Then the system was cooled to room temperature. After stirring for 8 h at ambient temperature, the reaction mixture was stored overnight at 4 °C and then filtered off on a sintered glass filter. The crystalline product was obtained and dried in a vacuum oven at an elevated temperature (50−55 °C).

Sample Treatment. Extraction of curcumin from samples was done according to the previously reported procedures (*15*): the samples of dried (100 °C for 24 h) curcumin spices (mustard and curry which were acquired in the commercial food establishment) were carefully ground in an agate mortar to obtain a fine intimately mixed powder. Then, 2 g of the powder was dissolved in a minimum volume of acetonitrile. Undissolved particles were removed by centrifugation. The clear centrifugate and combined acetonitrile washings were transferred into a 50 mL volumetric flask and diluted to the mark with acetonitrile. An appropriate volume of this solution was pipetted into a 10 mL colorimetric tube and its curcumin was determined according to the procedure described above. The curcumin content was calculated according to the linear regression equation.

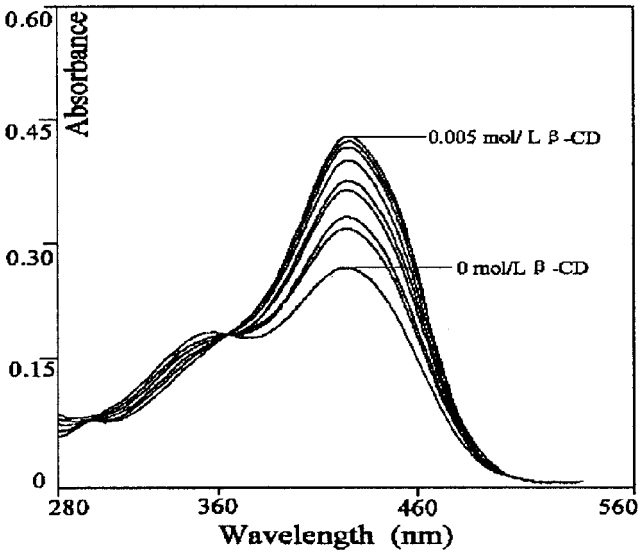

Figure 1. Absorption spectra of curcumin with various concentrations of β-CD: from bottom to top, 0; 5.0×10^{-4}; 1.0×10^{-3}; 1.5×10^{-3}; 2.0×10^{-3}; 2.5×10^{-3}; 3.0×10^{-3}; 4.0×10^{-3}; 5.0×10^{-3} mol/L; $C_{curcumin} = 1.0 \times 10^{-5}$ mol/L; pH = 2.4.

RESULT AND DISCUSSION

Absorption Spectra. Figure 1 displays absorption spectra of curcumin at different concentrations of β-CD. As the β-CD concentration is increased, the absorption maximum of curcumin at 426 nm is slightly red-shifted to 431 nm with a concomitant increase in the absorption intensity. An isosbestic point is observed around 370 nm. All these facts may be explained by partial shielding of the excitable electrons and chromophores in the β-CD cavity and therefore are rationalized as being indicative of complex formation.

Influence of pH. Curcumin is a phenol and therefore it is present in solution as an equilibrium between its protonated and deprotonated forms, according to the pH value (*23*) (**Figure 2**). Conclusions about the spatial distribution of the two phenolic hydroxyl of curcumin could be obtained by comparison of the pK_a values calculated in the presence and in the absence of β-CD (*22*, *24*). So, the changes in the absorption of both curcumin and the curcumin−β-CD complex solutions as a function of pH were analyzed.

We have chosen 470 nm as the detection wavelength at which the absorbance of curcumin changed most obviously in going from acid to basic media, both in the absence and in the presence of β-CD (**Figure 3**). The profiles of absorbance at 470 nm vs pH were used to calculate the deprotonation constants of curcumin in the ground state. These calculations were performed by using the program Sigma Plot according to the reported method (*25*). Through curve-fit, the deprotonation constants of curcumin in the absence of β-CD were obtained as follows: pK_{a1} = 8.10, pK_{a2} = 10.45, which were obviously different from

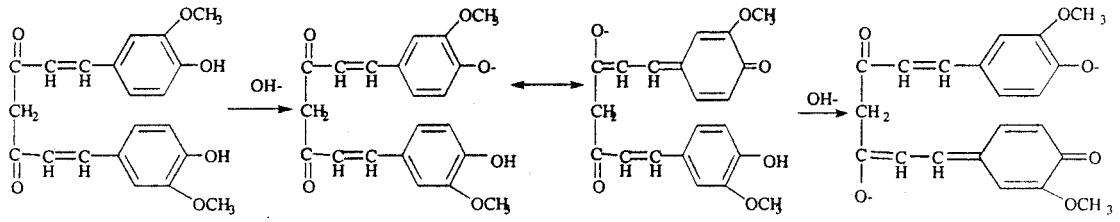

Figure 2. Protonation equilibrium between curcumin and its conjugate base.

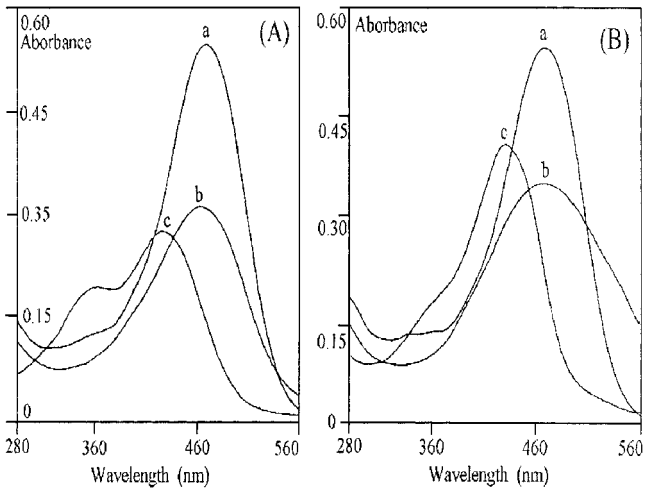

Figure 3. Absorption spectra of curcumin (A) and curcumin–β-CD inclusion complex (B) at different pH. (A): a. pH = 13.0, b. pH = 10.0, c. pH = 6.7, $C_{curcumin} = 1.0 \times 10^{-5}$ mol/L. (B): $C_{\beta\text{-CD}} = 6.0 \times 10^{-3}$ mol/L; other conditions as in (A).

those in the presence of β-CD: $pK_{a1} = 8.91$ $pK_{a2} = 11.00$ (**Figure 4**). It is apparent that both pK_{a1} and pK_{a2} in the presence of β-CD are larger than those obtained in the absence of β-CD. This suggests that the two phenolic hydroxyls of curcumin are located inside of the β-CD cavity in the inclusion complex (*22*, *24*).

By comparison of the corresponding spectra in **Figure 3**, it can be seen that the presence of β-CD has little effect on the absorbance of curcumin in strong basic media but obviously enhances the absorbance in acid media. So, the optimum pH value for the inclusion complex formation is in the lower pH range, where curcumin is in its acid form, but not its conjugate base, which has higher polarity and therefore is relatively difficult to enter the hydrophobic cavity of β-CD. Curcumin is exposed to hydrolytic degradative reactions in basic media and is therefore unstable, so it is more suitable to detect curcumin in the media whose pH value is maintained below 7 (*26*, *27*). The complex formed by the acid form of curcumin with β-CD has a maximum absorption at 431 nm. The analysis on the changes of the absorbance at 431 nm with the pH when pH is much less than pK_{a1} was carried out (**Figure 5**). It demonstrated that the absorbance was relatively high and almost remained constant over the pH range 1.9−3.1. Therefore, a pH 2.4 Tri-sodium citrate-HCl buffer system was selected.

Acetonitrile may not compete with the hydrophobic cavity of β-CD preferentially because of its high polarity ($\epsilon = 38.8$, 20 °C), and therefore is chosen as organic solvent to prepare the curcumin stock solution. The 1.0×10^{-5} mol/L solution of curcumin was prepared by injecting a 0.01 mL aliquot of the curcumin stock solution (1.0×10^{-2} mol/L) via a Gilsone microliter syringe into a 10 mL colorimetric tube. So, the sample solution was almost entirely aqueous and the percentage of acetonitrile present in the experimental solution (0.1% v/v) was

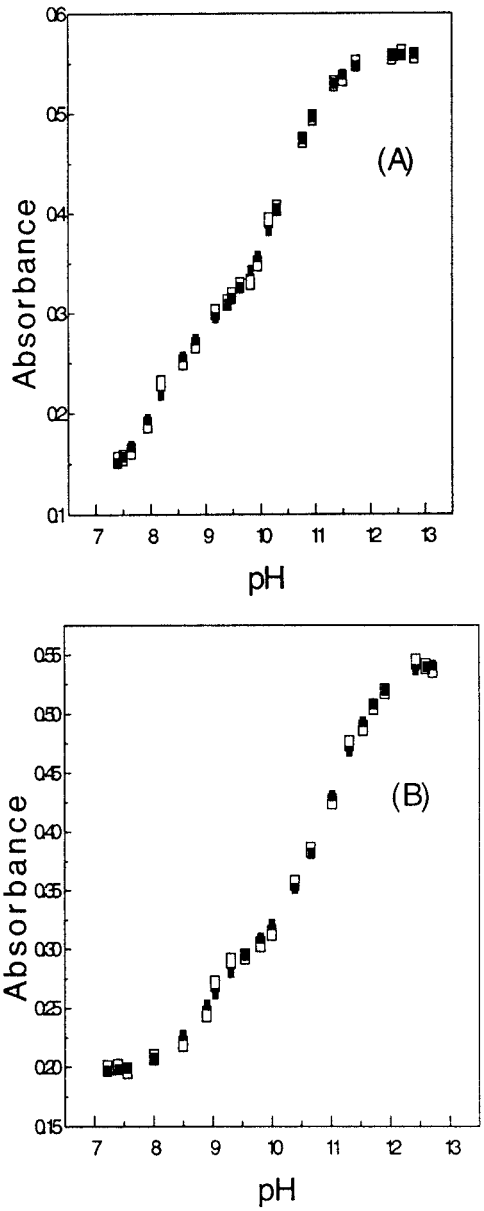

Figure 4. Absorbance of curcumin (A) and curcumin-β-CD inclusion complex (B) at 470 nm varying with pH. (A): $C_{curcumin} = 1.0 \times 10^{-5}$ mol/L, $t = 20$ °C, Ionic strength: 0.1M. ■ fit result, □ experimental result. (B): $C_{\beta\text{-CD}} = 6.0 \times 10^{-3}$ mol/L; other conditions as in (A).

considered to be too low to have any significant influence on the acid−base behavior of curcumin or the complex formation between curcumin and β-CD.

Infrared Spectrum. Inclusion complex formation may be proved by IR spectrometry because bands resulting from the included part of the guest molecule are generally shifted or their intensities are altered (*28*). If curcumin and β-CD form a inclusion complex, the noncovalent interactions between them such as van der Waals interactions, hydrophobic interactions

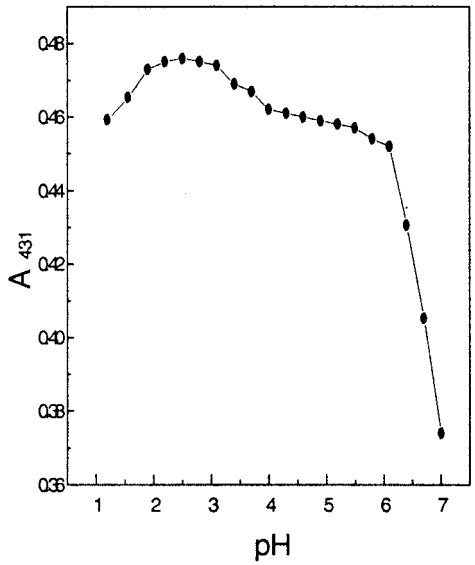

Figure 5. Influence of pH on the absorbance of curcumin–β-CD inclusion complex. $C_{curcumin} = 1.0 \times 10^{-5}$ mol/L; $C_{\beta\text{-CD}} = 6.0 \times 10^{-3}$ mol/L.

and hydrogen bonds will lower the energy of the included part of curcumin, reduce the force constants of the corresponding bonds, and therefore decrease its absorption frequency. By comparison of the IR spectrograms of curcumin (**Figure 6a**), β-CD (**Figure 6b**), the physical mixture of curcumin and β-CD (**Figure 6c**) and the inclusion complex of curcumin and β-CD (**Figure 6d**), we can see that the spectrum of c is essentially the combination of a and b, which indicates that physical mixture cannot lead to inclusion. We can also see that there are apparent differences between the spectra of c and d and that some characteristic IR peaks of curcumin change obviously. For example, the 1600 cm^{-1} absorption peak, which could be assigned to the stretching vibration of benzene ring skeleton, is red-shifted to 1580 cm^{-1}; the 1280 cm^{-1} absorption peak because of the Ar−O stretching vibration has a split, that is to say, apart from 1280 cm^{-1}, a new red-shifted absorption peak appears at about 1265 cm^{-1}. Based on these facts, it can be concluded preliminarily that the aromatic structures at each end of the curcumin molecule are included into the β-CD cavity. Although the alkene part of curcumin perhaps may not be included directly into the β-CD cavity, its absorption frequency may also be influenced indirectly because of its conjugation with the aromatic rings that are included by β-CD. So, the delocalization of π electron of the olefinic double bond is increased. As a result, the 1425 cm^{-1} peak, which could be attributed to the olefinic C−H in-plane bending vibration (δ_{C-H}), has a split and a red-shifted peak at about 1415 cm^{-1} comes into being. Therefore, IR spectrometry confirms the supramolecular inclusion complex formation between curcumin and β-CD.

Apparent Formation Constant. According to the molecule dimension, it is apparent that curcumin is too large (ca. 19 Å long and 6 Å wide) to be included entirely into one β-CD cavity (ca. 7.8 Å wide). It is reasonable to consider the complex formation with two molecules of β-CD. From the changes in the absorption spectra, an apparent formation constant value for the inclusion complex can be determined. These experimental data could not be fitted by considering the formation of

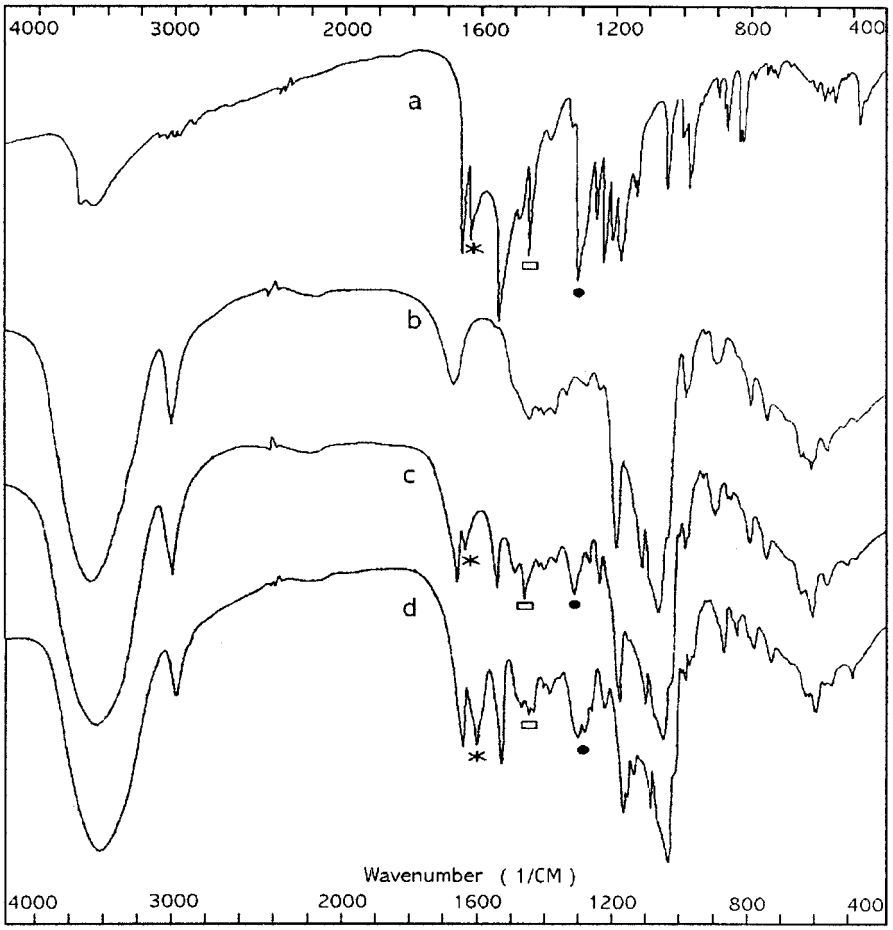

Figure 6. Infrared spectrograms of curcumin (a), β-CD (b), the physical mixture of curcumin and β-CD (c), and curcumin–β-CD inclusion complex (d).

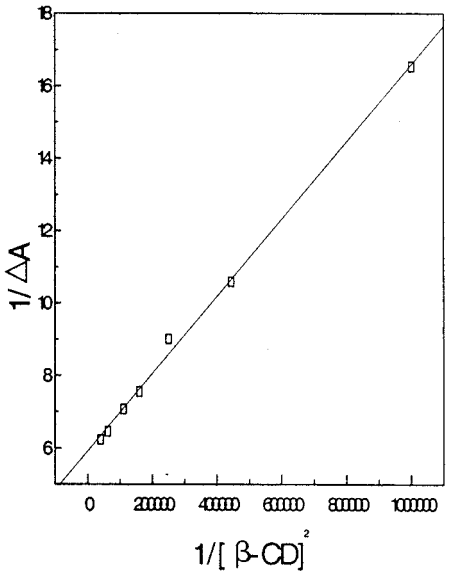

Figure 7. Double reciprocal plot for the β-CD–curcumin complex. A linear relationship is obtained when the data are plotted assuming a 1:2 stoichiometry of curcumin $(\beta\text{-CD})_2$ complex.

a 1:1 β-CD-curcumin complex, but by an 2:1 β-CD-curcumin inclusion complex according to the following reaction:

$$\text{curcumin} + 2\beta\text{-CD} \leftrightarrow \text{curcumin}(\beta\text{-CD})_2$$

The K value was determined by the typical double reciprocal plot (*24*)

$$\frac{1}{A - A_0} = \frac{1}{(A_\infty - A_0)KC_{CD}{}^2} + \frac{1}{A_\infty - A_0}$$

where A is the observed absorbance of the curcumin solution at each β-CD concentration tested; A_0 and A_∞ are the absorbance in the absence of β-CD and when all the curcumin molecules are complexed, respectively. It was taken into account that β-CD is in a large excess with respect to curcumin and therefore its free and analytical concentrations are similar; and the variations in the absorbance signals are proportional to the complex concentration, and at high β-CD concentration essentially all of the curcumin molecules are complexed.

The good linear relationship obtained when $1/(A - A_0)$ is plotted against $1/C_{CD}{}^2$ supports the existence of a 2:1 complex ($R = 0.9986$, **Figure 7**). Its apparent formation constant was determined to be 5.53×10^5 mol^{-2} L^2 through the nonlinear regression fit. The 2:1 host−guest inclusion complex could also be verified by the conclusion drawn above that both of the two phenolic hydroxyl are located inside the β-CD cavity.

In the inclusion complex, we supposed that each of the benzene rings of curcumin was included into one β-CD cavity under the driving of van der Waals interactions and hydrophobic interactions. Furthermore, curcumin is a molecule with electron donor groups able to form hydrogen bonds within the CD cavity, which may make the 2:1 complex more stable.

Inclusion Complex Thermodynamics. The thermodynamic parameters, standard free energy ($\Delta G°$), enthalpy ($\Delta H°$), and entropy ($\Delta S°$) for complexes of curcumin with β-CD were obtained from the van't Hoff equation: $\ln K = -\Delta H°/RT + \Delta S°/R$. The $\Delta H°$ and $\Delta S°$ of the complex formation were calculated from the slope and intercept by plotting $\ln K$ vs $1/T$ and $\Delta G°$ was obtained according to the equation $\Delta G° = \Delta H° - T\Delta S°$. The results are shown in **Table 1**. As can be seen, when the temperature increases from 20 to 65 °C, the $\Delta H°$ is negative, indicating that the complex dissociates when the temperature increases. The fact that $\Delta S°$ is positive may be attributed to the two reverse course: when curcumin molecules are included into the cavity of β-CD, the amount of independent curcumin molecules in the system reduces, which could make entropy decrease; at the same time, after guest molecules enter the cavity of β-CD, some water molecules are released, which leads to the increase of entropy. The net result of the two reverse courses is that the entropy of the system increases slightly.

Table 1. Changes of Curcumin–β-CD Complex as a Function of Temperature

	T (K)				
	293	308	318	328	338
K^a	13.22	12.69	12.43	12.03	11.67
$\Delta G^{\circ b}$ (kJ/mol)	−32.20	−32.50	−32.86	−32.81	−32.79
$\Delta H^{\circ c}$ (kJ/mol)	−28.09				
$\Delta S^{\circ d}$ (kJ/mol)	0.014				

[a] Apparent formation constant (K). [b] Standard free energy ($\Delta G°$). [c] Enthalpy ($\Delta H°$). [d] Entropy ($\Delta S°$).

Table 2. Effect of Foreign Ions on the Determination of 3.6839 μg/mL of Curcumin

foreign ions	tolerance level (μg/mL)
glucose, sucrose, glycin, SCN^-, Cl^-, I^-, K^+, Na^+, ClO_4^-, NH_4^+, SO_4^-, NO_3^-	1.2×10^4
Cd^{2+}, Zn^{2+}, Sr^{2+}, EDTA	1.0×10^4
Al^{3+}, Mg^{2+}, Ba^{2+}, $H_2PO_4^-$	8.0×10^3
MoO_4^{2-}	7.2×10^3
Co^{2+}, Sn^{2+}, AC^-, L-lysine	1.2×10^3
Cu^{2+}	1.0×10^3
CO_3^{2-}, Ni^{2+}, Mn^{2+}	8×10^2
B^{3+}	110
Pb^{2+}, $Cr_2O_7^{2-}$, Be^{2+}	20
Fe^{3+}, NO_2^-	5

Effect of Foreign Ions. A systematic study was carried out on the effects of the foreign ions on the determination of 3.6839 μg/mL (1.0×10^{-5} mol/L) of curcumin. A 1.2×10^4 μg/mL level of each potentially interfering ion was tested first. If interference occurred, the ratio was reduced progressively until interference ceased. The tolerance level was defined as an error not exceeding ±5% in the determination of the analyte. The results are summarized in **Table 2**. From **Table 2**, it can be seen that most of the foreign ions have no detrimental effect on the determination. In comparison with the interference results previously reported (*17*), the selectivity of our proposed method is a great improvement.

Analytical Parameters. The enhancement of the absorbance of curcumin produced through the complex formation might be very useful from an analytical point of view. Therefore, a spectrophotometric method for the determination of curcumin in bulk aqueous solution in the presence of β-CD was developed. For the purpose of comparison, calibration graph of curcumin in the absence of β-CD was also constructed. The analytical characteristics obtained are shown in **Table 3**.

As shown, the analytical characteristics in the presence of β-CD are significantly improved. Obvious decreases in both the limit of detection (LOD) and the limit of quantification (LOQ) are achieved with respect to the solutions without β-CD. The poor solubility of curcumin in aqueous solution results in a narrow linear range when it is determined. The solubility of

Table 3. Analytical Characteristics for Curcumin

analytical characteristics	in the absence of β-CD	in the presence of β-CD (7.5×10^{-3} mol/L)
linear regression equation[a]	$A = 0.0748C$ (μg/mL) − 0.0057	$A = 0.1264C$ (μg/mL) + 0.0039
linear range (μg/mL)	0–7.5	0–15
correlation coefficient	0.9986	0.9991
S_0[b]	3.4×10^{-3}	3.2×10^{-3}
LOD[c] (μg/mL)	0.136	0.076
LOQ[d] (μg/mL)	0.45	0.25
RSD[e]	1.46%	1.22%

[a] The number of data for each calibration graph correspond to eight different concentration levels, with three replicates for each level. [b] The standard deviation obtained from a series of 11 blanks solutions. [c] Limit of detection calculated according to the IUPAC definitions: $3S_0/K$, where K is the slope of the standard curve. [d] Limit of quantification calculated according to the IUPAC definitions: $10S_0/K$. [e] Relative standard deviation obtained from a series of 10 standards each containing 1.0×10^{-5} mol/L of curcumin.

Table 4. Determination of Curcumin in Curry and Mustard ($P = 0.95$)

	curcumin content	
samples	proposed spectrophotometric method (mg/g)	fluorometric method (*15*) (mg/g)
curry	1.56 ± 0.03	1.53 ± 0.04
mustard	2.96 ± 0.06	2.99 ± 0.05

Table 5. Results for the Recoveries of Curcumin from Samples

samples	sample content (μg/mL)	curcumin added (μg/mL)	curcumin found (μg/mL)	recovery (%)
curry	0.68	3.68	4.31	98.64
	0.81	2.95	3.68	97.29
	1.01	2.21	3.21	99.55
mustard	0.89	3.68	4.47	97.28
	0.89	2.95	3.81	98.98
	0.59	4.42	5.09	101.81

curcumin is enhanced through the inclusion complex formation with β-CD, which largely widens the linear range. So, it can be concluded that an improved sensitivity for the spectrophotometric determination of curcumin in the bulk aqueous solution can be achieved in the presence of β-CD.

Application. Samples of curry and mustard were submitted to the extraction procedure described above and further analyzed in triplicate according to the proposed method. The results are shown in **Table 4**. As shown, results obtained by the proposed spectrophotometric method agree with those of the fluorimetric method (*15*). At a confidence level (P) equal to 95%, there is no significant difference between the two methods.

To evaluate the extraction procedure and the accuracy of the method, a recovery assay was realized. The recoveries of curcumin added to sample solutions of different concentration are shown in **Table 5**. The recovery was 97.28−101.81%.

ABBREVIATIONS USED

β-CD, β-cyclodextrin.

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