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Study on the Supramolecular Interaction of Thiabendazole and β-Cyclodextrin by Spectrophotometry and Its Analytical Application

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The β-cyclodextrin−thiabendazole (β-CD−TBZ) inclusion complex was synthesized and its structure characterized by ^{1}H NMR and IR. The mechanism of the supramolecular interaction of TBZ and β-CD has been studied and discussed by spectrophotometry. The results showed that the phenyl ring of TBZ was included in the β-CD cavity to form a 1:1 host−guest complex with an apparent formation constant of 1.60×10^3 mol^{-1}·L. On the basis of the enhancement of the absorbance of TBZ produced through complex formation, a spectrophotometric method for the determination of TBZ in bulk aqueous solution in the presence of β-CD was developed. The linear relationship between the absorbance and TBZ concentration was obtained in the range of 8.86×10^{-7}−1.45×10^{-5} mol/L. The detection limit was 2.71×10^{-7}mol/L, and the relative standard deviation was 0.86%. The interference of 48 coexisting substances was slight. The proposed method has been successfully applied to the determination of TBZ in fruits with recoveries of 96−103%.

KEYWORDS: Thiabendazole; β-cyclodextrin; spectrophotometry; supramolecular complex

1. INTRODUCTION

Thiabendazole [2-(4-thiazolyl)-1*H*-benzimidazole, TBZ; **Chart 1**] has been widely used as a broad-spectrum bactericide for human and domestic animals (*1*) and also as a food additive (*2*), to maintain freshness in vegetables and fruits (*3*), as a pre- or postharvest fungicide (*4*), and in industry as a mildewproof agent. In view of its wide application, considerable works have been done for its detection and quantification. Various methods such as high-performance liquid chromatography (*5*), liquid chromatography−mass spectrometry (*6*), fluorometry (*7*), capillary electrophoresis (*8*), room temperature phosphorimetry (*9*), micellar electrokinetic capillary chromatography (*10*), capillary atomization mass spectrometry (*11*), polarography (*12*), and spectrophotometry (*13*) have been employed to determine TBZ in a variety of matrices such as serum, plasma, urine, saliva, pharmaceutical preparations, pesticide residues, and animal bodies. Most of these methods were carried out in an organic medium, needed expensive instrument, and required strictly controlled reaction conditions. Spectrophotometry has relatively low sensitivity and selectivity in aqueous solution despite its simplicity, quickness, and economy in analysis. Therefore, for routine quality control, the development of a novel, highly sensitive, and selective spectrophotometric method for the direct determination of TBZ in aqueous solution has important application value.

Cyclodextrins (CDs), the cyclic oligosaccharides consisting of six or more D-(+)-glucopyranose units (**Chart 2**), are well-known to have a hollow truncated cone with a hydrophobic cavity and a hydrophilic wall to form inclusion complexes with guest organic or inorganic molecules that possess suitable polarity and dimension. Therefore, as excellent enzyme models and molecule receptors, CDs have been widely used in many fields in science and technology (*14*). The formation of a supramolecular complex with CDs can alter the photochemical and photophysical properties of the guest molecules, and considerable attention has been focused on the luminescence application of the CDs. Drug molecules can demonstrate dramatically different physical, chemical, and biological properties through the formation of inclusion complexes with CDs (*15*, *16*), such as the enhancement of the solubility, stability, and bioavailability. CDs have been proved to be suitable candidates for targeted drug delivery and drug release in human

Chart 1. Chemical Structure of TBZ

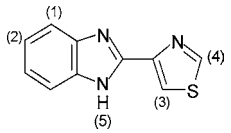
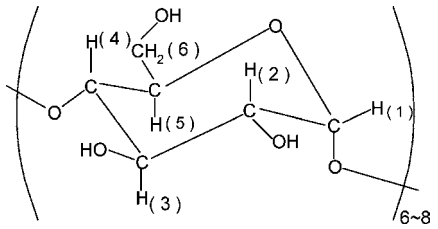


Chart 2. Structure of CD Oligosaccharide



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bodies, which can promote the digestion, absorption, and therapeutic effect of drugs. New methods with high sensitivity and selectivity for the determination of drugs in aqueous solution have been established on the basis of the significant enhancement in the absorbance or fluorescence intensity of drug molecules produced through complexation with CDs (*17*−*19*).

In the present work, a spectrophotometric study of the supramolecular interaction between TBZ and β-cyclodextrin (β-CD) was carried out. All of the data on the complexation of TBZ refer to the fact that TBZ was a good complex-forming guest, which was included in the cavity of β-CD to form a 1:1 inclusion complex. The apparent formation constant was estimated to be 1.60×10^3 mol^{-1}·L at room temperature, which was almost 20 times more than the reference (*20*) reported. The thermodynamic parameters ($\Delta G°$, $\Delta H°$, $\Delta S°$) for the formation of complexes were obtained from the van't Hoff equation. On the basis of the significant enhancement in the absorbance of TBZ produced through complexation with β-CD, a spectrophotometric method having improved sensitivity 4 times higher than that reported by spectophotometry (*13*) for the determination of TBZ in the bulk aqueous solution was developed. The proposed method has been successfully applied to the determination of TBZ in fruits with recoveries of 96−103%.

2. EXPERIMENTAL PROCEDURES

2.1. Instruments and Chemicals. All absorbance measurements were carried out on a UV-265 spectrophotometer equipped with 1.0 cm quartz cells (Shimadzu). pH measurements were made with a pHs-3C digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass−calomel electrode. Infrared spectra were obtained from a PE-983G IR spectrophotometer (Perkin-Elmer). ^{1}H NMR spctra data were recorded at a Varian Inova-300 nuclear magnetic resonance spectrometer (Varian; solvent, DMSO-d_6; internal standard, TMS). The sample chamber accommodated a thermostated cuvette holder, controlled to 20 ± 1 °C via a CS-50 constant temperature circulator (Chongqing Experimental Equipment Works, Chongqing, China). The specific rotary powers were determined on a WZZ-2 automatic polarimeter (Shanghai Physical Optics Instrument Factory, Shanghai, China). Melting points were determined on a Yamato MP-21 Micro Melting Point apparatus (Yanaco).

TBZ [purchased from the China Medicine (Group) Shanghai Chemical Reagent Corp.] was of analytical reagent grade. Its stock solution (5.00×10^{-4} mol/L) was prepared in acetonitrile and stored in the dark in amber bottles at 4 °C. β-CD [obtained from the China Medicine (Group) Shanghai Chemical Reagent Corp.] was purified by two recrystallizations in doubly distilled water, followed by vacuum-drying at 60 °C for 12 h and used at a concentration of 1.00×10^{-2} mol/L aqueous solution. NaAc−HCl was used as buffer solution (0.20 mol/L, pH 2.50). Other chemicals used were of analytical reagent or higher grade. Doubly distilled water was used throughout.

2.2. Synthesis and Characterization of β-CD−TBZ Inclusion Complex. *2.2.1. Synthesis of β-CD−TBZ Inclusion Complex.* According to the reaction routine reported in ref *21*, the calculated amount of TBZ (0.01 mol) to be complexed was dissolved in a minimum volume of ethanol at 60 °C and then added dropwise into the 1.5 equiv of β-CD aqueous solution at 60 °C upon continuous intensive stirring. The mixture solution was refluxed with vigorous agitation at 70 °C for ∼4 h, and then ethanol was removed at 80−82 °C. After the mixture was cooled to room temperature, it was stirred for 8 h at ambient temperature. The reaction mixture was allowed to store overnight at 4 °C and then centrifuged and filtered on a sintered glass filter. The product was obtained and washed sequentially with doubly distilled water and ethanol and then dried in a vacuum oven at an elevated temperature (60−65 °C); 7.32 g of a white crystalline sample was obtained in 69.41% yield.

2.2.2. Physical and Chemical Constants. The melting point, specific rotary power, and R_f of TBZ, β-CD, and the inclusion complex are shown in **Table 1**. The specific rotary power values were determined in methanol/water (1:2) at 25 °C. TLC was performed on silica gel plates (GF254), and the compounds were spread with acetic ether/dichloromethane (1:1); visualization was accomplished with iodine vapor.

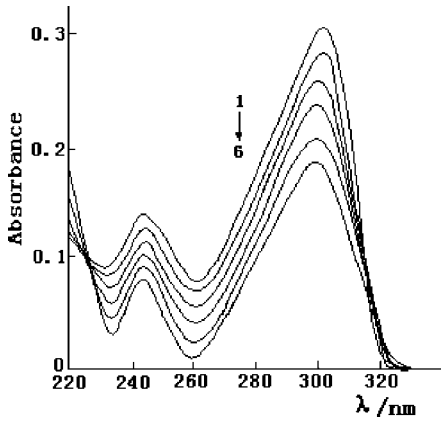

Figure 1. Absorption spectra of TBZ with various concentrations of β-CD: (1) 1.00×10^{-2}, (2) 8.00×10^{-3}, (3) 4.00×10^{-3}, (4) 3.00×10^{-3}, (5) 1.00×10^{-3}, (6) 0 mol/L of β-CD, $C_{TBZ} = 1.00 \times 10^{-5}$ mol/L, pH 2.50, 20 ± 1 °C.

Table 1. Physical and Chemistry Constants of TBZ, β-CD, and TBZ-β-CD Inclusion Complex

	mp (°C)	$[\alpha]_D^{25}$ ° mL dm^{-1} g^{-1}	R_f
TBZ	304−305	0	0.42
β-CD	without fixed mp, ≥200 °C decompose	161.29	0.11
inclusion complex	268∼270	−117.02	0.47

2.3. Experimental Procedure. *2.3.1. Determination of the Apparent Formation Constant.* Into a 10 mL colorimetric tube were added 0.20 mL of 5.00×10^{-4} mol/L TBZ, varied amounts of β-CD, and 2.00 mL of NaAc−HCl buffer solution (pH 2.50) sequentially. The mixed solution was diluted to 10 mL with doubly distilled water, ultrasonically oscillated for 15 min, and allowed to equilibrate at 20 ± 1 °C for 15 min. The absorbance at 303 nm was measured against a reagent blank. The apparent formation constant of the inclusion complex was obtained from the double-reciprocal plot (*22*).

2.3.2. Spectrophotometric Determination of TBZ. Into a 10 mL colorimetric tube were added an aliquot of TBZ stock solution containing $0.0-5.0 \times 10^{-6}$ mol of TBZ, 6.00 mL of 1.50×10^{-2} mol/L β-CD, and 2.00 mL of NaAc−HCl buffer solution (pH 2.50) sequentially. The mixed solution was diluted to 10 mL with doubly distilled water, ultrasonically oscillated for 15 min, and allowed to equilibrate at 20 ± 1 °C for 15 min. The absorbance at 303 nm was measured against a reagent blank.

The procedures of spectrophotometric determination of TBZ in the absence of β-CD were similar to those described above except that β-CD were not added.

2.3.3. Sample Treatment. The sample treatment was done according to the method of ref *23*: The pulp of fresh fruits was sliced and stirred into syrup. Into a 100 mL beaker were added 10 mL of syrup and a certain quantity of TBZ sequentially. The samples were extracted twice with 20 mL of ethyl acetate and filtered on a vacuum, and the residues were washed with 10 mL of ethyl acetate. Both extracts were mixed and dried in a rotary evaporator at 40 °C. The obtained solid was solubilized with 10 mL of acetonitrile and transferred into a 100 mL volumetric flask and then diluted to the mark with doubly distilled water. The TBZ content was calculated according to the linear regression equation.

3. RESULTS AND DISCUSSION

3.1. Absorption Spectra. Figure 1 displayed absorption spectra of TBZ at different concentrations of β-CD. As the β-CD concentration increased, the absorption maxima of TBZ at 244

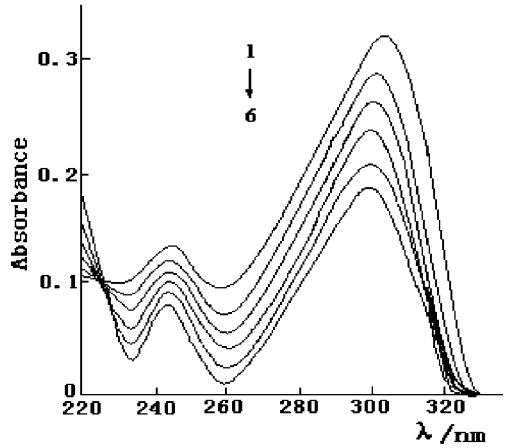

Figure 2. Absorption spectra of TBZ at different ratios of 2-propanol/water: 2-propanol content in the total solvent (v/v) was (1) 100%, (2) 60%, (3) 40%, (4) 25%, (5) 10%, and (6) 0%, $C_{TBZ} = 1.00 \times 10^{-5}$ mol/L.

and 300 nm slightly red-shifted 2 and 3 nm, respectively, with a concomitant increase in the absorption intensity.

To demonstrate that these spectral changes were not due to a solvent effect caused by a high concentration of β-CD, the effect of glucose on the spectra of TBZ was tested. The addition of glucose (in an equivalent mass of 9.00×10^{-3} mol/L β-CD) to a 1.00×10^{-5} mol/L of TBZ aqueous solution induced neither spectral shifts nor intensity changes in the absorption spectra of TBZ, which contrasted with those found when β-CD was added and confirmed that there was some specific interaction between β-CD and TBZ. Therefore, we concluded that the β-CD-induced changes observed in the absorption spectra of TBZ indicated the formation of supramolecular complexes between the two molecules (*24*).

Because the polarity in the hydrophobic cavity of β-CD was similar to that of alcohols, we used 2-propanol/water as medium to obtain the absorption spectra of TBZ at different 2-propanol/water ratios. It was found that the maximum absorption wavelength of TBZ red-shifted from 244 and 300 to 248 nm and 305 nm, respectively, and its absorbance intensity was gradually enhanced as the percentage of 2-propanol in the mixed solvent increased (**Figure 2**). That is, the spectra of TBZ in different alcohol/water ratios were almost the reprint of **Figure 1**. The facts suggested that the microenvironment around TBZ molecules in the presence of β-CD was similar to that in alcohols and thus indicated the formation of an inclusion complex between β-CD and TBZ.

To confirm this conclusion, we studied the influence of naproxen (*25*), a kind of substance that had been verified to be included by β-CD's cavity, on the absorption spectra of the β-CD−TBZ system (**Figure 3**). It could be found that the absorbance of the β-CD−TBZ system gradually decreased with increasing amount of naproxen. This phenomenon could explain why naproxen molecules competed to occupy the β-CD's cavity and thus caused redistribution of TBZ molecules into the aqueous phase. From these facts, it could be confirmed that TBZ molecules indeed entered into the cavities of β-CD and formed supramolecular inclusion complex.

The changes that occurred in the absorption spectra may be explained by partial shielding of the excitable electrons and chromospheres of TBZ in the β-CD cavity: the cavity of β-CD has a relatively high electronic cloud density, whereas the isopropyl in 2-propanol is also an electron-donating group, so both β-CD and 2-propanol can produce the electron-donating effect that will increase the π electron density of the conjugated

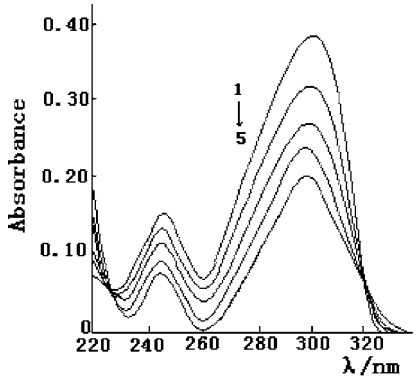

Figure 3. Influence of addition of naproxen on the absorption spectra of β-CD–TBZ system: (1) 6.00×10^{-5}, (2) 1.50×10^{-4}, (3) 1.80×10^{-4}, (4) 2.00×10^{-4}, (5) 2.50×10^{-4} mol/L of naproxen, $C_{\beta-CD} = 1.00 \times 10^{-2}$ mol/L, $C_{TBZ} = 1.30 \times 10^{-5}$ mol/L, pH 2.50, 20 ± 1 °C.

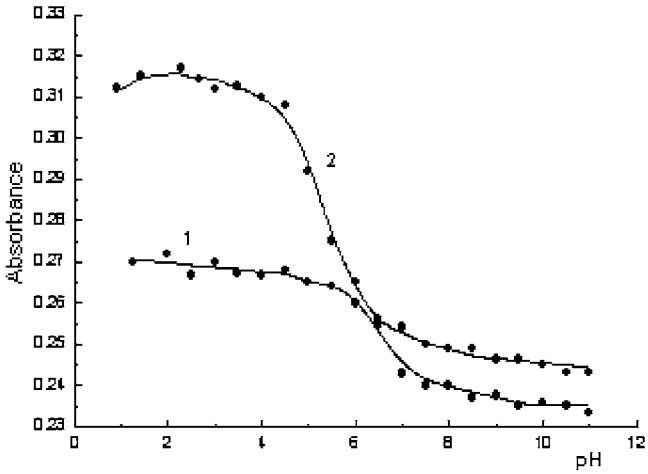

Figure 4. Absorbance of TBZ (1) and TBZ–β-CD inclusion complex (2) varying with pH: (1) $C_{TBZ} = 1.00 \times 10^{-5}$mol/L, 20 ± 1 °C; (2) $C_{\beta-CD} = 1.00 \times 10^{-2}$mol/L, other conditions as in plot 1.

system in TBZ and promote the electron transition, thus enhancing the absorbance of TBZ.

3.2. Influence of pH and Volume of Buffer Solution. Because of the instability of cyclodextrin at very low pH, the use of a strongly acidic solution containing β-CD was avoided (*26*). Thus, the pH effect on the system was studied over the range of 1.50−11.0 (**Figure 4**, plot 2). As could be seen, the absorbance intensity was relatively high and almost remained constant over the pH range of 2.00−3.20; therefore, a pH of 2.50 was fixed with the use of 0.20 mol/L NaAc−HCl buffer solution.

Because TBZ was a weak alkali, comparison of the deprotonation constant pK_a of TBZ in the absence and presence of β-CD could provide us with the information of the shielding degree experienced by TBZ. If there were dramatic changes of TBZ's pK_a when β-CD was added to the solution, it would be concluded that the guest molecule had been included in β-CD's cavity partially or wholely; otherwise, the guest molecule had not entered the β-CD cavity and was in the bulk aqueous medium. Therefore, the absorbance at 303 nm versus pH was determined to calculate the pK_a of TBZ, and the calculations were performed by using the program Sigma Plot according to the reported method (*27*): two forms of TBZ existed in solution at a certain pH range; thus, an equilibrum could be written:

$$TBZ + H_2O \rightleftharpoons TBZH^+ + OH^- \quad (1)$$

The alkali constant K_b could be written as

$$K_b = [TBZH^+][OH^-]/[TBZ] \quad (2)$$

The pH of the system was altered at a fixed TBZ concentration, so it could be seen that in a certain pH range the absorbance intensity changed with pH. C_1 and C_2 were used to represent the concentrations of TBZ and $TBZH^+$, respectively, so formula 2 could be rewritten as

$$K_b = \frac{C_2}{C_1}[OH^-] \quad (3)$$

and formula 4 could be obtained:

$$A = A_1\frac{C_1}{C_1 + C_2} + A_2\frac{C_2}{C_1 + C_2} = A_1\frac{1}{1 + \frac{C_2}{C_1}} + A_2\frac{1}{1 + \frac{C_1}{C_2}} \quad (4)$$

A in formula 4 referred to the total absorbance intensity of the system at 303 nm at varied pH, and A_1 and A_2 referred to the absorbance intensities of TBZ and $TBZH^+$, respectively, when the concentration of TBZ or $TBZH^+$ equaled the total concentration. Formulas 5 and 6 could be obtained from formula 3 as and then formula 7 could be obtained by means of combining

$$\frac{C_1}{C_2} = \frac{[OH^-]}{K_b} = 10^{pK_b - pOH} = 10^{pH - pK_a} \quad (5)$$

$$\frac{C_2}{C_1} = 10^{pK_a - pH} \quad (6)$$

formulas 4−6

$$A = A_1\frac{1}{1 + 10^{pK_a - pH}} + A_2\frac{1}{1 + 10^{pH - pK_a}} \quad (7)$$

Different A and pH values were measured to be applied for the curve-fitting of formula 7, and the pK_a of TBZ in the absence of β-CD was obtained to be 5.94 ± 0.12, which was obviously different from that in the presence of β-CD (6.87 ± 0.15) (**Figure 4**). The curve-fitting results indicated that TBZ entered the β-CD cavity to form a inclusion complex. Meanwhile, by comparison of the corresponding spectra in **Figure 4**, it could be seen that the presence of β-CD had little effect on the absorbance of TBZ in strong basic media but obviously enhanced the absorbance in acid media; thus, the optimum pH value for the inclusion complex formation should be in the low pH range, which provided additional evidence for the choiceof pH 2.50.

As the volume of the buffer added (from 1.00 to 3.00 mL) had little effect on the absorbance intensity, 2.00 mL of buffer solution was used in subsequent experiments.

3.3. Influence of β-CD Concentration and Reaction Time. The effect of β-CD concentration is shown in **Figure 5**, which demonstrates that the absorbance of TBZ−β-CD increased with increasing concentration of β-CD. When the concentration of β-CD added increased from 8.00×10^{-3} to 1.00×10^{-2} mol/L, the absorbance was relatively high and almost remained constant, so 1.00×10^{-2} mol/L of β-CD was used in subsequent experiments.

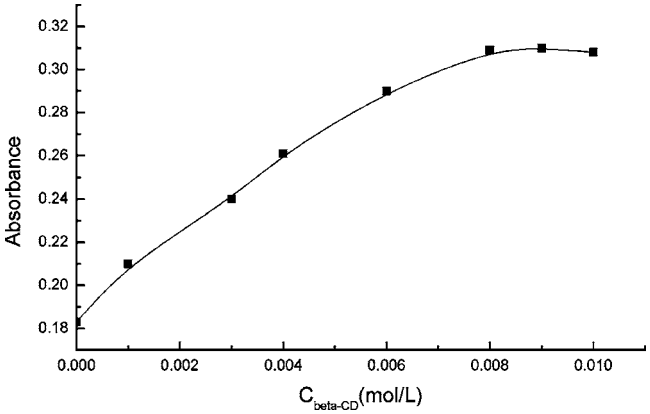

Figure 5. Absorbance of TBZ–β-CD inclusion complex with various concentrations of β-CD: $C_{TBZ} = 1.00 \times 10^{-5}$ mol/L, pH 2.50 (2.00 mL), 20 ± 1 °C.

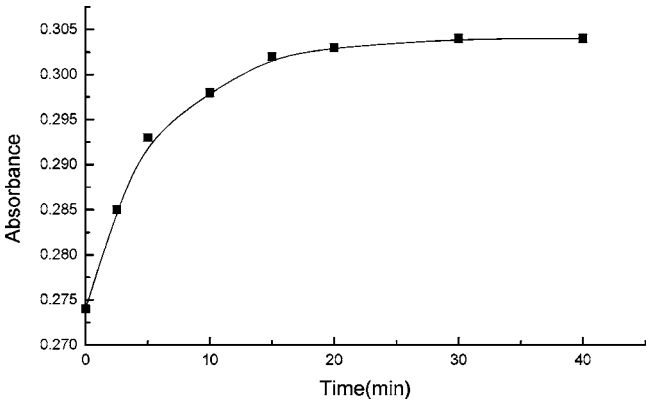

Figure 6. Absorbance of TBZ–β-CD inclusion complex at various times: $C_{\beta-CD} = 1.00 \times 10^{-2}$mol/L; $C_{TBZ} = 1.00 \times 10^{-5}$ mol/L, pH 2.50 (2.00 mL), 20 ± 1 °C.

The effect of interaction time was studied. The results (**Figure 6**) showed that the absorbance of the TBZ−β-CD inclusion complex reached a maximum after the reagents had been added for 15 min and remained constant for at least 30 min. Hence, after inclusive reaction was carried out for 15 min, the subsequent absorbance measurements were made at room temperature within 30 min.

3.4. IR Specrum. As we had known, the characteristic IR absorption frenquency of CD covered the 400−3800 cm^{-1} absorption band, so some characteristic IR absorption peaks of small organic guests in the spectra of the inclusion complex or the host−guest physical mixture might be covered up and hard to recognize. Meanwhile, some covered characteristic IR absorption peaks of the guest in the spectra of the host−guest physical mixture could appear and red or blue shift in the spectra of the inclusion complex. Thus, the variation of the shape, shift, and intensity of the absorption peak of the guest or host could provide enough information for the occurrence of the inclusion (*28*). By comparison of the IR spectra of TBZ (**Figure 7**, spectrum a), β-CD (**Figure 7**, spectrum b), the physical mixture of TBZ and β-CD (**Figure 7**, spectrum c), and the inclusion complex (**Figure 7**, spectrum d), it could be seen that spectrum c was essentially the combination of spectra a and b, which indicated that the physical mixture cannot lead to inclusion; there were apparent differences between the spectra c and d, and some characteristic IR absorption peaks of TBZ and β-CD changed obviously in the inclusion complex: the 1028−1151 cm^{-1} absorption band in the inclusion complex assigned to the characteristic C−O−C bond antisymmetric stretching vibration

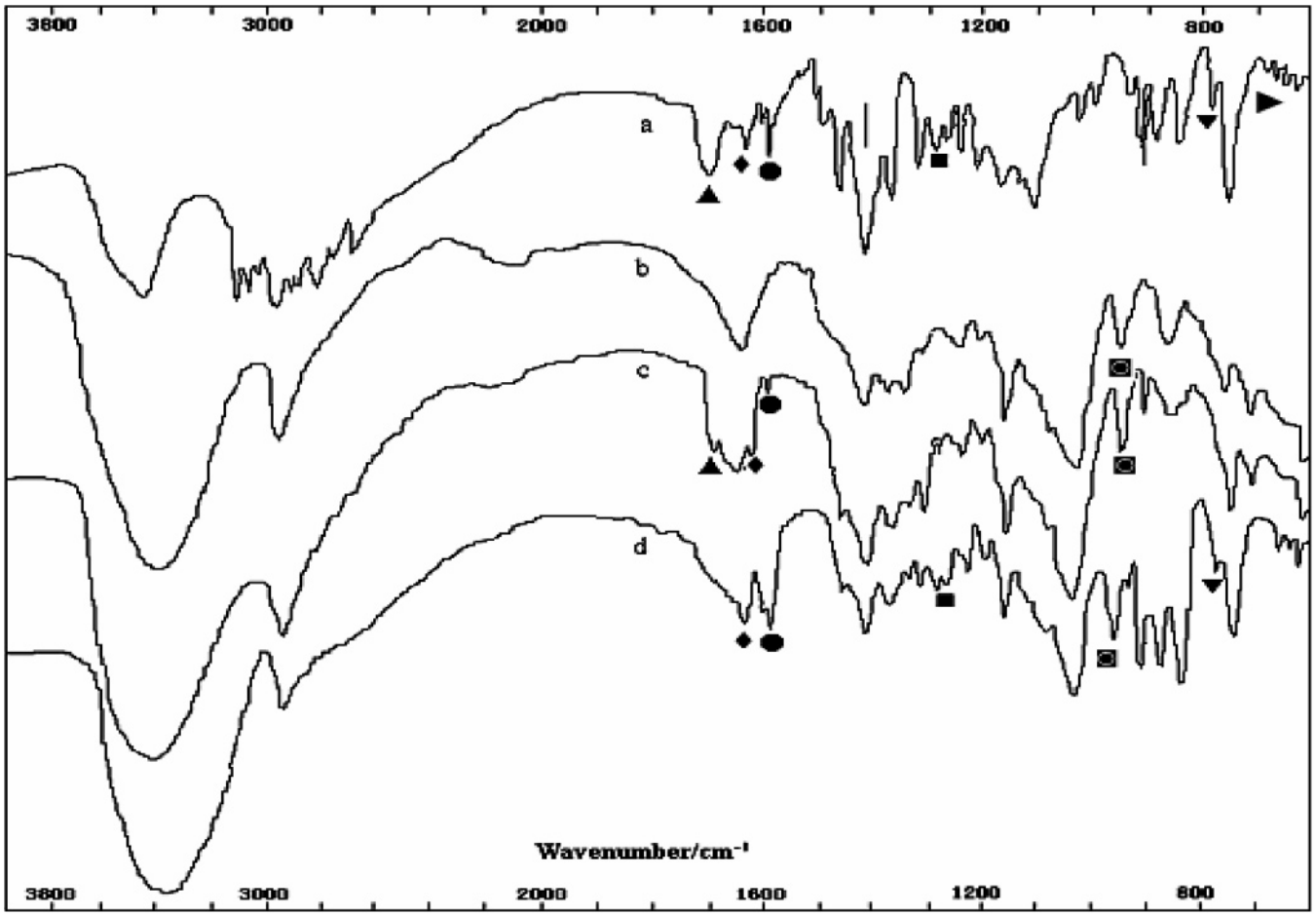

Figure 7. Infrared spectra of TBZ (a), β-CD (b), the physical mixture of TBZ and β-CD (c), and the TBZ–β-CD inclusion complex (d).

Table 2. Chemical Shifts δ of Protons in β-CD, TBZ, and TBZ–β-CD Inclusion Complex

	β-CD						TBZ			
	H_1	H_2	H_3	H_4	H_5	H_6	H_1	H_2	H_3	H_4
$\delta_{\beta-CD}(\delta_{TBZ})$	4.83	3.28	3.64	3.34	3.59	3.64	7.25	7.60	8.52	9.36
$\delta_{\beta-CD-TBZ}$	4.80	3.26	3.54	3.33	3.50	3.56	7.18	7.53	8.51	9.35
$\Delta\delta^a$	–0.03	–0.02	–0.10	–0.01	–0.09	–0.08	–0.07	–0.07	–0.01	–0.01

[a] $\Delta\delta = \delta_{\beta-CD-TBZ} - \delta_{\beta-CD}$ (δ_{TBZ}).

and C−C/C−O bond stretching vibration of β-CD (*29*); the 1300 cm^{-1} absorption peak (■), which could be assigned to the skeleton stretching vibration of heterocycle, namely, the thiazole ring, appeared in the inclusion complex; the 951 cm^{-1} absorption peak (gray box) due to the α-1,4-bond skeleton vibration of β-CD blue-shifted to 958 cm^{-1}; the benzene ring skeleton vibration absorption peak at 1580 cm^{-1} (●) and benzene ring −C═C− bond stretching vibration absorption peak at 1600 cm^{-1} (◆) of TBZ red-shifted to 1570 and 1585 cm^{-1}, respectively; the 770 cm^{-1} (▼) absorption peak assigned to four adjoining hydrogens at the benzene ring of TBZ blue-shifted to 776 cm^{-1}; the 1691 cm^{-1} (▲) and 673 cm^{-1} (rightward triangle) absorption peaks of TBZ disappeared in the inclusion complex. On the basis of these facts, it could be concluded preliminarily that the benzene ring of TBZ was included in the β-CD cavity to form a supramolecular inclusion complex.

3.5. ^{1}H NMR Spectrum. According to the ^{1}H NMR spectra of TBZ, β-CD, and the inclusion complex, apparent changes in chemical shifts of different protons could be observed (**Table 2**): unlike the dramatic upfield shifts of β-CD's interior H-3, H-5, and H-6 protons, which resulted from the shielding effect exerted by the inclusion of TBZ's benzene ring into β-CD's cavity, the shifts of β-CD's outside H-2 and H-4 protons could be neglected; the apparent shift changes of β-CD's H-3 indicated that the guest molecule entered β-CD's cavity along its wide rim, which possessed secondary hydroxyls (*30*); H-1 and H-2 of TBZ's benzene ring protons had dramatic chemical shift changes and both shifted upfield 0.07 ppm; meanwhile, other prontons of TBZ changed little. All of the facts showed obviously that TBZ's benzene ring was included in β-CD's cavity, which was in good agreement with the results obtained from IR spectra.

3.6. Determination of Apparent Formation Constant. Because the TBZ molecule was too large to be included entirely in β-CD's cavity, it was reasonable to consider the formation of inclusion complexes with a TBZ−β-CD complex ratio of 1:1 or 1:2. From the changes in the absorption spectra, an apparent formation constant for the inclusion complex could be determined. There was the following equation if a 1:1 TBZ−β-CD complex was formed:

$$1/(A - A_0) = 1/[(A_\infty - A_0)KC_{CD}] + 1/(A_\infty - A_0) \quad (8)$$

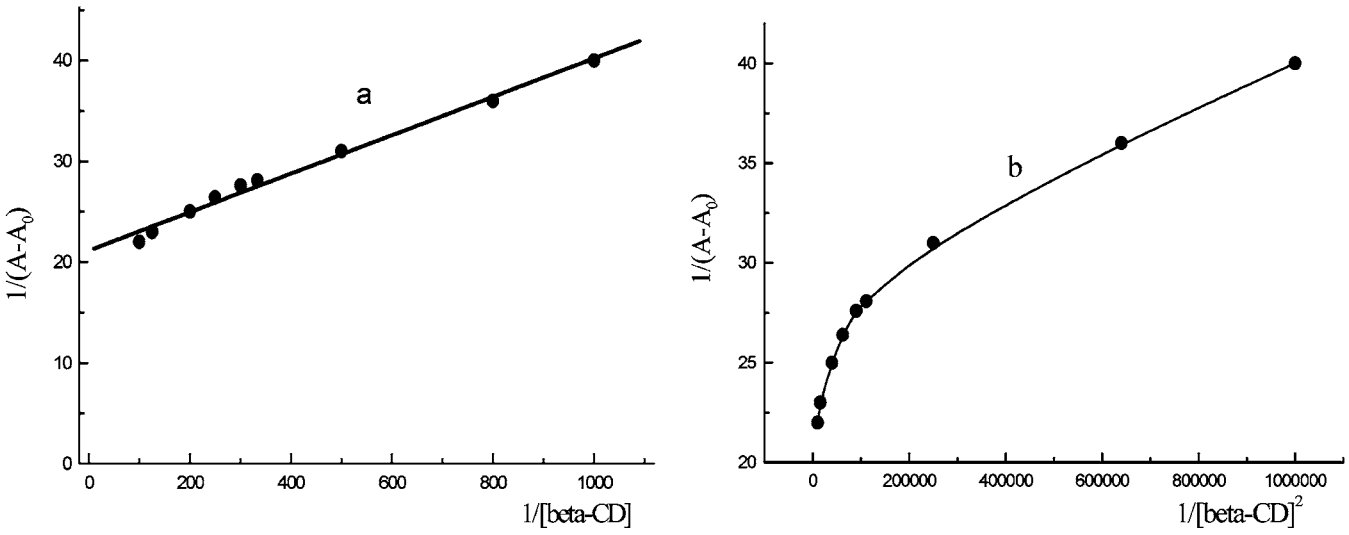

Figure 8. Double-reciprocal plot obtained from $1/(A - A_0)$ plotted against $1/[\beta\text{-CD}]$ (**a**) and $1/[\beta\text{-CD}]^2$ (**b**).

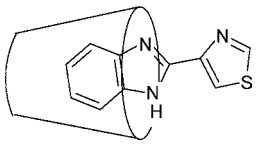

Figure 9. Chemical construction of the TBZ–β-CD inclusion complex.

If a 1:2 TBZ−β-CD was formed, the corresponding equation should be

$$1/(A - A_0) = 1/[(A_\infty - A_0)KC_{CD}^2] + 1/(A_\infty - A_0) \quad (9)$$

where A was the observed absorbance of the TBZ solution at each β-CD concentration tested; A_0 and A_∞ were the absorbances in the absence of β-CD and when all of the TBZ molecules were complexed, respectively. C_{CD} referred to the concentration of β-CD; K was the apparent complex constant. It was taken into account that (1) β-CD was in large excess with respect to TBZ, and therefore its free and analytical concentrations were similar; (2) the variations in the absorbance signals were proportional to the complex concentration; (3) at high β-CD concentration essentially all of the TBZ molecules were complexed. The K value was determined by the typical double-reciprocal plot (*22*).

As shown in **Figure 8**, the experimental data could not be fitted by considering the formation of a 1:2 TBZ−β-CD inclusion complex. A good linear relationship obtained when $1/(A - A_0)$ was plotted against $1/C_{CD}$ and supported the formation of a 1:1 complex ($r = 0.9989$). The apparent formation constant was determined to be $(1.60 \pm 0.12) \times 10^3$ mol^{-1}·L through the nonlinear regression fit, almost 20 times more than the reference (*20*) reported. The reason for the difference perhaps is that in ref *20*, TBZ exists in a pH 7.0 solution as a neutral molecule (*31*), but in an acidic solution of pH 2.5 (this paper), the protonation occurs on the imidazole ring (*31*), causing a resonance structure to induce a conformation that better fits the shape and size of cyclodextrin's cavity. Thus, the equilibrium constant of this paper exhibits a larger value than reported by ref *20*.

The obtained results from ^{1}H NMR, IR, and the complex mechanism studied above could provide sufficient information about the chemical construction of the TBZ−β-CD inclusion complex as shown in **Figure 9**.

3.7. Inclusion Complex Thermodynamics. The thermodynamics parameters, standard free energy ($\Delta G°$), enthalpy ($\Delta H°$), and entropy ($\Delta S°$), for complexes of TBZ with β-CD were obtained from the van't Hoff equation: $\ln K = -\Delta H°/RT + \Delta S°/R$. The $\Delta H°$ and $\Delta S°$ values of the complex formation were calculated from the slope and intercept by plotting $\ln K$ versus $1/T$, and $\Delta G°$ was obtained according to the equation $\Delta G° = \Delta H° - T\Delta S°$. The regression equation was $\ln K = 2037.18T^{-1} + 0.4258$ ($r = 0.9971$). The calculated results are shown in **Table 3**. As can be seen, when the temperature increased, $\Delta H°$ was negative, indicating that the complex dissociated. The fact that $\Delta S°$ was positive could be attributed to the two reverse courses: when TBZ molecules were included in the cavity of β-CD, the amount of independent TBZ molecules in the system reduced, which decreased the entropy; at the same time, after guest molecules entered the cavity of β-CD, some water molecules were released, which led to the increase of entropy. The net result of the two reverse courses was that the entropy of the system increased slightly.

3.8. Analytical Parameters. The enhancement of the absorbance of TBZ produced through the complex formation might be very useful from an analytical point of view. Therefore, a spectrophotometric method for the determination of TBZ in bulk aqueous solution in the presence of β-CD was developed. For the purpose of comparison, the calibration graph of TBZ in the absence of β-CD was also constructed. The analytical characteristics obtained are shown in **Table 4**.

As can be seen, the analytical characteristics in the presence of β-CD were significantly improved because β-CD could

Table 3. Apparent Formation Constant (K), Standard Enthalpy ($\Delta H°$), Entropy ($\Delta S°$), and Free Energy ($\Delta G°$) Changes of TBZ–β-CD Inclusion Complex as a Function of Temperature

	293 K	298 K	303 K	308 K	313 K	318 K	323 K	328 K	333 K
ln K	7.38	7.26	7.15	7.04	6.93	6.83	6.73	6.59	6.48
$\Delta G°$ (KJ·mol^{-1})	17.91	17.92	17.94	17.96	17.98	18.00	18.01	18.03	18.05
$\Delta H°$ (KJ·mol^{-1})					16.87				
$\Delta S°$ (J·mol^{-1}·K^{-1})					3.540				

Table 4. Analytical Parameters

analytical characteristic	without β-CD	proposed method
linear regression equation	$A = 1.77 \times 10^4 c$ (mol/L) − 0.018	$A = 4.54 \times 10^4 c$ (mol/L) − 0.142
linear range (mol/L)	7.45×10^{-6}–4.13×10^{-5}	8.86×10^{-7}–1.45×10^{-5}
correlation coefficient	0.9947	0.9991
standard deviation, S_0[a]	5.2×10^{-3}	4.1×10^{-3}
relative standard deviation, RSD[b] (%)	1.2	0.86
limit of detection, LOD[c] (mol/L)	8.8×10^{-7}	2.7×10^{-7}
limit of quatification, LOQ[d] (mol/L)	2.9×10^{-6}	9.0×10^{-7}

[a] S_0 obtained from a series of 11 blank solutions. [b] RSD obtained from a series of 11 standards each containing 1.00×10^{-5}mol/L TBZ. [c] LOD calculated according to the IUPAC definition: $3S_0/K$, where K is the slope of the standard curve. [d] LOQ calculated according to the IUPAC definition: $10S_0/K$.

Table 5. Effect of Foreign Interferences on the Determination of 1.00×10^{-5} mol/L TBZ

tolerance ratio in mass	foreign ions and materials
3500	Na^+, NH_4^+, Cl^-, I^-, starch, sucrose, lactose, glucose, glycin, NaCl
3000	K^+, Sr^{2+}, Ca^{2+}, Mg^{2+}, Al^{3+}, NO_3^-, SCN^-, CO_3^{2-}
2500	Zn^{2+}, Fe^{2+}, boracic acid, gelatin
2000	SO_4^{2-}, sorbitol, gum acacia power, sodium acetate
1000	Cu^{2+}, Mn^{2+}, Co^{2+}, Fe^{3+}, $H_2PO_4^-$, PO_4^{3-}, mannitol, methyl cellulose
500	Ni^{2+}, Br^-, ethanol, acetate acid
250	Be^{2+}, Ba^{2+}, ClO_4^-, NO_2^-, Ac^-
80	Cd^{2+}
30	Pb^{2+}, B^{3+}, $Cr_2O_7^{2-}$, MnO_4^{2-}

Table 6. Recovery of TBZ in Real Sample ($n = 5$)

sample	TBZ added (10^{-6} mol/L)	TBZ av found (10^{-6}mol/L)	recovery (%)	RSD (%)
banana	1.38	1.34	97	2.1
	1.70	1.72	101	3.1
	2.83	2.72	96	1.8
pineapple	2.50	2.46	98	2.5
	4.38	4.51	103	2.9
	5.20	5.16	99	2.1
apple	5.50	5.35	97	2.4
	4.50	4.59	102	2.0
	3.50	3.50	100	1.8

dramatically enhance the solubility, stability, and sensitivity of the analytical system. Obvious decreases in both the limit of detection (LOD) and the limit of quantification (LOQ) were achieved with respect to the solutions without β-CD.

3.9. Effect of Foreign Interferences. A systematic study was carried out on the effects of foreign interferences on the determination of 1.00×10^{-5} mol/L of TBZ. A 3500-fold mass excess of each interference over TBZ was tested first; if interferences occurred, the ratio was reduced gradually until the interferences ceased. The criterion for interference was fixed at a ±5% variation of the average absorbance intensity calculated for the established level of TBZ. The results are shown in **Table 5**, and it was obvious that the proposed method had excellent selectivity.

3.10. Application. To evaluate the accuracy of the proposed method, a standard adding method was performed to detect trace amounts of TBZ in fresh fruits. The results are shown in **Table 6**. As can be seen, the recovery was 96−103%.

3.11. Conclusions. The mechanism of the supramolecular interaction between TBZ and β-CD was studied and discussed. On the basis of the significant enhancement in the absorbance of TBZ produced through complexation with β-CD, a spectrophotometric method with improved sensitivity and selectivity for the determination of TBZ in bulk aqueous solution was developed. The proposed method has been successfully applied to the determination of trace amounts of TBZ in fruits.

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