

## The Reaction of Allyl Isothiocyanate with Hydroxyl/Water and $\beta$ -Cyclodextrin Using Ultraviolet Spectrometry

Zi-Tao Jiang\*, Qing-Feng Zhang, Hui-Lin Tian and Rong Li

Department of Food Science and Engineering, Tianjin University of Commerce, 300134 Tianjin, PR China

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### Summary

The reaction of allyl isothiocyanate (AITC) with hydroxyl/water and  $\beta$ -cyclodextrin ( $\beta$ -CD) in different acidic-alkaline media has been investigated by ultraviolet spectrometry. The kinetic parameters of the reaction were measured. It was found that after AITC translating into thiourea, the absorption peak shifted from 240 to 226 nm and the molar absorptivity increased about 16 times. The reaction can be seen as a pseudo first order reaction because the concentration of hydroxyl was constant.  $\beta$ -CD can inhibit the reaction of AITC with hydroxyl/water, *i.e.* the hydrolysis of AITC. The formation constant ( $K_a$ ) and thermodynamic parameters of the complex reaction were calculated.  $K_a$  decreased with the increase of temperature. The experimental results indicated that the inclusive process was an exothermic and enthalpy-driven process accompanied with a negative entropic contribution.

*Key words:* allyl isothiocyanate, hydroxyl,  $\beta$ -cyclodextrin, complexation

### Introduction

Allyl isothiocyanate (AITC) is one of the most important isothiocyanates (ITCs), which are enzymatic hydrolysates of glucosinolates that exist in the vegetables from *Cruciferous* family, such as horseradish (*Armoracia rusticana*) and wasabi (*Wasabia japonica* Matsum.). AITC is also a major flavour component with pungent taste and is easily decomposed in aqueous media. Horseradish and wasabi are used as an indispensable material for producing pungent spices and sauces, which are very popular in Hungary, England, Japan, Korea and China. Epidemiological studies have shown that a large intake of cruciferous vegetables can markedly reduce the risk of developing a variety of malignances (1,2). ITCs have high biological activities and have been used as an antibacterial agent. ITCs can also inhibit the platelet aggregation (3) and prevent both DNA-damage and the cancer caused by some dietary carcinogens such as polycyclic aromatic hydrocarbons, heterocyclic amines and nitrosamines (4). It is well known that the activation of dietary carcino-

gens is primarily catalyzed by phase I enzymes, such as cytochrome reductases, which are terminal oxidases of an enzymatic system that catalyzes the metabolism of a variety of foreign compounds and endogenous substrates. ITCs can inhibit the activity of phase I enzymes and/or induct that of phase II enzymes including quinone reductase, the glutathione S-transferases, epoxide hydrolase, and glucuronosyltransferase, detoxify these harmful carcinogens and convert them to hydrophilic metabolites (4–6). However, owing to electrophilic character of the ITCs' functional group, isothiocyanic group, ITCs react with some nucleophilic agents including amino, hydroxyl, thiol,  $\beta$ -dicarbonyls, carboxylic acids and water (7,8). Therefore, their lack of stability makes them very difficult to be used as food additives in aqueous media. Ohta *et al.* (9–11) carried out some related studies on improving their stability, and found that cyclodextrin (CD) may inhibit the decomposition in an aqueous solution.

$\beta$ -CD is made up of seven D-glucose monomers linked by  $\alpha(1,4)$  bonds, exhibiting the shape of a truncat-

\*Corresponding author; Phone: ++86 22 26 675 771, Fax: ++86 22 26 669 611, E-mail: ztjiang@yahoo.com

ed hollow cone. The cavities of  $\beta$ -CD are relatively more hydrophobic than water, whereas the external faces are hydrophilic (12).  $\beta$ -CD is very important in supramolecular chemistry because of its almost unique ability to form inclusion complexes with many different types of substrate compounds, widely used for improving the solubility and stability of the guest molecules (13,14). Recently,  $\beta$ -CD was approved for use in food by FDA, which has increased the interest in its food-related applications (15).

In the present study, the decomposition reaction mechanism of AITC in the aqueous solutions has been studied and UV-visible absorption spectrophotometry was used for the kinetic analysis. Furthermore, the formation constants of  $\beta$ -CD including AITC were measured based on the kinetic analysis.

## Materials and Methods

### Materials

AITC was separated from horseradish oil and further purified by a nitrogen protection distillation to collect 150–152 °C fractions in our laboratory, the content of which was more than 96 % by GC analysis. Other chemicals were of analytical reagent grade or better without further purification.  $\beta$ -CD was purchased from Tianjin Chemical Factory (Tianjin, China). Deionized water was used throughout the experiment.

A thermostatic shaker, model HZS-H was used to perform all inclusion procedures. A Unico UV-2102 spectrophotometer with matched 10-mm quartz cells was used to measure the absorbance.

### Determination of rate constant ( $k$ )

A volume of 10 mL of 2 mmol/L AITC solution and 10 mL of different concentrations of sodium hydroxide were added to a 50-mL stoppered conical flask. The final concentrations of sodium hydroxide in the reaction solutions were 2, 4, 6, 8 and 10 mmol/L. The contents of AITC in the reaction solution were determined at 5-min intervals according to the following process: 1 mL of the reaction solution was transferred to a 10-mL volumetric flask at 5-min intervals and diluted to the mark with deionized water, and then its absorbance was measured at 226 nm. The rate constants ( $k$ ) were calculated according to Eq. 1:

$$\ln \left[ \frac{(A_{\infty} - A_0)}{(A_{\infty} - A_t)} \right] = k \cdot t \quad /1/$$

where  $A_0$  and  $A_{\infty}$  were the absorbance of AITC and thiourea, respectively.  $A_t$  was the absorbance at time  $t$  (min),  $k$  was the rate constant,  $A_{\infty}$  was directly determined at  $[\text{AITC}] = 1 \text{ mmol/L}$  and  $[\text{OH}^-] = 1 \text{ mol/L}$ . Because  $[\text{OH}^-]$  was 1000 times of  $[\text{AITC}]$ , therefore, AITC can translate into thiourea completely. The slope of the straight line obtained by plotting of  $\ln[(A_{\infty} - A_0)/(A_{\infty} - A_t)]$  versus  $t$  was utilized to evaluate  $k$ .

### Determination of formation constants of AITC with $\beta$ -CD ( $K_a$ )

A volume of 10 mL of 2-mmol/L AITC solution and 10 mL of different concentrations of  $\beta$ -CD solution containing 4 mmol/L of sodium hydroxide were added to a 50-mL stoppered conical flask. The final concentrations of  $\beta$ -CD in the reaction solutions were 1, 2, 3, 4 and 5 mmol/L. The contents of AITC in the reaction solution were determined at 5-minute intervals according to the following process: 1 mL of the reaction solution was transferred to a 10-mL volumetric flask at 5-minute intervals and diluted to the mark with deionized water, and then its absorbance was determined at 226 nm. The formation constants of AITC with  $\beta$ -CD ( $K_a$ ) were calculated according to Eq. 2:

$$\frac{A_{\infty} - A'_{\infty}}{A'_{\infty}} = K_a \cdot \left( [\text{CD}]_0 - \frac{A_{\infty} - A'_{\infty}}{A_{\infty}} \cdot c_0 \right) \quad /2/$$

where  $A_{\infty}$  and  $A'_{\infty}$  were the absorbance of the monosubstituted thiourea in the absence or presence of  $\beta$ -CD, respectively, when AITC was completely translated into the monosubstituted thiourea.  $[\text{CD}]_0$  was the initial concentration of  $\beta$ -CD,  $c_0$  was the initial concentration of AITC,  $A_{\infty}$  and  $A'_{\infty}$  can directly be determined at different concentrations of  $\beta$ -CD. The slope of the straight line, *i.e.*  $K_a$ , was obtained by plotting of  $(A_{\infty} - A'_{\infty})/A'_{\infty}$  vs.  $\{[\text{CD}]_0 - c_0(A_{\infty} - A'_{\infty})/A_{\infty}\}$ .

## Results and Discussion

### Spectra of AITC in different pH

Ultraviolet absorption spectra of AITC in different pH are seen in Fig. 1. AITC has a maximum absorption at 240 nm in neutral and acidic solutions (pH=2–8). The molar absorptivity of AITC is very small at 240 nm, which is about 780 L/(mol·cm). However, when AITC was translated into the corresponding substituted thiourea in alkaline conditions (pH=9–12), whose molar absorptivity raises to 12 600 L/(mol·cm) and the maximum absorbance wavelength shifts to 226 nm, the molar absorp-

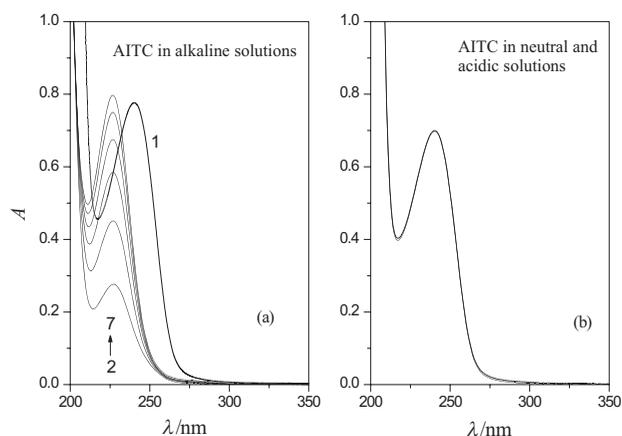
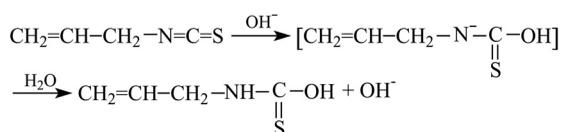


Fig. 1. Ultraviolet absorption spectra of AITC at different pH and reaction time  $c(\text{AITC})/(\text{mmol/L})$ : (1) 1, (curves 2→7) 0.1;  $t/\text{min}$ : 5, 10, 15, 20, 25 and 30; (a) pH=9–12, and (b) pH=2–8

tivity of the corresponding substituted thiourea was 16 times of AITC. It was observed that the absorbance of the solution rises with the increase of reaction time, which suggested that the formation of thiourea is gradual. Furthermore, the hydrolysis of AITC is easier in alkaline conditions than that in neutral and acidic solutions.

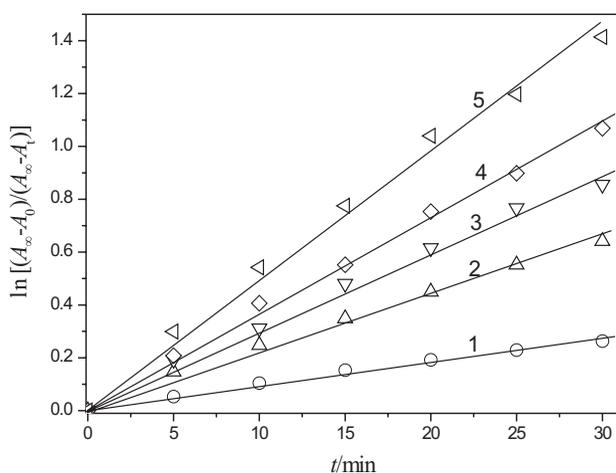
#### Kinetic analysis of AITC with hydroxyl/water

The reaction mechanism of AITC with hydroxyl/water has been proposed and shown in Scheme 1. Since there was no stronger nucleophilic agent in neutral and acidic conditions, AITC was very stable. In alkaline solutions, the hydroxyl could react directly with AITC to form the unstable intermediate, which captured a proton from the water immediately to become the stable thiourea. Because the concentration of hydroxyl was constant, the reaction of AITC with hydroxyl/water can be seen as a pseudo first order reaction (9):

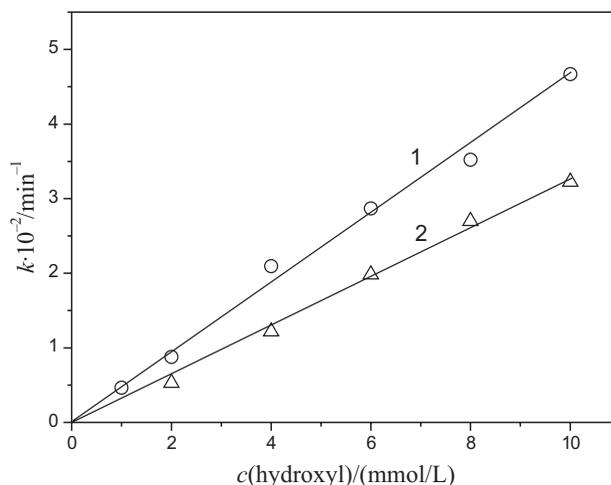


**Scheme 1.** The reaction mechanism of AITC with hydroxyl/water

The slopes of the straight lines obtained by plotting of  $\ln[(A_\infty - A_0)/(A_\infty - A_t)]$  versus  $t$  (Fig. 2) were also the rate constants,  $k$ , at different concentrations of hydroxyl. The high correlation suggested that the reaction could be really seen as a pseudo first order reaction. The  $k$  increased with the increase of the concentration of hydroxyl. As can be seen from Fig. 3,  $k$  versus  $[\text{OH}^-]$  gave a good line relationship. The linear equation was  $k=0.0587+0.456[\text{OH}^-]$ , the correlation was 0.996. Ohta *et al.* (10) determined the  $k$  of the reaction of AITC with hydroxyl/water in alkaline solutions at 5 °C by GC, and also found that the plot of  $k$  versus  $[\text{OH}^-]$  was a straight line. The mechanism of the reaction can also be proved by such an experimental fact that adding sodium hydroxide to



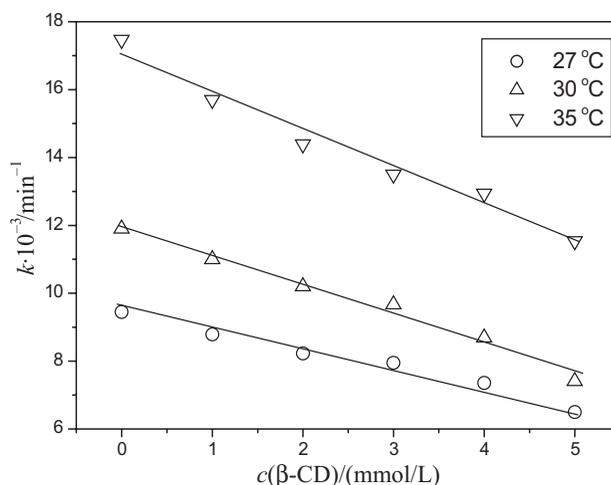
**Fig. 2.** Plot of  $\ln[(A_\infty - A_0)/(A_\infty - A_t)]$  versus time  $t$  (min) for different hydroxyl concentrations: (1) 2, (2) 4, (3) 6, (4) 8, (5) 10 mmol/L



**Fig. 3.** Plot of  $k$  versus  $[\text{OH}^-]$  at 27 °C for different  $\beta$ -CD concentrations: (1) 0, (2) 5 mmol/L

ethanol or hexane, the spectra of AITC remained unchanged. It was because the unstable intermediate could not capture the proton from ethanol and hexane and the reaction was stopped.

Fig. 4 shows the values of  $k$  at different temperatures in different  $\beta$ -CD concentrations. As can be seen,  $k$  increased drastically with the increase of temperature, which implied that the nucleophilic addition reaction between AITC and hydroxyl/water was endothermic. Using the well-known procedure through the Arrhenius equation, the activation energy of this reaction was calculated and the value was 59.0 kJ/mol. The relatively small activation energy revealed that the reaction is very easy to carry out and the nucleophilic ability of hydroxyl is very strong. We also calculated that the activation energies of reactions between AITC and piperidine, morpholine or diethyl amine were 76.0, 62.9 and 76.3 kJ/mol (16), respectively. The differences of the activation energies revealed that the nucleophilic abilities of the reagent are different. It was well known that the nucleophilic ability of hydroxyl was stronger than of amine.



**Fig. 4.** The rate constants,  $k$  at different temperature ( $[\text{OH}^-]=2$  mmol/L) for different  $\beta$ -CD concentrations

### Effect of $\beta$ -CD on the reaction of AITC with hydroxyl/water

It can be seen from Fig. 5 that the increases of absorbance are slower with the increases of the concentrations of  $\beta$ -CD. This implies that part of AITC was complexed by  $\beta$ -CD and isothiocyanic group of AITC was protected from the nucleophilic attack of hydroxyl by the steric hindrance of the torus. For comparison, the apparent rate constants ( $k_{\text{obsd}}$ ) were calculated (Fig. 3). The plot of  $k_{\text{obsd}}$  versus  $[\text{OH}^-]$  also gives a straight line, but the values of  $k_{\text{obsd}}$  are smaller than the  $k$  in the absence of  $\beta$ -CD. This result indicates the formation of inclusion complexes of AITC and  $\beta$ -CD. Fig. 4 shows that the plot of  $k_{\text{obsd}}$  versus  $\beta$ -CD concentrations is a straight line at different temperatures. This implies that the effect of  $\beta$ -CD on the reaction between AITC and hydroxyl/water is linear and can be calculated.

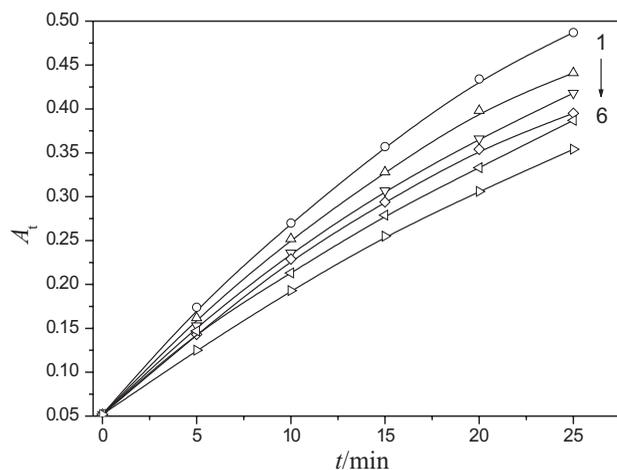
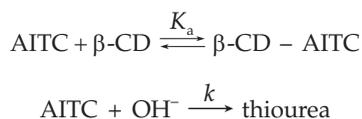


Fig. 5. The effect of  $\beta$ -CD on the reaction of AITC with hydroxyl/water at 35 °C  
 $[\text{OH}^-] = 2 \text{ mmol/L}$ ,  $c(\beta\text{-CD})/(\text{mmol/L})$ : 0, 1, 2, 3, 4, 5 (curves 1→6)

### Formation constants of AITC with $\beta$ -CD

The  $K_a$  is a parameter which only relies on temperature (17). At the same temperature, it should not make any difference whether the  $\beta$ -CD is present or not. The experiment results show that the reaction of AITC with hydroxyl is slower in the presence of  $\beta$ -CD than that in the absence of  $\beta$ -CD, which is because part of AITC was complexed by  $\beta$ -CD. When  $\beta$ -CD is added, AITC reacts according to the following two reactions:



Although the rate of the complex reaction is very fast and can reach the equilibrium in several seconds (12), at the beginning of the reaction there is an amount of AITC that is not complexed by  $\beta$ -CD. Therefore, it is reasonable that hydroxyl attacks first the naked AITC in the solution.

The straight lines obtained by plotting  $A_t$  versus  $(1 - \exp(-kt))$  at different concentrations of  $\beta$ -CD were utiliz-

ed to evaluate  $K_a$  (from the slope in Fig. 6). In addition, the thermodynamic parameters were calculated in Table 1 using the equations described (17).

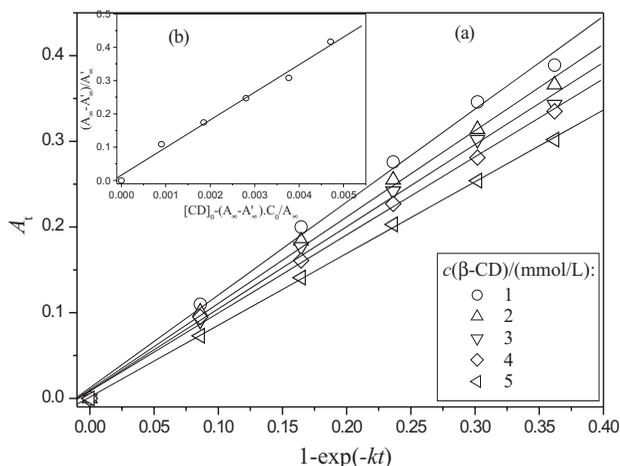


Fig. 6. (a): plot of  $A_t$  versus  $(1 - \exp(-kt))$  at 35 °C for calculating  $A'_\infty$  at different  $\beta$ -CD concentrations; (b): plot of  $(A_\infty - A'_\infty)/A'_\infty$  versus  $\{[\text{CD}]_0 - c_0(A_\infty - A'_\infty)\}/A'_\infty$  for calculating formation constants of AITC with  $\beta$ -CD ( $K_a$ )

Table 1. Formation constants ( $K_a$ ) and thermodynamic parameters for the binding of AITC to  $\beta$ -CD in 0.2-mmol/L sodium hydroxide medium

Temperature °C	$K_a$ L/mol	$R$	$-\Delta G$ kJ/mol	$-\Delta H$ kJ/mol	$-\Delta S$ J/kmol
27	$103 \pm 4$	0.986	11.56		
35	$90 \pm 4$	0.995	11.52	13.6	6.8
40	$82 \pm 3$	0.993	11.47		

### The stabilization of AITC in the complex

The stabilization of the complex (microcapsule) of  $\beta$ -CD including AITC was determined at room temperature and at 85 °C. Fig. 7 shows the plot of residual AITC versus time at 85 °C. AITC was a volatile component,

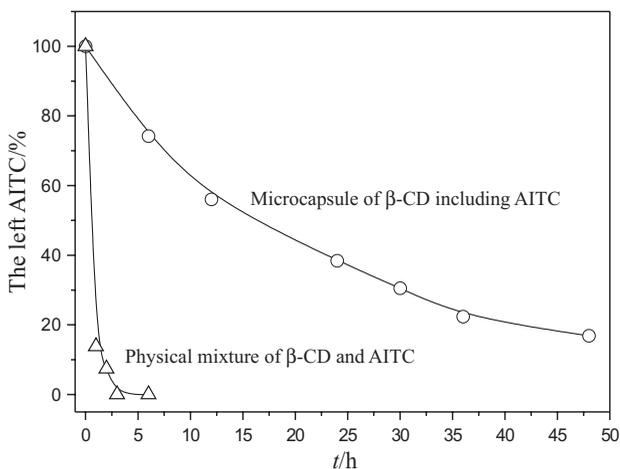


Fig. 7. Changes in AITC contents in the microcapsule at 85 °C

however, it was determined that there were more than 17 % left in the microcapsule after 48 h. Compared to the control sample (the physical mixture of  $\beta$ -CD and AITC), the content of AITC in 1 h decreased to 13 % and after 3 h AITC was unable to be determined. The result suggested that after AITC was included in the cavities of  $\beta$ -CD, the steric hindrance of  $\beta$ -CD torus gave a good protection against evaporation.

## Conclusions

Based on the fact that the  $\beta$ -CD can inhibit the hydrolyzation of AITC, we assumed that the isothiocyanic group is included in the cavities of  $\beta$ -CD. This presupposition is also reasonable in mechanics. The cavities of  $\beta$ -CD have high density of electron cloud and they present some characteristics of Lewis base (12). The inclusion of the electrophilic isothiocyanic group in  $\beta$ -CD cavities would make the dipole-dipole interaction between AITC and  $\beta$ -CD stronger and increase the van der Waals force. This would improve the stabilization of the complexes. Finally, the following conclusions can be obtained: (i)  $K_a$  decreases with the rise in temperature, which indicates that the complexation process is favourable at lower temperature. As the temperature increases, the affinity of the  $\beta$ -CD for AITC decreases. (ii) The negative value of  $\Delta G$  suggests that the complexation is a spontaneous process and thermodynamically favoured. (iii) The complexation processes have negative  $\Delta H$  values, implying that the processes are exothermic. The complex formation is entropically unfavourable as the inclusion process is accompanied by a decrease in entropy. In theory, the negative enthalpy change arises from the van der Waals force and the release of high-enthalpy water molecules from the cavities of  $\beta$ -CD, while the negative entropy change is the steric barrier caused by molecular geometrical shape and the limit of  $\beta$ -CD cavities for the freedom of shift and rotation of guest molecule (12). In this system,  $\Delta H$  and  $\Delta S$  are negative in the experimental temperature range, which indicates that the inclusion process is enthalpy-controlled and van der Waals force plays an important role in the process. (iv) The values of  $K_a$  between  $\beta$ -CD and AITC are relatively small. This is in accordance with the hydrophobicity and configuration of AITC molecules. AITC has relatively high solubility ( $w=0.2$  %) in water (18), so the hydrophobic interaction is not strong. The configuration of AITC is rod-like linear molecules, so the size-fit relation between  $\beta$ -CD and AITC is not very appropriate and it causes the van der Waals force to be weak. However, hydrophobic interaction and van der Waals force were considered to be the most important binding forces for the formation of inclusion complexes of CDs (12). It was assumed that using  $\alpha$ -CD (the internal diameters of the  $\alpha$ - and  $\beta$ -CD cavities are 0.47 and 0.6 nm, respectively) to get a better size-fit relation would attain a better complexation, which has been approved by Ohta *et al.* (11).

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