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ORIGINAL ARTICLE

Preparation, quantitive analysis and bacteriostasis of solid state iodine inclusion complex with β-cyclodextrin

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Abstract A solid state iodine/β-cyclodextrin inclusion complex (iodine/β-CD) was prepared by the saturated water solution method. The iodine/β-CD binding constant was 1,286 M^{-1} and the stoichiometric ratio was 1:1(I_2:β-CD). In order to determine the iodine content in the complex powder, a novel iodimetry method was investigated, and its accuracy and expeditiousness were verified by high performance liquid chromatography (HPLC). Release behaviors of iodine/β-CD powder were investigated by iodimetry and thermogravimetric (TG) analyzer respectively. The loss percentage of iodine was 5.8wt% for 7 days exposing under room temperature, and a time versus release curve was established. The TG diagram not only proved the stability of I_2 in the iodine/β-CD complex, but also clarified the thermal decomposition behavior of the iodine/β-CD. For verifying the germicidal activity of iodine encapsulated by β-CD, the *Aspergillus niger*, a kind of common fungus in the food, was taken as an evaluation media. The results indicate that by the method of disc diffusion, the discs with 1.5wt% iodine showed an obvious bacteriostasis effect for *Aspergillus niger*.

Keywords Iodine · β-cyclodextrin · Inclusion complex · Preparation · Germicidal activity

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Introduction

Iodine has a broad-spectrum ability of killing pathogens including: bacteria, fungus, virus and amebic protozoa etc. It has low toxicity and no side effects for mammalians. Due to the properties of easy sublimation, lower water-solubility, bad smell, corrosivity and strong dyeing character, however, its applications have been greatly restricted. In order to solve these problems, the iodine compositions with surfactants such as polyether polyol or povidone have been widely investigated [1–3]. The results have revealed that water-solubility and molecule stability of iodine composition have been greatly improved. Besides, antimicrobial activity and low toxicity have been retained. However, most of surfactants do not come from reproducible natural products, and even have environmental hazard. Moreover the combination of iodine and surfactants is not strong enough to give a relative ideal release time and thermal stability which could restrict the usage of iodine. So, a novel kind of environmental friendly natural reproducible materials need to be further investigated in the iodine composition researches.

Cyclodextrins(CDs) are torus-shaped cyclic oligosaccharides made up of α-1,4 linked D-glycopyranose with 6(α-), 7(β-), 8(γ-) units. They come from the decomposition of starch with cyclodextrin glucanotransferase(CGTase) and have non-toxicity which has been confirmed by Szejtli [4]. The inside cavity of β-CD is hydrophobic and the outside is hydrophilic. It is well know that CDs can effectively encapsulate some hydrophobic molecules to form inclusion complexes, which present higher water-solubility, the protection, and slow release of the guest molecules [5, 6]. So, some hydrophobic drugs were encapsulated into the cavity of CDs in order to enhance the water-solubility [7–10] or controlling release [5, 11–13]. Iodine, a hydrophobic molecule, can be transferred into the cavity of CDs to obtain iodine/

CDs complex with improving solubility and molecule stability [14]. But the thermal releasing behaviors and germicidal activity of the encapsulated iodine have not been studied yet.

Aspergillus niger, a fungus, is one of the most common species of the genus Aspergillus. It causes a disease called black mold on certain fruits and vegetables such as grapes, onions, and peanuts, and is a common contaminant of food [15]. Therefore, we take it as an example to study the bacteriostasis effect of iodine encapsulated by β-CD.

Accuracy and expeditiousness are needed in the works of determination of the guest molecule's content in the complex. Some methods, such as titration, UV-spectrophotometer, HPLC and fluorescence [16–18], are usually used. With regard to solid state complex, a proper dissolution is a key issue in the process of determination.

The aim of this work was to prepare the solid state iodine/β-CD powder and prove its bacteriostasis effect. The binding constant was determined and the saturated water solution method was used in the preparation of the solid state iodine/β-CD powder. The iodine content in the complex was quantified by two methods: one is iodemetry and the other is HPLC. In order to test the stability of the iodine encapsulated by β-CD, release experiments were performed under finite-time and different temperatures.

Experimental

Materials

β-CD was purchased from TCI Shanghai., which was dried for 24 h under 105 °C, Iodine (I_2) (guarantee reagent) and potassium iodide (analytical reagent) were offered from Kermel Co., Ltd. (Tianjin, China). Unless special notations, all the chemicals were analytical reagent grade and without further purification. *N*, *N*-Dimethylformamide (DMF), sodium acetate, acetic acid, disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium hydrogen carbonate, and sodium hydroxide were purchased from Tianjin Dongli Chemical Reagent Plant. Alcohol, methanol, acetone, and chloroform from Shanghai Chemical Reagent Plant. Distilled water was used throughout the entire study and ultrapure water prepared by Milli-Q century system (Millipore, American) was used in the chromatography researches. The iodine solution (I_2 solution) was prepared by the cosolvent of potassium iodide (KI) with the mass ratio of 1:10(I_2: KI).

Ultraviolet–visible spectra of iodine/β-CD solution with different pH value

The absorption spectra were recorded using a UV–Vis dual-beam spectrophotometer, Persee 1901 (China). All measurements were carried out using standard 1 cm thick quartz cuvette which was strictly sealed during the determination. Buffer solutions with different pH value (pH = 1–12) were prepared by hydrochloric acid, acetic acid-sodium acetate, disodium hydrogen phosphate-sodium dihydrogen phosphate and sodium hydrogen carbonate-sodium hydroxide.

0.028 mM iodine and 0.028 mM β-CD were respectively dissolved in different buffer solutions, then stirred for 3 h. After stored for 12 h, their UV–Vis absorption spectra were measured with the scanning scope of 270–400 nm and the buffer solutions were used as the references.

Determination of binding constant by Hildebrand-Benesi equation

Iodine solutions at a constant concentration of 0.024 mM were added to β-CD solutions with concentration of 1.66, 2.50, 3.32, 4.15, 4.98 mM, respectively. The buffer solution (pH = 5), as a diluent, was used in this process. The mixtures were stirred for 3 h and stored overnight at 25 °C in thermostatic water-circulator bath. Then, buffer solution (pH = 5) was used as reference, the measuring wavelength was fixed to 289 nm and the UV–Vis spectra of the aforementioned solutions were recorded in the range of 270–400 nm. The binding constant was calculated by Hildebrand-Benesi Eq. 1 [19]:

$$\frac{1}{\Delta A} = \frac{1}{K\Delta\varepsilon C_{I_2} C_{\beta-CD}} + \frac{1}{\Delta\varepsilon C_{I_2}} \quad (1)$$

where ΔA is a change of absorbance after addition of $\beta - \mathrm{CD}(\Delta A = A_{\mathrm{iodine}/\beta-CD} - A_{I_2})$, C_{I_2} and $C_{\beta-CD}$ are the total molar concentrations of iodine and β-CD, respectively, K is the binding constant and $\Delta\varepsilon$ is the difference of the molar absorptivity between iodine/β-CD and I_2. If the resulting plot of $1/\Delta A$ against $1/C_{\beta-CD}$ yields a straight line, the stoichiometric ratio of 1:1 (I_2 and β-CD) is expected, and the binding constant K can be calculated by the Eq. 2:

$$K = \frac{\mathrm{slope}}{\mathrm{intercept}} \times 1,000 \quad (2)$$

Preparation of solid state iodine/β-CD complex

50 mL I_2 solution (58 mM) was dropped into 150 mL β-CD solution (pH = 5, 19.3 mM) in 250 mL conical flask. The mixture in the conical flask was sealed by parafilm and stirred for 3 h with magnetic stirrer, then stored for 12 h in ice-water-bath (273 K or desired temperature controlled in thermostatic water-circulator bath) in order to encapsulate the iodine completely. After storage, some brown precipitation was collected by vacuum filtrating. Then the precipitate was washed by deionized water (100 mL) and KI solution (1.2 mM, 100 mL),

respectively. The final product was spread evenly on watch glass and dried at 45 °C for 24 h in a vacuum drying oven.

Quantification of iodine in the complex powder

Method of iodimetry

0.2 g iodine/β-CD was weighed precisely in iodine flask, then added to 4 mL DMF and made sure the iodine/β-CD dissolved completely. Thereafter, 140 mL deionized water was added and titrated by $Na_2S_2O_3$ standard solution (0.01 M). Titration was continued until the solution turned out light yellow and added 3 mL starch-iodine indicator (0.5wt %). In the meantime, the solution turned out blue and continued to titrate until turned out colorless. Every sample was titrated thrice. The iodine percentage is given by formula 3:

$$\text{Iodine percentage} = 0.1269 \times \frac{C_{Na_2S_2O_3} \times V_{Na_2S_2O_3}}{m} \times 100\% \quad (3)$$

where $C_{Na_2S_2O_3}$ denotes the concentration of sodium thiosulfate standard solution, and $V_{Na_2S_2O_3}$ is the volume of sodium thiosulfate standard solution added during titration (mL), m represents the weight of the iodine/β-CD.

Method of high performance liquid chromatograph (HPLC)

The HPLC system belonged to Agilent 1,100 series and consisted of a RID detector (G1362A, Agilent), a Rheodyne manual injector fitted with a 20 μL loop and a Agilent-Hypersil ODS column (250 × 4.6 mm, 5 μm). 15%(v/v) methanol and 85%(v/v) deionized water was used as mobile phase at a flow rate of 0.7 mL/min. Before injecting, following samples were prepared: A. 100 μg/mL β-CD solution was prepared with 15%(v/v) methanol solution. B. 0.0283 g iodine/β-CD powder dissolved in 1.00 mL DMF was weighed and diluted with mobile phase in 50 mL volumetric flask. C. 1.00 mL DMF was diluted with mobile phase in 50 mL volumetric flask. D. 0.0263 g standard I_2 dissolved in 2.00 mL DMF was weighed and diluted with mobile phase in 100 mL volumetric flask. Every sample was injected thrice.

Release experiment of solid state iodine/β-CD

Release of iodine by finite-time

Solid state iodine/β-CD inclusion complex was spread evenly on the surface of watch glass (11 cm) and put into hermetic draught cupboard. The iodine content was determined using iodimetry, and the titer of sodium thiosulfate to solid state iodine/β-CD complex was 0.01227 g/mL. The time interval for determination was 0, 2.3, 24, 48, 72, 96, 120, 144, and 166 h, respectively.

Release of iodine by different temperatures

TG curves were recorded on Perkin-Elmer Pyirs1 thermogravimetric analyzer between 30 and 400 °C at a constant heating rate of 5.0 °C/min under pure nitrogen gas. The solid samples were iodine/β-CD inclusion complex and β-CD. The β-CD powders was predried under 105 °C for 24 h and placed in desiccators for cooling down to room temperature. The iodine/β-CD inclusion complex was predried under 45 °C by vacuum drying for 12 h and also placed in desiccators for cooling down.

Bacteriostasis experiments by iodine/β-CD with disc diffusion method

Quantitative filter papers with a diameter of 1 cm were used as some experimental diffusion discs. The discs were sterilized by electric sterilizer (BILON-D-1, Depai biology Corp. China), and then dipped into iodine/β-CD solutions with 0.5, 1.0, and 1.5wt % iodine for 10 min. They were dried at 45 °C under vacuum condition for standby.

The sterile pipette was used to transfer 0.1 mL bacterial suspensions of *Aspergillus niger* (3 mg/mL) to spread evenly on a plate which was pour with complete medium (beef extract, peptone, agar). Every plate was placed on 3 discs with the same iodine weight percentage and cultivated for 24 h in order to examine the effects of bacteriostasis [20–22].

Results and discussion

Influence of pH value and determination of binding constant

Figure 1 shows the ultraviolet–visible spectra of iodine/β-CD inclusion complex with different pH solvent. It is observed that the shape of ultraviolet spectrum of the iodine/β-CD does not have any change among the pH range from 1 to 9, but the absorbance is changed regularly, especially at the wavelength of 289 nm. At the pH value of 10 and 11, there are no spectra at all. This phenomena can be explained as follows: I^-, IO_3^- and β-CD do not have any UV spectrum in the wavelength scope of 270–400 nm, but only I_2 has. So the change of absorbance is due to the change of iodine content which depends on the dismutation reaction equilibrium under different alkali environment (Eq. 4). The stronger alkalinity is, the lower the content of iodine is.

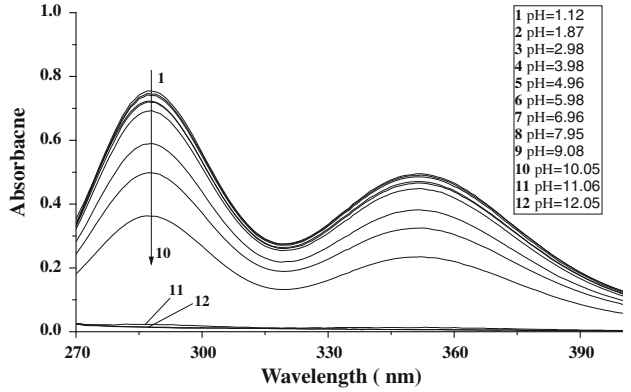

Fig. 1 Ultraviolet–visible spectra of iodine/β-CD inclusion complex under various pH values

$$I_2 + 6OH^- \rightleftharpoons IO_3^- + I^- + 3H_2O \quad (4)$$

β-CD takes place the hydrolysis to produce glucose or acyclic maltose in the acidic condition [4, 23], therefore, pH of the solutions was kept to be 5, as a preferred value.

Compared with I_2, the spectrum shape of iodine/β-CD does not change, but the absorbance increases slightly with the addition of β-CD [24] (Fig. 2). This result demonstrates that the distribution of the energy states of the I_2 molecule would not be changed when I_2 was encapsulated by β-CD, that is, the probability of the electron transition would not be changed either [25]. Lambert–Beer Law (Formula 5) [26] is given:

$$A = 0.4343 N_A a_i l c \quad (5)$$

where N_A denotes Avogadro's number and l denotes the length of a cuvette. c is the concentration of analyte and a_i is the product of the value of sectional area and the probability of the electron transition of the molecule.

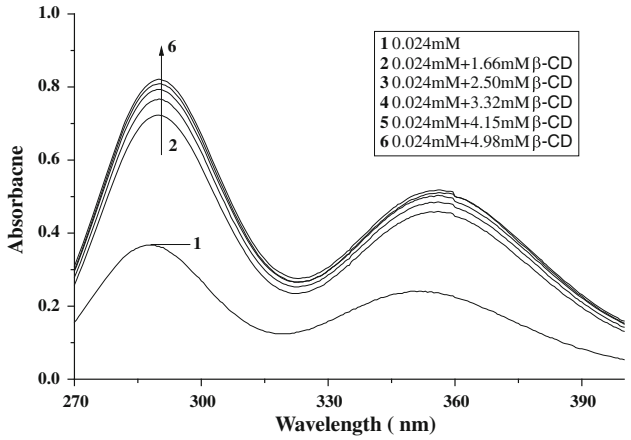

Fig. 2 Ultraviolet–visible spectra of I_2 and iodine/β-CD inclusion complexes

Table 1 Data for the determination of stability constant

$C_{\beta\text{-}CD}$/mM	0	1.66	2.50	3.32	4.15	4.98
A	0.367	0.723	0.766	0.791	0.806	0.819
$1/C_{\beta\text{-}CD}$		2.81	2.51	2.36	2.28	2.21
$1/C_{\beta\text{-}CD}$/mM^{-1}		0.60	0.40	0.30	0.24	0.20

According to Lambert–Beer Law and the analysis discussed above, the absorbance (A) depends on the value of sectional area (a_i). Compared with I_2 the sectional area of iodine/β-CD complex became large, therefore, resulting in the increase the value of the absorbance.

Based on Hildebrand-Benesi Eq. 1 and experimental results listed in Table 1, the binding constant can be calculated [19]. First of all we can obtain the regression Hildebrand-Benesi equation ($y = 1.4894x + 1.9157$) with correlation coefficient (r) 0.9998, and then the binding constant (K) (1,286 M^{-1}). Because of the linearity of the equation, the stoichiometric ratio of 1:1(I_2: β-CD) is fixed. For this reason, the ratio of β-CD and iodine was chosen 1:1 (molar ratio) during the preparation of iodine/β-CD complex powder. On the other hand, due to high binding constant (1,286 M^{-1}), the encapsulation is a spontaneous process and the iodine/β-CD complex shows the ideal thermal stability and time release behavior, which are discussed in the following sections.

Comparison of the methods between the modified iodimetry titration and HPLC

A properly quantitive method of the iodine content is needed in the study of the release behaviors of iodine/β-CD complex. Accuracy and expeditiousness are the key issues in the determination. A suitable solvent is the guarantee of the accuracy, because it can completely dissolve the complex and reduce the error in the processes of weighing, titration and the determining by HPLC. In our experiment of iodimetry, dissolving iodine/β-CD powder in water, potassium iodide solution, alcohol, acetone and chloroform did not give a satisfying result, except methanol and DMF. For obtaining a obvious titration endpoint, the methanol concentration must be kept at no more than 5%(v/v), but such concentration can not dissolve the complex powder unless 50%(v/v). However, if the solvent is DMF, these phenomena will not happen. Nowdays It has not been reported that DMF, as a solution, was applied to dissolve out the iodine molecule encapsulating in the iodine/β-CD complex powder. Furthermore, DMF not only accelerates the dissolving rate of the iodine/β-CD complex powder and shortens the assay time, but also increases the weighing dosage of the complex powder and reduces the weighing error. Based on this experiment procedure, the titration data are given in Table 2.

Table 2 Titration data of iodine/β-CD titrated by 0.0103 mol/L sodium thiosulfate

	Weight of iodine/β-CD powder (g)	Volume of sodium thiosulfate (mL)	Iodine percentage (%)	Average of iodine percentage (%)
1	0.2147	28.17	17.15	17.32
2	0.2024	27.00	17.44	
3	0.2035	27.05	17.37	

The same sample was also determined by the method of HPLC. The chromatograms are shown in Fig. 3. Compared the chromatogram B with A, according to the retention time, we can confirm the peak of 6.840 min is β-CD (Fig. 3B). Then, the same comparison of C with D, the peak of 2.283 min is iodine (Fig. 3B). According to retention data showed in Table 3 the iodine concentration of iodine/β-CD inclusion complex solution was 97.9 μg/mL. Finally, we obtained the percentage of iodine in the iodine/β-CD powder, 17.30wt%, which is coincided with the result of iodimetry.

The accuracy of the iodimetry was corroborated by the method of HPLC, furthermore, the method of iodimetry is more convenient and wider-range use in applications.

The preparation of solid state complex powder

Saturated water solution method was used in the preparation of solid state complex powder. Since iodine sublimate quickly at the temperature of 318 K and the freezing point of water is 273 K, the inclusion balance temperature was selected between 273 and 303 K with a temperature interval of 10 K. The results are shown in Fig. 4. With the increase of inclusion balance temperature, the iodine content decreased linearly. This results indicate that the encapsulation is an exothermic process. Therefore, lower temperature is beneficial to the encapsulation of iodine. Furthermore, from Fig. 5, when the stirring time was longer than 1 h, the inclusion process was in the balance and the iodine content reached 17.5%.

Time stability and release behavior analysis of solid state iodine/β-CD

Figure 6 shows time stability of iodine/β-CD complex. The iodine lost percentage is no more than 5.8wt % when it was placed for a week under an open system. This result may

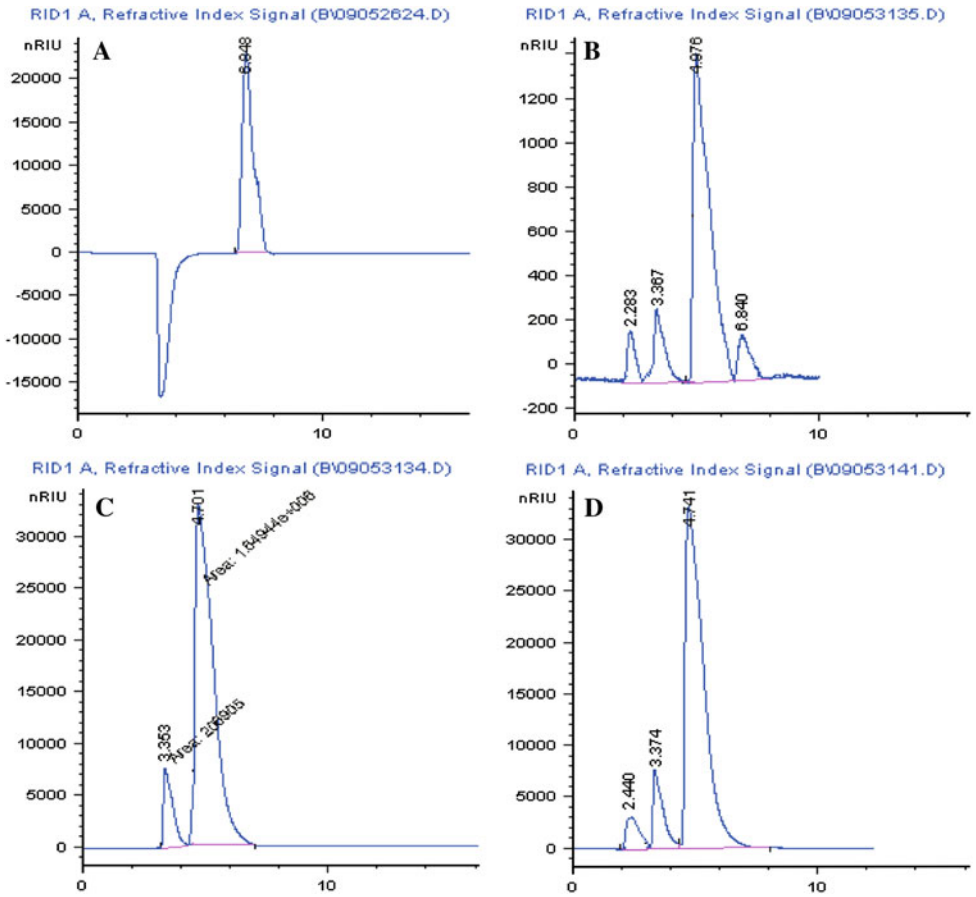

Fig. 3 HPLC diagram: **A** β-CD; **B** iodine/β-CD complex dissolved in DMF; **C** DMF; **D** iodine dissolved in DMF

Table 3 Retention data of iodine standard solution and iodine/β-CD inclusion complex

Analyte	Retention time (min)	Area	Average area	Concentration (μg/mL)
Iodine standard solution	2.440	20519.0	20331.7	263.0
	2.311	20236.0		
	2.366	20240.0		
Iodine/β-CD inclusion complex solution	2.283	7529.6	7568.8	97.9
	2.290	7593.5		
	2.289	7583.4		

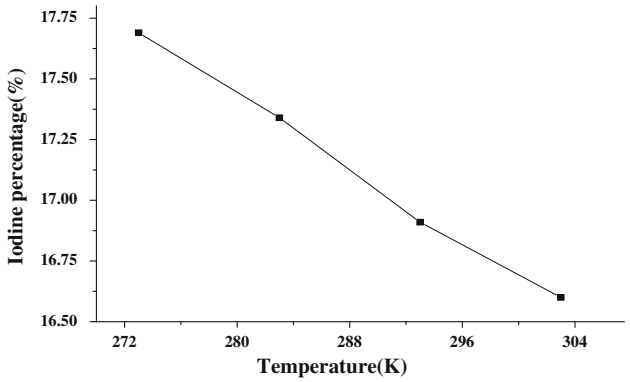

Fig. 4 Influence of balance temperature on iodine percentage

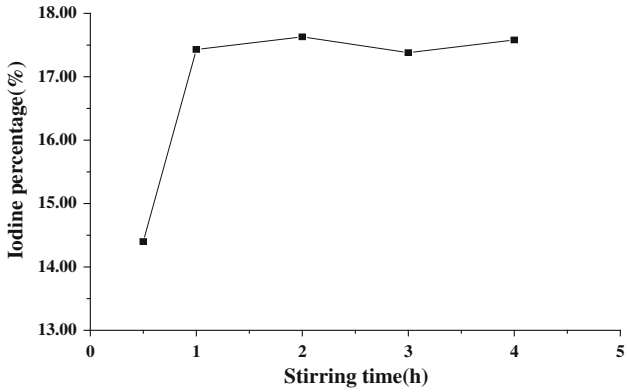

Fig. 5 Influence of stirring time on iodine percentage

prove that iodine complex powder could not only release iodine molecules, which have the ability of bacteriostasis, but also could control the releasing rate of iodine. Using the researches of Tze-Lon Neoh [14], the model of Avrami's Eqs. (6, 7) could be applied to correlate the release time-course of spray dried complex of CDs.

$$R = \exp(-[kt]^n) \tag{6}$$

$$R = \frac{\text{Amount of iodine remained after prescribed time}}{\text{Initial amount of iodine}} \tag{7}$$

Then, formula (8) could be leaded out by the formula (6)

$$\ln(-\ln R) = \ln k + n \ln t \tag{8}$$

$n = 0.32$ and $k = 5.78 \times 10^{-4}$ s^{-1} could be obtained by linear regression. The values is similar with the work of

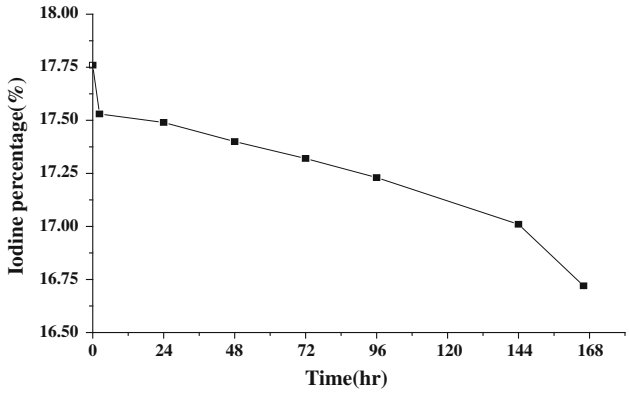

Fig. 6 Curve of solid state iodine/β-CD stability versus time

Tze-Lon Neoh [14] and the release behavior is also belong to a diffusive release process. The slight differences probably come from the experiment conditions. This research was carried out under the atmosphere of a relative open system and more closed to practiced application.

Thermal stability and decomposition behavior analysis of solid state iodine/β-CD

Figure 7 illustrates TG curves of β-CD and iodine/β-CD. The weight loss process of β-CD could be divided into two stages. The first weight loss in the range of 25–82 °C is

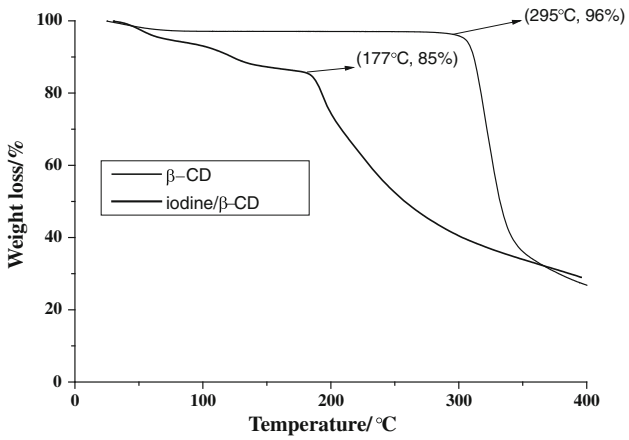

Fig. 7 TG diagrams of β-CD and iodine/β-CD

Fig. 8 Inhibitory effect of *Aspergillus niger* by I_2/β-CD solution with different iodine weight percentage content: **A** 0.5%; **B** 1.0%; **C** 1.5%

attributed to the release of water molecule combined on the β-CD powder, even though the powder of β-CD is seriously protected from recombination of water by our preparation [27]. The second weight loss started at the temperature of 297 °C, it is due to the decomposition of β-CD.

Compared with the TG curve of β-CD, the weight loss process of iodine/β-CD complex could be divided into three stages. The first weight loss in the range of 39–79 °C is attributed to the release of adsorption iodine and small amount of combined water from β-CD, even though the solid state of iodine/β-CD complex powder is predried at 45 °C for 24 h in vacuum drying oven and placed in desiccators for cooling down. The second weight loss in the range of 79–177 °C is attribute to the release of the iodine from the cavity of β-CD, probably caused by the channel crystal lattice collapse of the complex. The relative strong combination of the iodine/β-CD complex protects the iodine molecule from subliming early. Compared with the sublimation of iodine at the temperature of 45 °C, the iodine encapsulated by β-CD demonstrates a good thermostability. The third weight loss which started at the temperature of 177 °C is attributed to the decomposition of β-CD. Since iodine probably reacts with the small amount of absorption water in the complex to produce hydroiodic acid and hypoiodous acid at higher temperatures. The fact has proved that acid can effectively catalyze the hydrolysis of β-CD [23]. Therefore, the early decomposition of β-CD molecule in the complex. Besides, the total weight loss of the aforementioned two stages is almost 15%, which is similar with the weight percentage of iodine in the iodine/β-CD complex powder, so the third stage is further proved to be the weight loss of β-CD.

The inhibitory effect of iodine/β-CD on *Aspergillus niger* by disc diffusion method

Figure 8 gives the inhibitory effect of iodine/β-CD on *Aspergillus niger* with 0.5, 1.0, and 1.5wt% iodine. Comparing the three pictures, it is seen that the discs treated with iodine/β-CD solutions containing 1.5wt % iodine (Fig. 8C) were given a clear inhibitory effect with an average bacteriostasis diameter of 2.9 cm. This result demonstrates a strong inhibitory effect on *Aspergillus niger*. The discs treated with iodine/β-CD solution containing 1.0wt% iodine (Fig. 8B) showed kind of inhibitory effect, but the ones of 0.5wt% (Fig. 8C) hardly gave a satisfactory result.

Conclusion

Based on the stoichiometric ratio of iodine and β-CD in the complex (1:1) and the binding constant (1,286 M^{-1}) obtained from the linear regression of Hildebrand-Benesi equation, the encapsulation is a spontaneous process. Due to lower temperature is beneficial to the preparation of the complex with high iodine content, the encapsulation is also a exothermic process, and the iodine content reached 17.32wt %. In order to determine the iodine content in the complex powder, two methods were investigated, and the method of iodimetry shows not only its accuracy but also expeditiousness and convenience. Using the iodimetry and TG analysis, the time and thermal stabilities of the iodine/β-CD complex are clearly enhanced The bacteriostasis experiment results demonstrate that the discs treated with iodine/β-CD solution containing 1.5wt% iodine presents a strong inhibitory effect on *Aspergillus niger*.

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