Physicochemical properties of catechin/β-cyclodextrin inclusion complex obtained via co-precipitation

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ABSTRACT

Inclusion complex of catechin (CAT) with β-cyclodextrin (β-CD) was prepared using co-precipitation method to enhance antioxidant stability of CAT and the physicochemical properties of the inclusion complex were studied. The CAT/β-CD inclusion complex was analyzed through phase solubility study, Fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC), scanning electron microscopy (SEM) and X-ray diffraction (XRD). Phase solubility study indicated that CAT and β-CD formed 1:1 stoichiometric inclusion complex. Results of FT-IR indicated that CAT was stabilized in β-CD cavity by intra-molecular hydrogen bonds. Results of DSC and SEM proved that CAT/β-CD inclusion complex formed. XRD results showed that the formation of new solid crystalline phases in the CAT/β-CD inclusion complex. CAT was effectively protected through encapsulation into β-CD and the antioxidant stability of CAT was improved after encapsulation. In addition, the release behavior of CAT from the inclusion complex increased with increasing of the temperature.

1. Introduction

As plant polyphenols, catechin (CAT) usually exists in wine, tea, cocoa and fruit products (Arts, Hollman, & Kromhout, 1999; Liu, Lu, Kan, Wen, & Jin, 2014; Pascual-Teresa, Santos-Buelga, & Rivas-Gonzalo, 2000). Antioxidant and free radical scavenging are the important properties of CAT (Jullian, Miranda, Zapata-Torres, Mendizábal, & Olea-Azar, 2007). Numerous studies have demonstrated the roles of CAT in anti-platelet aggregation (Chang & Hsu, 2014), anti-inflammatory (Fan, Sang, & Jiang, 2017) and anti-bacterial properties (Silva et al., 2011). CAT is known to be a functional ingredient with great commercial value and has potential applications in foods and medicines. The Food and Drug Administration of USA has already issued safety certification for CAT (Hara, 2011). However, it is often difficult to incorporate CAT into functional foods and medicines due to its bitter, astringent taste, poor water solubility and physicochemical stability (Folch-Cano, Jullian, Speisky, & Olea-Azar, 2010; Ho, Thoo, Young, & Siow, 2017a).

A variety of encapsulation methods have been used to improve stability and solubility of certain ingredients in medicines and foods. Among all kinds of encapsulant systems, cyclodextrins (CDs) could be deemed as one of the simplest and most efficient one (Ciobanu, Landy, & Formenton, 2013). CDs are nontoxic cyclic oligosaccharides produced by hydrolysis of starch resulting from intramolecular transglycosylation reactions caused by CD glucanotransferase enzyme. The most popular CDs are α-CD, β-CD and γ-CD with 6, 7 and 8 units of (1-4)-linked α-D-glucopyranose, respectively (Valle, 2004; Wang et al., 2015). The special arrangement of molecules makes CDs exhibit a hydrophobic inner cavity and hydrophilic outer surface. This feature enables CDs to be hosting both non-polar and polar guests, no matter if they are polymers or small molecules (Davis & Brewster, 2004; Wang et al., 2015).
When guests are combined with CDs, the inclusion complex can increase the solubility of the guest, improve taste and odor, protect against oxidation, light and heat decompositions and reduce volatilization (Ho et al., 2017a; Marques, 2010). Moreover, the release profile of guest molecules can also be controlled (Wang, Cao, Sun, & Wang, 2011). Among the three major kinds of CDs, the most commonly used is β-CD which matches with most of the regular guests within the molecular weight range of 200 ~ 800 g/mol and its cost is also quite reasonable (Waleczek, Marques, Hempel, & Schmidt, 2003). There were some previous works related to the complexation between CAT and CDs (Ho et al., 2017a, 2017b; Jullian et al., 2007; Krishnaswamy, Orsat, & Thangavel, 2012; Żyżelewicz, Oracz, Kaczmarska, Budryń, & Grzelczyk, 2018). The results indicated that CAT can be encapsulated into the cavity of CDs. Compared with α-CD and γ-CD, β-CD usually gave the highest constants of stability (Jullian et al., 2007). However, in the previous studies, the CAT/β-CD inclusion complex was obtained by drying the solution (Ho et al., 2017a, 2017b; Krishnaswamy et al., 2012). Due to the high amount of solvent in the solution, drying usually takes a long time, which leads to high energy consumption. In addition, long-term high temperature drying will result in charring and thermal degradation of the inclusion complex (Hedges, 1998).

Co precipitation method is deemed to be one of the most commonly used methods to obtain CDs inclusion complexes, with the advantage of simplicity and efficiency (Ayala-Zavala et al., 2008; Hedges, 1998). In this technique, a certain amount of CDs is dissolved in water and ethanol solution containing guest is added with agitation (Bhandari, D’Arc, & Bich, 1998). During cooling, crystallization and precipitation occur and the inclusion complexes can be obtained after washed and dried (Marques, 2010). As the filter cake contains less water, the drying time for obtaining the inclusion complexes is shortened, which is more conducive to reducing energy consumption and thermal degradation of the inclusion complexes in the drying process. The inclusion complexes form new solid crystalline phases which improve physicochemical stability of the guest molecules, such as volatility (Zhang et al., 2015), oxidation (Tsai, Tsai, Wu, & Tsai, 2010; Wang et al., 2011) and thermal stability (Yang, Yao, Xiao, Chen, & Ji, 2016; Zhang et al., 2015). The release behavior could also be controlled by encapsulating guest molecules in new solid crystalline phases (Zhang et al., 2015). Stable physicochemical properties and controllable release behavior increase the potential applications of these inclusion complexes in foods and medicines.

Although the co precipitation method has the mentioned advantages, there are few reports in the scientific literature on the preparation of CAT/β-CD inclusion complex by the co precipitation method, the understandings of physicochemical properties, antioxidant stability, and releasing behavior of CAT/β-CD inclusion complex obtained by the co precipitation method still need to deepen. In this paper, CAT/β-CD inclusion complex was prepared using co precipitation method and characterized in terms of phase solubility study, differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM) and X-ray diffraction (XRD). Furthermore, the antioxidant stability and release profiles of CAT in the CAT/β-CD inclusion complex were investigated.

2. Materials and methods

2.1. Materials

(+)-CAT (purity >98%) and 2,2’-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diaminonium salt (ABTS) were obtained from Macklin Biochemical Co., Ltd. (Shanghai, China). β-CD was bought from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Other chemicals were all of analytical grade.

2.2. Phase solubility study

Analysis of phase solubility was implemented based on the method from Higuchi and Connors (1965). CAT (excess amount) was mixed with 20 mL of β-CD aqueous solutions in different concentration levels (0, 2, 4, 6, 8, 10, 12 and 14 mM). After mixing, the solutions were kept stirring for 24 h at room temperature in darkness. The resulting solutions were then screened with a hydrophilic membrane filter (0.45 μm). Then 1 mL of the filtrate was sampled and diluted 100 times with anhydrous ethanol, treated with ultrasound for 20 min and centrifuged at 2500 rpm for 10 min. The CAT content in the supernatant was analyzed using a UV-visible spectrophotometer (UV-2550, Shimadzu, Japan) at 230 nm. All experiments were performed in triplicate. The apparent stability constant ($K_c$) was calculated using Higuchi-Connors equation based on the straight-line part of the phase solubility diagram:

$$K_c = \frac{slope}{S_0 \times (1 - slope)}$$

where $S_0$ is the solubility of CAT when there is no β-CD.

2.3. Preparation of CAT/β-CD inclusion complex

CAT/β-CD inclusion complex was prepared using the co precipitation method (Wang et al., 2011) with a slight alteration. Based on the result of phase solubility studies, CAT and β-CD with a molar ratio of 1:1 were used for the inclusion. The β-CD solution was obtained by dissolving β-CD (3.41 g) in 50 mL ethanol/water mixture (1/2 in volume) at 60°C. CAT solution was prepared by blending CAT (0.87 g) with anhydrous ethanol (25 mL) in a centrifuge tube. After the β-CD solution cooled to 40°C, the CAT solution was added into the β-CD solution dropwise with magnetic stirring at 200 rpm. This mixture was kept continuously stirring for 5 h in dark and then maintained at 4°C overnight. After cold precipitation, CAT/β-CD inclusion complex was obtained via vacuum filtration. The precipitate was rinsed by using 30% ethanol aqueous solution to remove the CAT on surface of β-CD and then freeze-dried. The resulted inclusion complex powder was stored at 4°C for further analysis.

2.4. Physical mixture of CAT/β-CD

CAT (0.87 g) was mixed with β-CD (3.41 g) in a mortar by a plastic spoon to create a homogeneous mixture and used as control.
2.5. Determination of CAT in inclusion complex

CAT anhydrous ethanol solutions with different concentrations (1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 μg/mL) were prepared. Calibration curve of CAT was generated by measuring the absorbance at 230 nm with a UV-visible spectrophotometer. The regression equation was as follows:

\[ Y = 0.057X + 0.002, R^2 = 0.999 \]  

Where \( X \) is the absorbance and \( Y \) is the concentration (μg/mL) of CAT.

The content of CAT in inclusion complex was determined following the previously reported method (Wang et al., 2011) with slight modification. The sample of inclusion complex and anhydrous ethanol were mixed in a flask, treated with ultrasonic for 20 min, and then centrifuged at 2500 rpm for 10 min. The supernatant was measured at 230 nm and CAT content was determined using the calibration curve. The total recovery, inclusion ratio and loading capacity of CAT were calculated by the following equations (Wang et al., 2011; Wen et al., 2016):

\[
\text{Total recovery} \% = \frac{M_0}{M_1 + M_2} \times 100
\]  

\[
\text{Inclusion ratio} \% = \frac{M_1}{M_2} \times 100
\]  

\[
\text{Loading capacity} \% = \frac{M_3}{M_0} \times 100
\]

where \( M_0 \) is the weight of recovery inclusion complex, \( M_1 \) and \( M_2 \) are the initial weight of β-CD and CAT, \( M_3 \) is the weight of CAT in inclusion complex. All measurements were performed 3 times.

2.6. Characterizations

Spectra of FT-IR, from 4000 to 400 cm\(^{-1}\) were recorded using an FT-IR instrument (IRAffinity-1 SHIMADZU, Japan) with 4 cm\(^{-1}\) and 32 scans as resolution. The samples were ground with KBr (1:100, w/w) and then compressed into an ultrathin disc for the measurements.

Thermal properties of samples were analyzed with a DSC (DSC 7, Perkin-Elmer, USA). All samples (3–4 mg) were sealed in aluminum pan. The samples were heated from 30 to 260°C with rate 5°C/min under nitrogen atmosphere at a flow rate of 25 mL/min.

XRD patterns were recorded with Rigaku D/max2500 X-ray diffractometer (Rigaku Corporation, Tokyo, Japan) using Cu Ka radiation (\( \lambda = 1.542 \text{ Å} \)). The samples were scanned over a 2θ in the range of 5–30° at 2°/min.

Morphology of samples was observed by using SEM (Zeiss, MERLIN Compact, Germany). Carbon black tape was used to attach the samples on specimen stubs, and gold was sputter-coated on surface the sample before observation.

2.7. Antioxidant stability of CAT/β-CD inclusion complex

Antioxidant stability of CAT/β-CD inclusion complex was evaluated following to the method described by Ho et al. (2017a). Aqueous solutions of CAT, CAT/β-CD physical mixture and CAT/β-CD inclusion complex with concentration of 1 mg/mL were prepared in capped test tubes. The resulting solutions were then heated at different temperatures (65, 85, 100 and 120°C) for different times (30 and 60 min). After the temperature cooled down, the total antioxidant activity was measured using ABTS method described by Re et al. (1999) with slight alteration. ABTS solution (2 mL, 7.4 mM) was mixed with potassium persulfate solution (2 mL, 2.45 mM) in a 5 mL centrifuge tube. The mixture was kept in darkness for 12 h at room temperature. Ethanol was added into the obtained green ABTS\(^{+}\) solution to dilute it 50 times. Absorbance of the diluted ABTS\(^{+}\) solution at 734 nm was recorded. The diluted ABTS\(^{+}\) solution (2 mL) was mixed with 0.5 mL of the prepared aqueous solution of the samples in a 5 mL centrifuge tube and kept still for 6 min. The absorbance of the sample solution at 734 nm was recorded. All measurements were repeated in triplicate. The total antioxidant activity was calculated using the following equation (Wu et al., 2018):

\[
\text{Total antioxidant activity} \% = \left( \frac{A - A_i}{A} \right) \times 100
\]

where \( A \) is the absorbance of the diluted ABTS\(^{+}\) solution at 734 nm, \( A_i \) is the absorbance when sample was added. The sample (4 g) was placed in a dark box (60 cm×60 cm×130 cm) at room temperature (23 ± 1°C). The box was ventilated every 24 h to replenish fresh air. At designated time intervals (0, 5, 10, 15, 20, 25 and 30 days), 0.5 g sample was taken out to determine the total antioxidant activity according to the method described above.

Antioxidant retention (%) related to the total antioxidant activity of the initial sample was calculated by the following equation (Ho et al., 2017a):

\[
\text{Antioxidant retention} \% = \left( \frac{A_i}{A} \right) \times 100
\]

where \( A \) is the total antioxidant activity of the initial sample, \( A_i \) is the total antioxidant activity of treated sample.

2.8. Release profile

Release behavior of CAT from the CAT/β-CD inclusion complex was analyzed based on the method described by Ambrogi, Fardella, Grandolini, Perioli, and Tiraliti (2002) with slight modification. The maximum solubility of CAT in phosphate buffered solution (PBS, pH 7.4) at 25°C was measured, the obtained value was 198 mg/100 mL. The inclusion complex (400 mg) containing 63 mg of CAT was suspended in 100 mL of PBS in an Erlenmeyer flask. The Erlenmeyer flask was placed in a shaking water bath at 25, 35, 50°C and reciprocated with a speed of 100 rpm. At designated time intervals, 3 mL of the release medium was sampled and replaced by fresh PBS to maintain the constant volume. The removed release medium was centrifuged at 12000 rpm for 2 min. Anhydrous ethanol was used to dilute the supernatant 100 times. CAT content was determined by
measuring the absorbance at 230 nm. Each measurement was repeated 3 times.

2.9. Statistical analysis

SPSS software (Version 16.0, Chicago, USA) was used for statistical analysis via analysis of variance (ANOVA). Duncan’s multiple range tests (p<0.05) were used to compare the means to identify which groups were significantly different from others.

3. Results and discussion

3.1. Phase solubility study

Phase solubility study not only can be used to analyze the stoichiometry but also to determine the binding constant (Li et al., 2018). Figure 1 shows phase solubility diagram of CAT with β-CD. It can be seen that the solubility of CAT increased in a liner way against increase of β-CD concentration, such linearity was considered as classic A1 type, which indicating an equal stoichiometry in the CAT/β-CD inclusion complex (Higuchi & Connors, 1965; Martínez-Alonso, Losada-Barreiro, & Bravo-Díaz, 2015). By analyzing the change of proton shift using the continuous variation, Jullian et al. (2007) confirmed that CAT and β-CD formed an inclusion complex with equal stoichiometry in aqueous medium. Żyżlewieicz et al. (2018) also obtained the same stoichiometric ratio using electrospray ionization mass spectrometry technique. The data in Figure 1 showed that the water solubility of CAT increased about 16 times when 14 mM β-CD was incorporated.

The stability constant (Kc) of CAT/β-CD inclusion complex represents the binding strength of β-CD and CAT. The value of Kc was 3542 M⁻¹, indicating that there is strong interaction between β-CD and CAT, and CAT can combine with β-CD in a stable way, because the ideal range of stability constant for complexes is between 100 and 5000 (Marques, 2010).

3.2. CAT/β-CD inclusion complex

Based on the results of phase solubility study, CAT and β-CD with equal molar ratio was chosen to prepare inclusion complex. The total recovery was 90.76 ± 0.58%, the inclusion ratio was 70.37 ± 1.02% and the loading capacity was 51.29 ± 2.02%, which means about 158 mg CAT can be encapsulated into 1 g β-CD. It was reported that the theoretical maximum loading of essential oils in β-CD is 8–12% (Wen et al., 2016). The above results indicated that the loading capacity of solid compounds in β-CD was higher than theoretical maximum loading of essential oils.

3.3. FT-IR analysis

Figure 2 shows the FT-IR spectrum of CAT, β-CD, and CAT/β-CD inclusion complex. The FT-IR spectrum of CAT (Figure 2(a)) was the typical feature of phenolic compounds. The broad absorption band at 3404 cm⁻¹ was due to -OH stretching vibrations, while the band at 1369 cm⁻¹ was due to plane bending vibrations of -OH (Liu et al., 2014). The bands between 1400 and 1600 cm⁻¹ were due to stretching vibrations of C = C in the aromatic ring (Chen, Tsao, Liu, Huang, &
The absorption bands between 1200 and 1300 cm\(^{-1}\) were due to C-O/C-C stretching vibrations (Liu et al., 2014). Figure 2(b) was the FT-IR spectrum of \(\beta\)-CD and there were obvious absorption bands at 3390, 2929, 1649, 1155 and 1029 cm\(^{-1}\), which were corresponding to O-H stretching vibrations, C-H stretching vibrations, H-O-H bending vibrations, C-O stretching vibrations and C-O-C stretching vibrations, respectively (Zhang et al., 2015). For the FT-IR spectrum of the CAT/\(\beta\)-CD inclusion complex (Figure 2(c)), it was very similar to that of \(\beta\)-CD instead of CAT due to the less amount of CAT in the complex. The same phenomena were observed by other researchers (Wang et al., 2011; Yuan, Jin, & Xu, 2012). It was noted that the absorption bands at 3390 and 2929 cm\(^{-1}\) for \(\beta\)-CD were shifted to 3379 and 2926 cm\(^{-1}\) for the inclusion complex, which could be attributed to the formation of intra-molecular O-H–O hydrogen bonds that stabilizes CAT in the cavity of \(\beta\)-CD (Aree & Jongrungruangchok, 2016).

### 3.4. DSC analysis

DSC was used to identify the formation of the CAT/\(\beta\)-CD inclusion complex. When guest molecules entered \(\beta\)-CD cavity, thermal peak usually shifted or disappeared (Wang et al., 2011). As shown in Figure 3(a), CAT showed two endothermic peaks at 133.92 and 172.75°C, respectively. The results were similar to Liu et al. (2014), the peak at 133.92°C was due to the endothermic melting and the peak at 172.75°C was related to the melting point of CAT. The DSC curve of \(\beta\)-CD (Figure 3(b)) showed a wider endothermic peak at 86.67°C which is caused by the dehydration of water molecules bound to \(\beta\)-CD (Wang et al., 2011; Yang et al., 2016; Zhang et al., 2015). The DSC curve for CAT/\(\beta\)-CD physical mixture (Figure 3(c)) was similar to that of \(\beta\)-CD, meaning \(\beta\)-CD was in free status. However, for the CAT/\(\beta\)-CD inclusion complex, a different DSC curve was observed (Figure 3(d)). Compared to \(\beta\)-CD, the endothermic peak of the CAT/\(\beta\)-CD inclusion complex shifted to low temperature, its broadening and reduction in ΔH provided an evidence that disorder happened, which might be caused by the CAT/\(\beta\)-CD inclusion complex formation (Vertzoni, Kartezi, Reppas, Archontaki, & Valsami, 2006). These results suggested that some of the water molecules bound to \(\beta\)-CD were replaced by CAT molecules. In other words, CAT was successfully embedded into \(\beta\)-CD cavity. In addition, the disappearance of the two endothermic peaks of CAT in the DSC curve of the CAT/\(\beta\)-CD inclusion complex indicated that the CAT was encapsulated in the cavity of the \(\beta\)-CD, not just the physical mixture. This is evident when comparing the thermograms of the physical mixture and the inclusion complex (Abarca, Rodriguez, Guarda, Galotto, & Bruna, 2016). These results evidenced that the CAT was protected within the cavity of the \(\beta\)-CD.

### 3.5. XRD analysis

Figure 4 shows the XRD patterns of CAT, \(\beta\)-CD, CAT/\(\beta\)-CD physical mixture and CAT/\(\beta\)-CD inclusion complex. CAT displayed some distinct peaks at 2θ = 10.2, 12.2, 14.8, 16.4, 19.5, 21.0, 23.0, 24.1, 26.0 and 27.9°, indicating its crystalline nature. Similar crystalline peaks were also observed by other researchers (Liu et al., 2014). Some sharp peaks at 2θ = 9.0, 10.6, 12.5, 15.3, 18.8, 20.7, 22.7 and 27.0° were observed for \(\beta\)-CD, which was similar to that obtained by others (Krishnaswamy et al., 2012; Wang et al., 2011; Yang et al., 2016; Zhang et al., 2015), proving the crystalline characteristic of \(\beta\)-CD. This pattern agrees with the cage-type packing in the molecular organization of \(\beta\)-CD (Abarca et al., 2016; Kayaci, Sen, Durgun, & Uyar, 2014). The crystalline peaks of \(\beta\)-CD were detected in the physical mixture of CAT and \(\beta\)-CD, indicating that there was no difference of the crystalline form \(\beta\)-CD, and similar phenomena were also observed by other researchers (Zhang et al., 2015). However, for the CAT/\(\beta\)-CD inclusion complex, some peaks which were originally related to \(\beta\)-CD or CAT did not appear and new peaks at 6.6, 8.6, 11.7, 12.6, 15.8, 17.7 and 19.9° were observed, suggesting the formation of CAT/\(\beta\)-CD inclusion complex and presence of new crystalline phases in the inclusion complex. The XRD pattern of the CAT/\(\beta\)-CD inclusion complex in this work was different from that of the CAT/\(\beta\)-CD inclusion complex obtained by Krishnaswamy et al. (2012). The reason for this difference is that the inclusion complex was prepared by different methods.
probably is the difference in preparation methods of the inclusion complexes, co-precipitation and molecular inclusion. In addition, sharp peaks at 9 and 10.6° disappeared and new peak at 11.7 and 19.9° appeared, which is related to the cage-type packing in the molecular structure of β-CD changed to channel-type packing, and this is a strong evidence of inclusion complex formation (Abarca et al., 2016).

3.6. Morphology observation

Figure 5 shows morphology of CAT, β-CD, CAT/β-CD physical mixture and CAT/β-CD inclusion complex. It was observed that CAT was needle-shaped crystals, which was in accordance with the observation of Liu et al. (2014). The β-CD presented in irregular particles, which was agreement with the report of Zhang et al. (2015). For the CAT/β-CD physical mixture, typical crystals of CAT and β-CD were observed indicating the mixing didn’t change the morphology of CAT and β-CD. Interestingly, the shape of the CAT/β-CD inclusion complex, multi-tier regular parallelogram, was totally different from that of CAT and β-CD. These observations also proved the formation of CAT/β-CD inclusion complex.

3.7. Antioxidant stability of CAT/β-CD inclusion complex

Table 1 shows the antioxidant retention (%) of CAT, CAT/β-CD physical mixture and the CAT/β-CD inclusion complex heated in water at 65, 85, 100 and 120°C for 30 and 60 min, respectively. The data in Table 1 suggested that with increase of temperature, the antioxidant retention (%) decreased considerably. For the samples heating at 85, 100, 120°C for 30 and 60 min, significant differences (p<0.05) in the antioxidant retention (%) was observed except the CAT/β-CD inclusion complex heated at 100°C. The antioxidant retention (%) of CAT/β-CD inclusion complex was higher than that of CAT and CAT/β-CD physical mixture and still maintained about 80% after heating at 120°C for 60 min, while the antioxidant activity of CAT only retained about 60%. These results indicated that complexation of CAT with β-CD protects CAT against oxidative processes. Ho et al. (2017a) reported that CAT/β-CD inclusion complex has higher antioxidant retention (%) than CAT after heating and the antioxidant activity of CAT/β-CD inclusion complex retained more than 80% after heating at 120°C for 60 min. Li, Taylor, Ferruzzi, and Mauer (2012) reported that, CAT degraded with increasing temperature, which leads to

![Figure 5. SEM images of CAT (a), β-CD (b), CAT/β-CD physical mixture (c) and CAT/β-CD inclusion complex (d).](image_url)

![Figure 5. Imágenes SEM del CAT (a), β-CD (b), mezcla física CAT/β-CD (c) y del complejo de inclusión CAT/β-CD (d).](image_url)

| Sample                        | Heat treatment time (min) | 65°C | 85°C | 100°C | 120°C |
|-------------------------------|---------------------------|------|------|-------|-------|
| CAT                           | 30                        | 96.57 ± 1.14<sup>bc</sup> | 84.20 ± 0.93<sup>c</sup> | 76.61 ± 1.81<sup>b</sup> | 70.63 ± 0.80<sup>c</sup> |
|                              | 60                        | 96.48 ± 1.28<sup>b</sup>  | 80.09 ± 2.96<sup>cde</sup> | 69.88 ± 3.90<sup>c</sup> | 63.83 ± 3.49<sup>d</sup> |
| CAT/β-CD physical mixture     | 30                        | 95.82 ± 0.83<sup>bc</sup> | 82.56 ± 0.46<sup>cde</sup> | 75.40 ± 1.91<sup>b</sup> | 69.20 ± 1.30<sup>c</sup> |
|                              | 60                        | 95.10 ± 0.91<sup>c</sup>  | 78.33 ± 0.77<sup>e</sup>  | 69.18 ± 3.85<sup>c</sup> | 63.80 ± 2.08<sup>d</sup> |
| CAT/β-CD inclusion complex    | 30                        | 98.65 ± 1.07<sup>a</sup>  | 97.96 ± 0.87<sup>e</sup>  | 91.44 ± 0.88<sup>a</sup> | 87.49 ± 1.89<sup>b</sup> |
|                              | 60                        | 97.77 ± 0.94<sup>ab</sup> | 92.23 ± 1.03<sup>b</sup>  | 87.24 ± 2.11<sup>a</sup> | 80.84 ± 1.04<sup>b</sup> |

Values are given as mean ± standard deviation of three measurements (n = 3).
Different letters (a–d) in the same column indicate significantly different (p < 0.05).
Los valores representan la media ± desviación estándar de tres mediciones (n = 3).
Las diferentes letras (a-d) en la misma columna indican diferencias significativas (p < 0.05).
a negative impact on antioxidant activity. The results of this work were consistent with these previous ones.

CAT is easily oxidized when exposed to air (Ho et al., 2017a). Figure 6 showed antioxidant retention (%) of CAT, CAT/β-CD physical mixture and CAT/β-CD inclusion complex exposure to air. After 30 days of exposure, the antioxidant retention (%) of CAT was only 18.37%, and the antioxidant retention (%) of the CAT/β-CD physical mixture was 33.33%. This difference in antioxidant retention is probably because a portion of β-CD in the physical mixture sample covered CAT and preserved it from oxidation in a certain extent. For the CAT/β-CD inclusion complex, the antioxidant retention (%) was 78.06% after 30 days exposure, indicating that the CAT was effectively preserved due to the formation of inclusion complex. The current result was similar to a previous one, in which the antioxidant activity of CAT/β-CD inclusion complex retained about 79% after 28 days exposure to air (Ho et al., 2017a).

3.8. Release of CAT from the CAT/β-CD inclusion complex

In order to investigate the effect of temperature on release of CAT from the inclusion complex, the temperature was selected from room temperature (25°C) to 50°C which does not affect the biological activity of the CAT. Figure 7 presented release profiles of CAT from the CAT/β-CD inclusion complex in PBS medium at 25, 35 and 50°C. All profiles showed a similar burst release at 30 min with release of 18%, 36% and 58% at 25, 35 and 50°C, respectively. After then, the cumulative release increased gradually before reaching a plateau. The release reached 100% after 360 min at 50°C, but the maximum release was only 71.2% and 39.6% after 360 min at 35 and 25°C. Tang, Sun, Zhao, Pu, and Zhang et al. (2018) reported that release of mesalazine from the mesalazine/hydroxypropyl-β-CD inclusion complex with stability constant of 2162.7 M⁻¹ was about 95% in PBS at 37°C for 120 min. Comparing to this release of mesalazine, the release of CAT from the CAT/β-CD inclusion complex in PBS medium at 35°C as shown in Figure 7 was much lower. This is probably because the stability constant of the CAT/β-CD inclusion complex (3542 M⁻¹) is much higher than that of the mesalazine/hydroxypropyl-β-CD inclusion complex. Additionally, the release behavior of CAT from the inclusion complex increased with increasing of the temperature. Therefore, the CAT/β-CD inclusion complex has temperature-controlled release property, which makes it possible to apply to drug delivery systems with temperature sensitivity.

4. Conclusions

By using co-precipitation method, CAT was successfully embedded into the cavity of β-CD. Results of phase solubility study suggested that β-CD can form 1:1 inclusion complex with CAT and aqueous solubility of CAT was considerably increased once it was complexed with β-CD. The stability constant of CAT/β-CD inclusion complex was 3542 M⁻¹. The formation of CAT/β-CD inclusion complex was proved via DSC, FT-IR, SEM and XRD. The results of FT-IR indicated that CAT was stabilized in β-CD cavity by intra-molecular hydrogen bonds. The results of XRD suggested a formation of a new crystalline phase in the CAT/β-CD inclusion complex. Moreover, the antioxidant stability of CAT was enhanced after encapsulated in β-CD.

Disclosure statement

No potential conflict of interest was reported by the authors.

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