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REVIEW ARTICLE

In vitro dissolution study on inclusion complex of piperine with ethylenediamine-β-cyclodextrin

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Abstract

The co-evaporation method was used to synthesize the EN-β-CD@Piperine inclusion complex with a molar ratio of 1:1. The properties and structures of the inclusion complex were characterized by various methods to investigate the inclusion mode and interactions between host and guest. The results of molecular modeling were theoretically analyzed to determine the inclusion mechanism of inclusion complexes. Finally, the vitro dissolution release studies showed that the water solubility of piperine was significantly enhanced when EN-β-CD was combined with piperine. Therefore, the EN-β-CD@Piperine inclusion complex provides a promising development for the clinical application of piperine in the future.

Keywords Natural medicine · Piperine · Cyclodextrin inclusion complex · Molecular docking · Dissolution study · ROESY NMR

Introduction

Natural products from the herbal remedy, medicinal plants, functional foods and their ingredients have been used to treat various diseases from ancient times to modern times [1]. Over the last few decades, more and more researchers have been focused on the research on traditional medicine [2, 3]. Black pepper is widely incorporated into various foods and its medicinal properties have been well quoted in various traditional medicine [4]. Piperine is an alkaloid present in types of plants, such as black pepper, long pepper and other Piper species [5, 6]. Piperine has biological and pharmacological applications, such as antimetastatic, antiulcer, anti-inflammatory [7, 8], antifungal [9, 10]. However, its poor water solubility cause limitations in pharmaceutical activities and it is inevitable to use a higher dose of drugs to obtain the anticipated pharmacological response. Therefore, it is imperative to improve the solubility of piperine to avoid dose escalation and toxicity [11, 12]. Various formulation approaches such as cyclodextrin complexation [13, 14], emulsion, floating microspheres, self-emulsifying drug delivery system and nanoliposome of piperine are used to investigate the enhancement of solubility/dissolution rate and in vivo bioavailability. Among the various approaches, cyclodextrin complexation is the method of choice that enhances water solubility of the drugs.

Cyclodextrins (CDs), a series of cyclic oligosaccharides, are composed of six (α), seven (β) or eight (γ) D-glucose units linked by α-1,4 bonds, named as α-CD, β-CD and γ-CD, respectively [15, 16]. Among these various types of CDs,β-cyclodextrins (β-CD) are widely used due to low cost, and its cavity size makes it possible to form inclusion complex with various guest molecules through hydrogen bonding, van der Waals interaction and hydrophobic interactions [17, 18]. One of the main problems in pharmaceutical industry is poor water solubility of the drugs which lead to decrease in bioavailability. Through inclusion complex formation of drugs, β-CD can overcome this problem and enhance the solubility and stability of hydrophobic drug molecules [19, 20]. But, native β-CD have poor water solubility and toxicity which hinder its further application in pharmaceutical formulations [21, 22]. So far, chemically modified β-CD derivatives such as hydroxypropyl-β-CD(HP-β-CD) [23, 24], sulfobutylether-β-CD (SBE-β-CD) [22, 25] and ethylenediamine-β-cyclodextrin (EN-β-CD) [26, 27] have much higher water solubility and less toxic than unmodified β-CD. However, to the best of our knowledge, no scientific study about the impact on EN-β-CD to

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enhance solubility and dissolution rate of piperine have not been reported.

In this work, we have synthesized a novel inclusion complex of EN-β-CD and piperine that improved aqueous solubility and bioavailability. UV/Vis spectroscopy was measured to evaluate the formation of EN-β-CD@Piperine inclusion complex in aqueous solution. The structures and properties of EN-β-CD@Piperine inclusion complexes were characterized by ^{1}H and 2D ROESY NMR, FT-IR, XRD and TGA. Finally, encapsulation efficiency (EE) and in vitro release analysis of inclusion complex were investigated.

Experiment

Materials and chemicals

Piperine (PC > 97%) was obtained from Shanghai Aladdin Pharmaceutical Corporation. β-CD was purchased from Zibo Qianhui Biotechnology Company, p-toluenesulfonyl chloride, *N*,*N*-dimethylformamide (DMF), acetone, ethylenediamine, Anhydrous ethanol, concentrated hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from Tianjin Damao Pharmaceutical Corporation. All experiments were carried out in deionized water. All solvents and reagents were analytical grade. All chemicals and reagents were without further purification.

Synthesis of mono[6-O-(p-toluenesulfonyl)]-β-CD

A solution of sodium hydroxide (6.00 g) in deionized water (200 mL) was added dropwise to a solution of β-CD (10.00 g) in deionized water. The reaction mixture was stirred at 10 °C about 30 min. A solution of p-toluenesulfonyl chloride (3.36 g) was added dropwise into homogeneous solution forming white precipitate immediately. The reaction mixture was stirred for 5.0 h. The white precipitate was filtered and washed several times with acetone, and then was dried in a vacuum at 60 °C for 12 h.

Synthesis of mono(6-ethylenediamine)-β-CD

2.00 g of 6-OTs-β-CD were dissolved in DMF (14.00 mL), and then added dropwise to anhydrous ethylenediamine (20.00 mL). The reaction mixture was stirred at 65 °C for 4 h. Then, acetone (100.00 mL) was poured into the resultant solution forming white precipitate immediately. The white precipitate was recrystallized for several times with acetone. The obtained white precipitate was dried in vacuum at 40 °C for 12 h.

Preparation of EN-β-CD@Piperine

Piperine (0.5 mM, 0.143 g) and EN-β-CD (0.5 mM, 0.5885 g) were completely dissolved in a mixed solution of deionized water/anhydrous ethanol (10% (v/v)).The resulting mixture was stirred at 40 °C for 4 h. After evaporating the ethanol from the mixture, the uncomplexed piperine was filtered. The obtained filtrate was dried in vacuum at 40 °C for 12 h. The synthesis of EN-β-CD and the preparation route of the inclusion complex are shown in Fig. 1.

Determination of the standard curve of piperine

A series of piperine anhydrous ethanol solutions with their concentrations ranging from 0.10 to 0.80 Mm (0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80 mM) were configured. The absorbance was measured at 343 nm in UV at room temperature as x-coordinate and absorbance (A) as y-coordinate. We found the standard curve of piperine could be depicted by the equation:

$$A = 0.1299C - 0.01971 \quad R^2 = 0.9985 \tag{1}$$

Determination of encapsulation efficiency (EE)

The percentage of encapsulation efficiency (%) was determined according to the following equation:

$$EE(\%) = m_1 / m_0 \times 100\% \tag{2}$$

where m_1 is the weight of piperine in the EN-β-CD and m_0 is the total weight of piperine used in the encapsulation process.

Effect of molar ratio of EN-β-CD with piperine on encapsulation efficiency

Piperine and EN-β-CD were completely dissolved in a mixed solution of anhydrous ethanol and deionized water. The molar ratio of EN-β-CD with piperine is ranging from 20:1 to 10:1 (20:1, 18:1, 16:1, 15:1, 14:1, 12:1, 10:1).The reaction mixture was stirred at 40 °C for 2 h. The amount of piperine in the supernatant was measured as absorbance at 343 nm. All experiments were repeated in triplicate. Encapsulation efficiency (EE) was calculated.

Effect of reaction temperature on encapsulation efficiency

EN-β-CD was dissolved in deionized water, while the piperine was dissolved in anhydrous ethanol, and then added dropwise to the mixed solution. The encapsulation efficiency of EN-β-CD with piperine was analyzed at 25 °C,

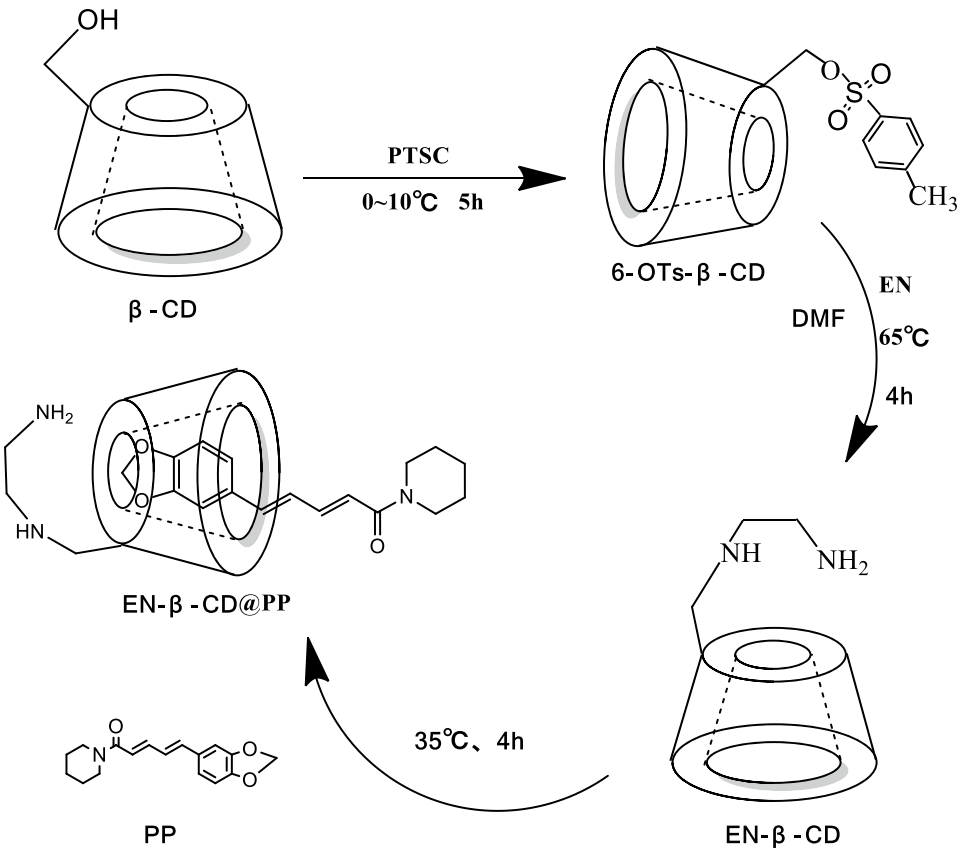

Fig. 1 The synthesis of EN-β-CD and the preparation route of the inclusion complex

35 °C, 45 °C and 65 °C for 2 h in the molar ratio of 15:1. The amount of piperine in the supernatant was measured as absorbance at 343 nm. The analyses were carried out in triplicate. Encapsulation efficiency (EE) was calculated.

Effect of reaction time on encapsulation efficiency

Piperine and EN-β-CD were completely dissolved in a mixed solution of anhydrous ethanol and deionized water. The reaction mixture was stirred at 35 °C for 2, 3, 4, 5, 6 and 7 h, respectively. The molar ratio of EN-β-CD with piperine was 15:1. The amount of piperine in the supernatant was measured as absorbance at 343 nm. All experiments were carried out in triplicate. Encapsulation efficiency (EE) was calculated.

Job's plot

The stoichiometry of EN-β-CD@Piperine inclusion complex was determined by Job's method of continuous variation with the UV spectrophotometer. The total concentration of piperine and EN-β-CD was held at 4.5×10^{-5} mol/L, and the molar fraction of n(EN-β-CD)/[n(Piperine) + n (EN-β-CD)] varied from 0 to 1. The absorbance was measured at 343 nm in UV for a series of solutions. The absorption of each piperine solution in the presence and absence of EN-β-CD were determined in the same condition.

FTIR analysis

Before the experiment, the piperine, EN-β-CD, EN-β-CD@Piperine inclusion complex and EN-β-CD/Piperine physical mixture were dried in a vacuum oven at 60 °C for 12 h. The samples were performed on FT-IR spectrophotometer, using KBr solid as background at a resolution of 4 cm^{-1} over the frequency range of 400–4000 cm^{-1}.

XRD analysis

The XRD patterns were measured using Bruker D8 diffractometer with Cu Kα radiation (40 kV, 100 mA), at a scanning rate of 6°/min. Powder samples were mounted on a vitreous sample holder and scanned with a step size of $2\theta = 0.02°$ between $2\theta = 3°$ and 50°.

^{1}H-NMR analysis

The β-CD, EN-β-CD and EN-β-CD@Piperine inclusion complex were prepared in D_2O, and ^{1}H NMR experiments for β-CD, EN-β-CD and EN-β-CD@Piperine inclusion complex were recorded on a Bruker Avance AV-III 400 MHz

NMR spectrometer at room temperature. Tetramethylsilane (TMS) was used as a standard. The structure of piperine (a) and the proton structure of EN-β-CD (b) are shown in Fig. 2.

2D ROESY analysis

The 2D ROESY spectra were performed using a 600 MHz instrument (Bruker Avance, Germany) operating at room temperature. The inclusion complex was prepared in D_2O. Chemical shifts were presented as ppm and tetramethylsilane (TMS) was used as the internal standard.

TGA analysis

The thermal properties of the piperine, EN-β-CD, EN-β-CD@Piperine inclusion complex and EN-β-CD and piperine physical mixture were characterized by thermogravimetric analyzer. Thermal analysis were performed with NETZSCH5 instrument, at a linear heating rate of 10 °C/min from the temperature ranged from 20 to 500 °C under nitrogen atmosphere (flow rate = 50 mL/min).

Molecular docking

Molecular docking studies were conducted using the software's Discovery Studio 3.5. β-CD was downloaded from the RCSB protein data bank. The protein around β-CD (Fig. 3a) was deleted to obtain the coordinates of β-CD (Fig. 3c). EN-β-CD (Fig. 3d) was constructed by modifying the $–HNCH_2CH_2NH_2$ groups at the 6th position of the glucose moieties of β-CD. The geometry of piperine (Fig. 3b) was optimized using Gaussian 09 software with the DFT-B3LYP method and 6-31G basis set. All water molecules were removed, and only the polar hydrogens were added. Using Discovery Studio program (version 3.5), a yellow box was generated around β-CD. We generated the inclusion complexes by docking the guest piperine into the EN-β-CD cavity. The Discovery Studio program (version 3.5) gave several possible docked models for the most probable structure based on the geometric shape complementarity score.

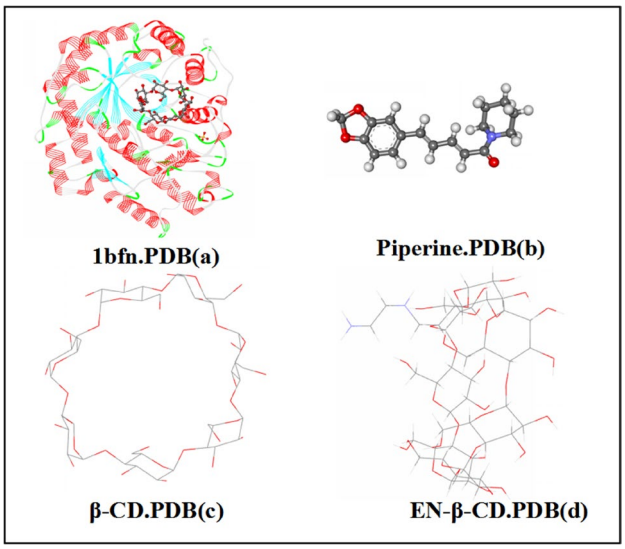

Fig. 3 The structure of host and guest in molecular docking

In vitro release studies

In vitro release studies were carried out using a modified paddle-type dissolution apparatus. 1.5 mg of EN-β-CD@Piperine inclusion complex or piperine were accurately weighed into a conical flask. The dissolution medium was phosphate-buffered saline (pH 6.8). The experiment was carried out at 100 rpm in a dissolution medium (900.00 mL) maintained at 37 ± 1 °C. The samples were taken at predetermined time intervals (1, 3, 5, 7, 10, 15, 20, 25, 30, 45, 60 min), and then filtered through a 0.45 um membrane.

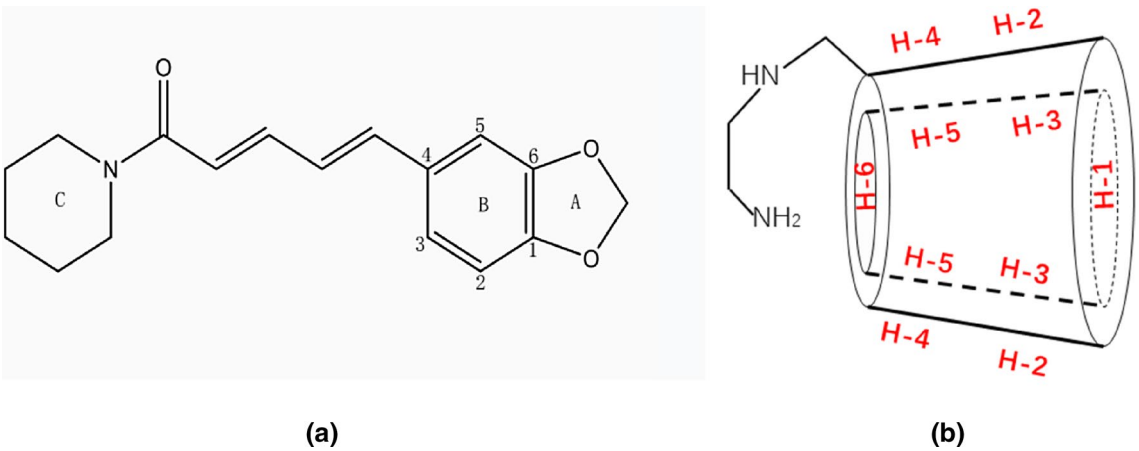

Fig. 2 The structure of piperine (**a**) and the proton structure of EN-β-CD (**b**)

The fresh medium with an equal volume was added immediately to maintain release media. The piperine contents in the release medium were analyzed by the UV-spectrophotometer at 343 nm.

Results and discussion

Effect of molar ratio of EN-β-CD with piperine on encapsulation efficiency

Figure 4 shows the effect of molar ratio of EN-β-CD with piperine on encapsulation efficiency. When the molar ratio of EN-β-CD with piperine increased from 20:1 to 15:1, the encapsulation efficiency increased from 34.75 to 72.72%. This could be explained by the vacant capacity of cavity of EN-β-CD to entrap piperine. However, it is obvious that the encapsulation efficiency declined from 72.72 to 38.24% with molar ratio of EN-β-CD with piperine increased from 15:1 to 10:1. This occurred because of the fact that the saturation of piperine encapsulate into EN-β-CD.

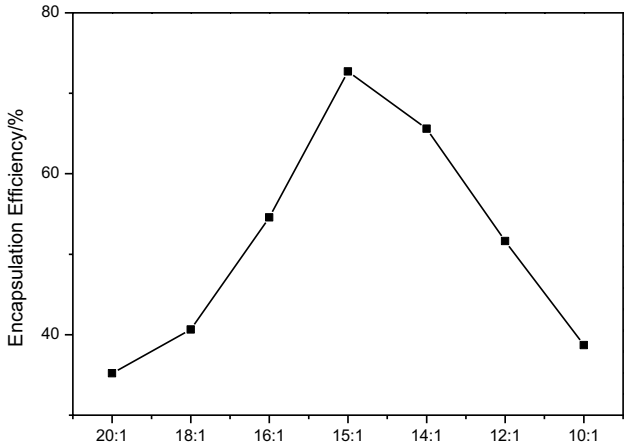

Fig. 4 Effect of molar ratio of EN-β-CD with piperine on encapsulation efficiency

Effect of reaction temperature on encapsulation efficiency

The encapsulation efficiency (EE) of EN-β-CD@Piperine prepared at different reaction temperature is shown in Fig. 5. It can be observed that the reaction temperatures increased from 25 to 35 °C, the encapsulation efficiency increased from 72.18 to 79.32%. The results can be explained by the fact that EN-β-CD could effectively load piperine into the cavity to increase the drug encapsulation efficiency. However, it is obvious that the encapsulation efficiency declined from 79.32 to 38.32% with further increase of the reaction temperature from 35 to 65 °C. This observation could be attributed to increase in temperature, influencing the process of EN-β-CD interaction with piperine.

Effect of reaction time on encapsulation efficiency

Effect of reaction time on encapsulation efficiency was investigated and result is shown in Fig. 6. As can be seen from Fig. 6, the encapsulation efficiency increases rapidly with time prolonging and then increases slowly, and when time reached 4 h, the encapsulation efficiency reaches the maximum eventually. The initial rapid encapsulation efficiency might be attributed to piperine effectively attached to the EN-β-CD. However, when the encapsulation efficiency of reaction time is more than 4 h, the system reached equilibrium. It is concluded that the saturation of piperine encapsulate into EN-β-CD.

Characterization of inclusion complex

The FT-IR spectra of piperine, EN-β-CD, EN-β-CD and piperine physical mixture and EN-β-CD @Piperine inclusion

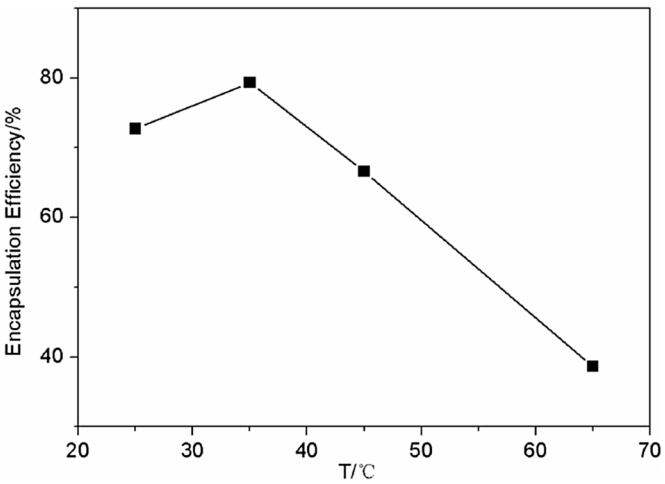

Fig. 5 Effect of reaction temperature on encapsulation efficiency

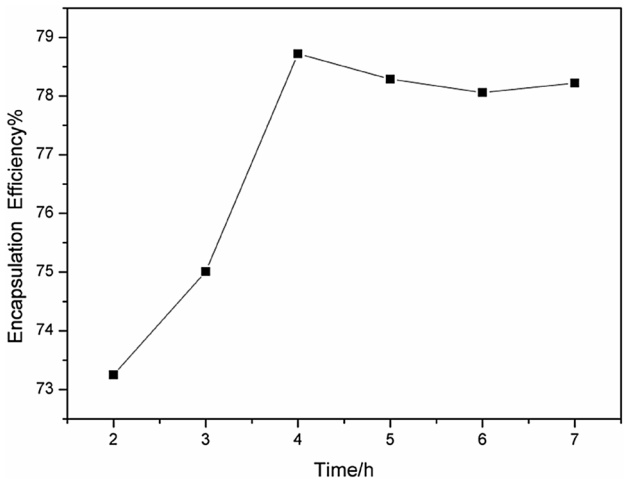

Fig. 6 Effect of reaction time on encapsulation efficiency

complex are presented in Fig. 7. Infrared spectra of piperine have obvious characteristic absorption peaks at 2939, 1633, 1193 and 997 cm^{-1}, which correspond to C-H stretching vibration, N–H bending vibration, bis-epoxy ether characteristic peak and monoepoxy C–O–C in the molecular structure of piperine. The infrared spectrum of EN-β-CD has obvious characteristic absorption peaks at 2927, 1658, 1080 and 858 cm^{-1}, corresponding to N–H bending vibration in the molecular structure of EN-β-CD, C=O stretching vibration, C–N stretching vibration, and NH_2 symmetrical bending vibration. The infrared spectrum of EN-β-CD@Piperine inclusion complex was compared with the infrared spectrum of piperine and EN-β-CD, and the 2939 cm^{-1} peak of C–H stretching vibration in the molecular structure of piperine disappeared. When the piperine molecule is embedded in the cavity of the EN-β-CD, mutual chemical interaction is formed between the two molecules, so that the C–H stretching vibration in the piperine molecule is weakened and disappears. Furthermore, the C–O–C characteristic peak in the piperine molecule is red shifted from 997 to 1002 cm^{-1}, which may be due to the formation of hydrogen bonds in the EN-β-CD cavity by the epoxy group. The physical mixture of EN-β-CD and piperine shows that it is only a simple superposition of the characteristic absorption peaks of the host–guest molecule. Therefore, the inclusion complex was successfully synthesized.

TGA analysis can be applied to determine the thermal stability between cyclodextrins and guest molecules. The TGA spectra of piperine, EN-β-CD, EN-β-CD and piperine physical mixture and EN-β-CD@Piperine inclusion complex are illustrated in Fig. 8. We found that piperine (Fig. 8a) begun to mass loss at about 210 °C. The TG curves showed that the weight loss of piperine is about 62.00% at 344 °C, and the mass loss at 500 °C is nearly 83.00%. In the case of EN-β-CD (Fig. 8b) two important mass losses were observed. The first zone is around 100 °C, with a mass loss of 5.00%, while the second stage occurs at approximately 500 °C with a mass loss of 67.00%. For the inclusion complexes, EN-β-CD@Piperine (Fig. 8c) began to mass loss quickly at about 100 °C, and a second zone is around 200 °C, with a mass loss of 18.00%. Three stage is approximately 325 °C with degradation of 78.00% of the total mass. In contrast, the TG curves of the EN-β-CD and piperine physical mixture (Fig. 8d), there are two decomposition-related peaks at 210 °C and 325 °C, which is the superposition of EN-β-CD and piperine. The results showed that the inclusion complex formed by EN-β-CD@Piperine had higher decomposition temperature than the host–guest molecule, and the thermal stability of piperine changed, indicating that

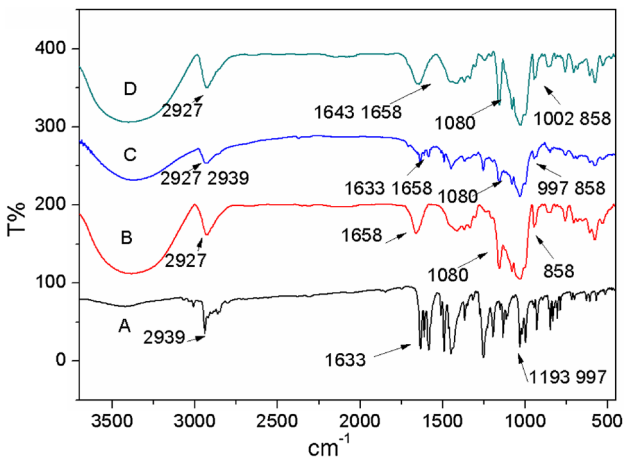

Fig. 7 FTIR spectra of piperine (**a**), EN-β-CD (**b**), EN-β-CD and piperine physical mixture (**c**) and EN-β-CD@Piperine inclusion complex (**d**)

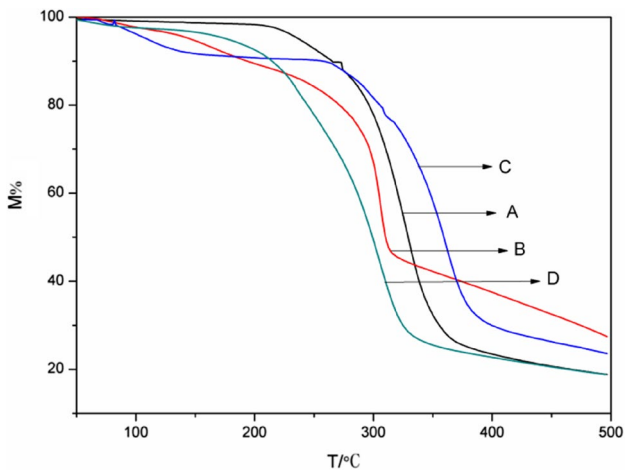

Fig. 8 TG curves of piperine (**a**), EN-β-CD (**b**), EN-β-CD@Piperine inclusion complex (**c**), EN-β-CD and piperine physical mixture (**d**)

the host–guest molecule formed a relatively stable composite structure.

The XRD patterns of piperine, EN-β-CD, EN-β-CD@Piperine inclusion complex and EN-β-CD and piperine physical mixture were listed in Fig. 9. The diffraction angle of piperine (Fig. 9a) showed sharper peaks at 14.34, 19.83, 27.71, 25.92°, indicating that the piperine material has a crystalline form. But, compared to piperine, the XRD pattern of EN-β-CD (Fig. 9b) has a flatter curve, indicating that it is amorphous. The XRD pattern of the EN-β-CD was amorphous nature that lacked crystalline peaks. The XRD pattern of the EN-β-CD@Piperine inclusion complex noted an amorphous halo pattern. These results suggested that the piperine molecule inserted into EN-β-CD cavity. The XRD patterns of EN-β-CD and piperine physical mixture was investigated, indicating that the physical mixture has properties of both amorphous EN-β-CD and some crystalline peaks of piperine. Therefore, the XRD results indicate that a novel inclusion complex was synthesized.

The ^{1}H-NMR spectrum of β-CD, EN-β-CD and EN-β-CD@Piperine inclusion complex were shown in the Fig. 10. The characteristic peak δ(ppm) and proton chemical shift values of EN-β-CD and EN-β-CD@Piperine inclusion complex compared with β-CD were shown in Table 1. It can be seen that the proton chemical shift value of the inclusion complex was significantly different from the chemical shift of the host molecule, and the chemical shift variations were calculated by the following formula: $\Delta\delta = \delta(\text{free}) - \delta(\text{complex})$. The values of H-4 and H-6 in the inclusion complex were small and the values of H-1, H-2, H-3 and H-5 showed significant chemical shifts. It is fairly noteworthy that the H-3 protons shifted 0.042 ppm and the H-5 proton shift 0.019 ppm. Because both H-3 and H-5 protons are located in the interior of EN-β-CD cavity, the H-5 protons are near the narrow side of the cavity and the H-3 protons are located on the wide side. It was confirmed that piperine molecule should penetrate into the EN-β-CD cavity from the wide side.

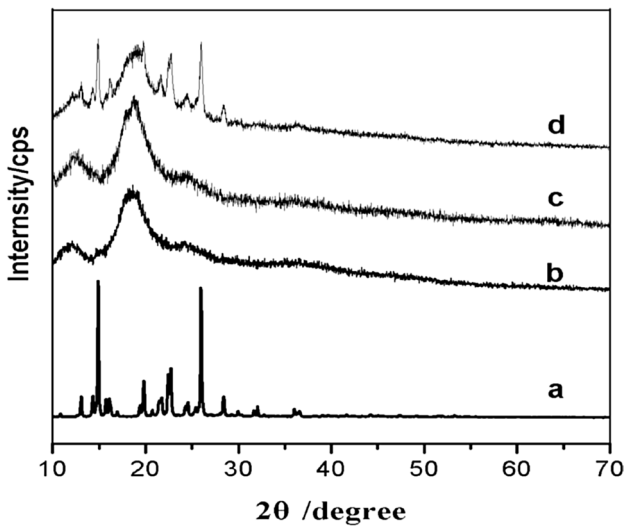

Fig. 9 XRD patterns of piperine (**a**), EN-β-CD (**b**), EN-β-CD@Piperine inclusion complex (**c**) and EN-β-CD and piperine physical mixture (**d**)

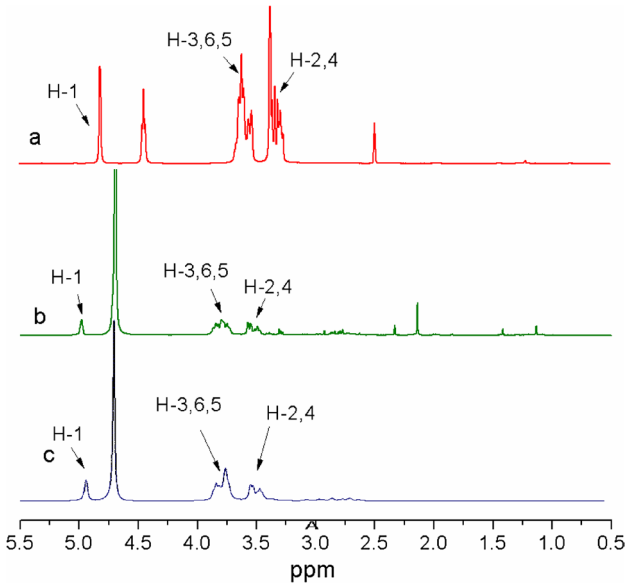

Fig. 10 ^{1}H-NMR spectrum of β-CD (**a**), EN-β-CD (**b**) and EN-β-CD@Piperine inclusion complex (**c**)

2D ROESY spectroscopy provides information about the spatial proximity between EN-β-CD and piperine atoms complex systems. Two protons locating in space within 0.4 nm produce a nuclear overhauser effect (NOE) cross correlation in NOE spectroscopy (NOESY) or ROESY. To get additional conformational information, we obtained 2D ROESY of the inclusion complex of piperine with EN-β-CD. The ROESY spectrum of the EN-β-CD@Piperine inclusion complex (Fig. 11) displayed appreciable correlation of H-1 and H-5 protons of piperine with H-3, H-5/H-6 protons of EN-β-CD, as well as key correlations between the H-2 protons of piperine and the H-3, H-5/H-6 protons of EN-β-CD. These results indicate that B ring of piperine was included

Table 1 Chemical shift data of EN-β-CD and EN-β-CD@Piperine inclusion complex

EN-β-CD@Piperine	δ(free)	δ(complex)	Δδ
H-1	4.977	4.944	0.033
H-2	3.567	3.531	0.036
H-3	3.866	3.824	0.042
H-4	3.514	3.508	0.006
H-5	3.822	3.803	0.019
H-6	3.752	3.750	0.002

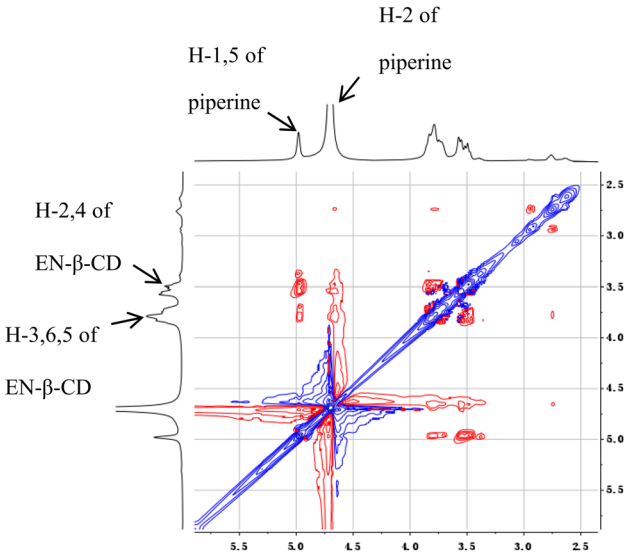

Fig. 11 ROESY spectrum of EN-β-CD@Piperine inclusion complex in D_2O

the EN-β-CD cavity. Based on these observations, together with the 1:1 stoichiometry suggested by Job's plot, we deduced that the benzene ring of piperine was merged into the EN-β-CD cavity from vast side [28, 29].

Molecular docking

Molecular docking was employed to determine the interaction between EN-β-CD and piperine. Discovery Studio program (version 3.5) was used to find docking transformations with good dominant conformation, as shown in Fig. 12. Comparing with ten dominant conformations, it can be found that the other nine dominant conformations are benzodioxolane groups of the piperine entering into the EN-β-CD cavity from the wide side, except for conformation-9. Interestingly, this structure dominates the entire simulation time. The stable EN-β-CD@Piperine inclusion complex are formed by hydrogen bonds. On the other hand, conformation-2 and conformation-8 indicate that the guest molecules enter the EN-β-CD cavity from the wide side, and the benzene ring and the outer ethylenediamine group can also undergo P–π conjugation. In addition, the lowest binding energy of the conformation-1 indicates that it was the most stable among the ten dominant conformation. Molecular modeling study is consistent with the results of the ^{1}H and 2D ROESY NMR [30, 31] (Table 2).

Stoichiometry (Job's plot)

The Job's plot is known as method to determine the stoichiometry in host–guest molecules. Figure 13 presents the Job's plot for the EN-β-CD@Piperine inclusion complex using the

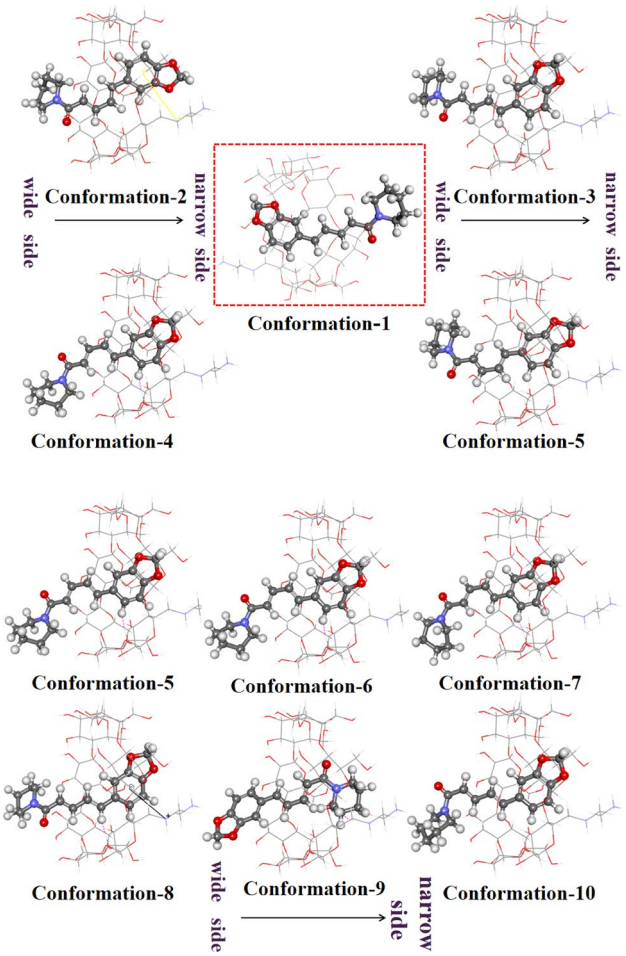

Fig. 12 Various possible dominant conformation of EN-β-CD@Piperine inclusion complex

UV–Vis spectroscopy. x-coordinate is the molar fraction of n(EN-β-CD)/[n(Piperine) + n (EN-β-CD)], and y-coordinate is the absorbance intensity of piperine in the presence and absence of EN-β-CD. The stoichiometric ratio of inclusion complex can be judged from the highest point of the curve. If the highest point of the curve is 0.5, the stoichiometric ratio of the inclusion complex is 1:1, if the highest point is 0.67 or 0.75, the stoichiometric ratio is 1:2 or 1:3. From the curve of the ratio of the amount of continuously changing substances in the EN-β-CD@Piperine inclusion complex system, it can be seen that the maximum value of ΔA in the curve corresponds to a ratio of 1:1, which clearly proves EN-β-CD@Piperine inclusion compound with a ratio of 1:1 [32, 33].

In vitro release studies

The aim of this study was to investigate the inclusion properties of EN-β-CD with the piperine to modify drug release and to increase the oral bioavailability. Comparing release rate of EN-β-CD@Piperine inclusion complex and pure

Table 2 Scoring results of dominant conformations between EN-β-CD and piperine

Stoichiometry (Job's plot)				
Serial number	Absolute energy	Conf. number	Relative energy	LibDock Score
Conformation-1	76.627	1	1.356	92.529
Conformation-2	81.948	24	6.677	92.478
Conformation-3	75.348	13	0.077	92.366
Conformation-4	82.533	33	7.261	91.779
Conformation-5	81.948	24	6.677	91.038
Conformation-6	86.723	24	6.677	90.923
Conformation-7	75.348	37	11.452	90.592
Conformation-8	83.150	13	0.077	90.538
Conformation-9	75.809	43	7.879	90.447
Conformation-10	75.397	22	0.538	89.661

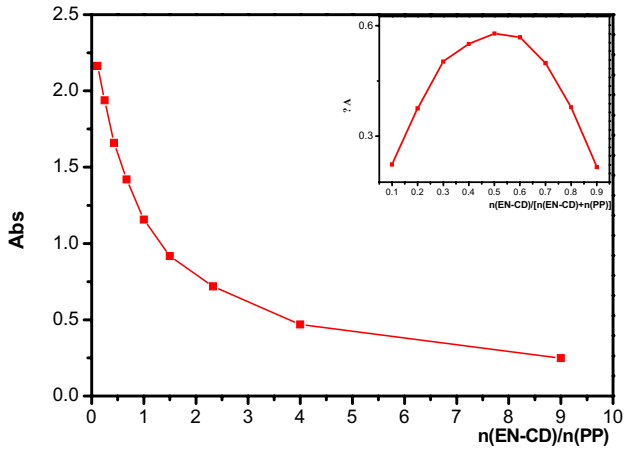

Fig. 13 Job's plot of EN-β-CD@Piperine system

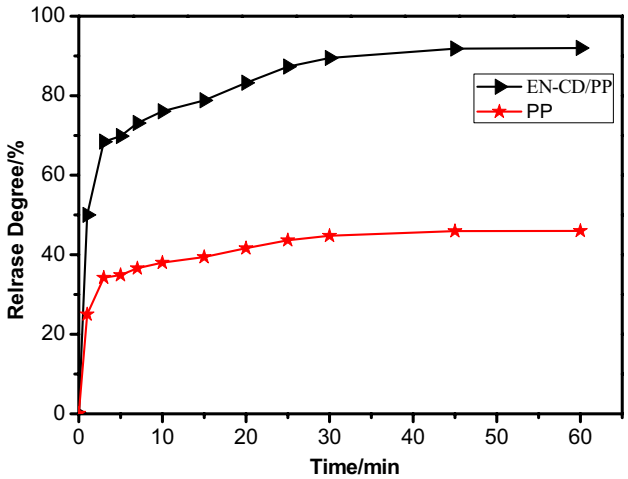

Fig. 14 Release profiles of piperine from EN-β-CD@Piperine inclusion complex

piperine, different release rates were observed in Fig. 14. For pure piperine, the release rate was 35% within 5 min. The dissolution rate of EN-β-CD@Piperine inclusion complex was more than 70% within 5 min. 100% of EN-β-CD@Piperine inclusion complex was released within 60 min whereas for piperine solution, approximately 40% was released during the same time. Corporately, EN-β-CD@Piperine inclusion complex exhibited significantly rapid release property. We deduced that the hydrophobic piperine penetrate into the EN-β-CD cavity, forming a relatively stable composite structure.

Conclusion

In this study, EN-β-CD@Piperine inclusion complex was prepared by a co-evaporation method. The inclusion complex structures and properties were confirmed through FT-IR, UV, XRD, TGA, ^{1}H and 2D ROESY NMR. The TGA curve indicated that EN-β-CD@Piperine inclusion complex has a higher decomposition temperature than pure piperine. The ^{1}H and 2D ROESY NMR curve indicated that piperine molecule should penetrate into the EN-β-CD cavity from the wide side. Molecular modeling confirmed that the complex of between EN-β-CD and piperine was 1:1. Besides, in vitro release experiments indicated that the water solubility of piperine was remarkably improved. Therefore, the EN-β-CD@Piperine inclusion complex could be beneficial to design of a new formulation of piperine for the application in the field of medicine.

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