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ORIGINAL ARTICLE

# Effect of external factors on the curcumin/2-hydroxypropyl-β-cyclodextrin: in vitro and in vivo study

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**Abstract** The effect of 2-hydroxypropyl-β-cyclodextrin (HPβCD) on solubility, stability and oral bioavailability of curcumin by external factors adjustment, was investigated with an aim of a simple, stable and effective formulation. The phase solubility studies showed the solubility of curcumin increased slightly with increasing pH. However, the apparent stability constant ($K_S$) were found to decrease with increasing pH from $1.29 \times 10^4$ $M^{-1}$ at pH 3.0 to $5.22 \times 10^3$ $M^{-1}$ at pH 7.0. The thermodynamic parameters were calculated for inclusion complex formation in aqueous solution. Interestingly, it could be concluded that the degrees of curcumin stability improved by HPβCD grew with increasing drug–cyclodextrin binding ability. Furthermore, in vivo study not only revealed that the bioavailability of curcumin after oral administration to rats was significantly improved by curcumin/HPβCD inclusion complex, but also showed more dramatic changes in the plasma concentration–time curve (1752.76–866.70 ng $mL^{-1}$ h) and the peak plasma concentration (370.10–178.11 ng $mL^{-1}$) of drug by formation of complexes in pH 3–7 solution.

**Keywords** Cyclodextrin · Curcumin · Phase solubility · Stability · Pharmacokinetics

**Abbreviations**

| | |
|---|---|
| CD | Cyclodextrin |
| HPβCD | 2-Hydroxypropyl-β-cyclodextrin |
| HPLC | High performance liquid chromatography |
| $S_0$ | Solubility in a medium in the absence of CD |
| $K_S$ | Apparent stability constant for the drug–CD interaction |
| $[C_0]$ | The initial concentration of drug |
| $[C_t]$ | The time-dependent concentration of drug |
| $\Delta H$ | Values of enthalpy change |
| $\Delta S$ | Values of entropy change |
| $\Delta G$ | Variation of Gibbs free energy |
| $k$ | Observed first-order rate constant of drug |
| $k_0$ | Observed first-order rate constant of drug in the absence of CD |
| $k_C$ | Observed first-order rate constant for the inclusion complex |
| $E_a$ | Activation energy (the amount of energy needed to initiate a chemical process, most often a reaction) |
| R.S.D. | Relative standard deviation |
| $C_{max}$ | Maximum plasma concentration |
| $T_{max}$ | Time required to reach $C_{max}$ |
| AUC | Total area under the plasma concentration–time |
| $AUC_{(0-24)}$ | Total area under the plasma concentration–time curve from 0 to 24 h |
| $AUC_{(0-\infty)}$ | Total area under the plasma concentration–time curve from time zero to infinity |

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## Introduction

Curcumin is a natural active ingredient in the rhizomes of the herb *Curcuma longa* L. [1]. It has been used not only as a dietary spice in foods in several Asian countries [2, 3], but also in pharmaceutical industry for many ailments because of its wide spectrum of pharmacological activities [4, 5]. It has been reported that curcumin has enormous potential in the prevention and treatment of cancer [6–9], HIV [10, 11], cystic fibrosis [12, 13], Alzheimer's disease [14] and Parkinson's disease [15]. However, as a hydrophobic polyphenol compound (see Fig. 1a), the poor aqueous solubility, relatively low bioavailability [16] and especially easy alkaline hydrolysis of curcumin have been highlighted as major problems of development into a modern drug.

Cyclodextrins (CDs) are cyclic oligosaccharides containing six or more (α-1,4)-linked α-D-glucopyranose units. The cavities of CDs are relatively hydrophobic compared to water, while the external faces are hydrophilic [17]. A variety of compounds especial with hydrophobic group never fail to be caged entirely or, at least partially, in cavities of CDs to form inclusion complexes through noncovalent interactions in aqueous solution [18–21]. This inclusion could lead to changes in the physicochemical properties of the guest, such as solubility, stability and bioavailability [22–27].

It has been well established that CDs and their derivatives as pharmaceutical excipients could overcome the undesirable properties of curcumin molecules through the formation of inclusion complexes [28–30]. The super molecular interactions of curcumin and CDs have been studied by spectrophotometry [31–33] or fluorescence methods [34, 35]. Tønnesen H. H. group has previously found that different CDs and their derivatives, such as *β*-CD, *γ*-CD, 2-hydroxypropyl-*β*-CD (HP*β*CD), hydroxypropyl-α-CD, hydroxypropyl-*γ*-CD, randomly methylated *β*-CD, sulfobutylether-*β*-CD, and so on could increase the solubility of curcumin and the hydrolytic stability of curcumin under neutral-alkaline conditions [36]. It could been concluded that increased stability of curcumin depended on the degree of protection by the different CDs, and the general order of the stabilizing effect was HP*β*CD > 2-*O*-methyl-*β*-CD ≫ hydroxypropyl-*γ*-CD in solution [37]. Yadav et al. [38] have developed a novel CD complex of curcumin that has superior attributes compared with free curcumin for cellular uptake and for anti-proliferative and anti-inflammatory activities. Chauhan et al. have concluded curcumin/*β*-CD enhanced curcumin delivery and improved its therapeutic efficacy in prostate cancer cells [39]. Among a variety of CDs, HP*β*CD (Fig. 1b) has been selected for encapsulation of curcumin in our study because it is good water-soluble and safe to human, which has been widely used in many pharmaceuticals [40].

It is well known that, external factors not only plays important role in in vitro evaluation of the complex [41–45], but also may have remarkable influence in in vivo study of the inclusion complex [46–48]. However, according to our knowledge, there is no report on the effect of HP*β*CD on pharmacokinetics controlled by pH of curcumin up to now. In the present study, we systematically studied the influence of the external factors (such as pH and temperature) on the apparent stability constant and inclusion thermodynamics between HP*β*CD and curcumin, which further affects the solubility, stability and pharmacokinetics of curcumin.

## Materials and methods

### Reagent and materials

Curcumin was purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Both curcumin reference (Batch No. 110823-200603) and emodin reference (Batch No. 110756-200110) were supplied by National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HP*β*CD (average mol. weight: 1,380–1,500) were purchased from Wacker Chemie AG (Burghausen, Germany). All experiment water was obtained from a Milli-Q system (Millipore Corp. model OM-140). Methanol of high-performance liquid chromatography grade was purchased from Concord technology Co., Ltd (Tianjin, China). All other chemicals were

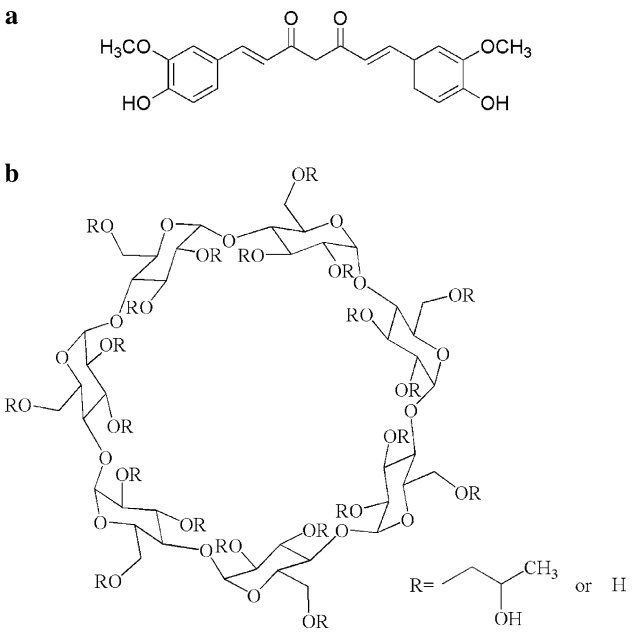



**Fig. 1** The structure of curcumin (**a**) and 2-hydroxybutenyl-*β*-cyclodextrin (**b**)

reagent grade. The buffers were prepared by phosphoric acid and disodium hydrogen phosphate. The ionic strength of the buffers was adjusted to $\mu = 0.08$ by addition of NaCl.

### Analysis of curcumin

Quantification of the curcumin concentration was carried out by HPLC on a Waters 515 HPLC system equipped with a 2487 UV-Detector (Waters Corporation, USA). Sample analysis was performed on a Waters SymmetryShield$^{TM}$ RP-$C_{18}$ column (150 mm × 3.9 mm, 5 μm). The mobile phase consisting of methanol–water–phosphoric acid (85:15:0.01, v/v/v) was prepared daily and filtered through Millipore membrane (0.45 μm) and degassed by ultrasonication before use. The UV absorbance of the sample (10 μL) was measured at the wavelength of 425 nm. The corresponding calibration curves were constructed, and linear response was found in the range from 0.0294 to 7.36 μg mL$^{-1}$.

### Phase solubility study

The phase solubility study was performed according to the method of Higuchi and Conners and determined at pH values ranging from 3.0 to 7.0 [49]. Excess amounts of curcumin were added to 10 mL buffer solutions containing HP$\beta$CD (ranging from 0 to 3 mM). The sealed tubes were kept in the dark and violently shaken for 12 h at 50 °C. After equilibrium was reached, aliquots were filtered (0.45 μm pore size) to remove undissolved solid. The filtrates were diluted with methanol (1:1, v/v) and centrifuged for 10 min before HPLC analysis. All the pH measurements were performed on a pH meter (DELTA 320, METTLER TOLEDO Corporation, Switzerland) equipped with a combination electrode, which was calibrated with primary buffer solution of pH 4.01, 6.86, and 9.18. The study was also investigated at different temperatures (30, 40, 45, 50, and 60 °C) in pH 7.0 buffer solutions. All studies were carried out in triplicate. The plots of molar concentration of the curcumin against the total molar concentration of HP$\beta$CD gave phase solubility diagrams.

### Hydrolytic stability

The hydrolytic stability of curcumin in HP$\beta$CD solution (5 mM) was monitored in pH 3.0–7.0 buffer solutions at 50 °C, respectively. The stability of curcumin in the absence of HP$\beta$CD was control groups. Stock solutions of the curcumin were prepared in methanol at a concentration of 0.368 mg mL$^{-1}$. Curcumin solutions (50 μL) were mixed into the above buffer solutions (10 mL). The samples were sealed and protected from light, then laid in a thermostat bath at predefined temperature. Samples (100 μL) were withdrawn at regular time intervals. These samples were diluted with methanol (100 mL), vortexed for 30 s, and centrifuged for 10 min. Aliquots (10 μL) were analyzed by HPLC. To avoid microbial degradation, all glassware and solutions were autoclaved prior to use. In the hydrolytic experiment, the influence of temperature was investigated at 30, 40, 45, 50, and 60 °C in pH 7.0 buffer solutions, respectively. All studies were carried out in triplicate.

### Analysis of curcumin in plasma

For blood sample analysis, HPLC experiments were performed on the Waters Alliance 2695 HPLC system with Waters 2487 UV detector. The analysis was performed on another column of the same type as mentioned above at 425 nm. The mobile phase consisted of 0.1% aqueous phosphoric acid (A) and methanol (B). A gradient programmer was: 0 min, 70% B; 15 min, 90% B. A constant flow rate of 1.0 mL min$^{-1}$ was maintained.

### The pharmacokinetics

Male Wistar rats with weights of 210 ± 10 g were obtained from Shanchuanhong Laboratory Animal Co., Ltd (Tianjin, China). The rats were divided into four groups stochastically and each group had six rats. All the experimental rats were fasted overnight before experiment. The animals were deprived of food 12 h before experimentation and 6 h after oral dosing. Fresh water was freely available during the entire experiment. Group 1 was oral administered with gum arabic solution (10%, pH 7.0), which contains 500 mg kg$^{-1}$ curcumin as a control experiment. The curcumin suspension was prepared by ultrasonication for 0.5 h before use. Group 2–4 were oral administered with curcumin/HP$\beta$CD (1:5, M/M) complex in the pH 3.0, 5.0, and 7.0 buffer solutions (equivalent to 500 mg kg$^{-1}$ curcumin), respectively. These complexes were made by shaking for 4 h. Blood samples were collected at 2 min, 30 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 5 h, 7 h, 10 h, and 24 h from orbital venous plexus after the administration. Plasma was immediately prepared by centrifugation for 10 min at 4000 rpm (Sigma low-temperature centrifuge). The plasma (200 μL) was mixed with 25 μL of an internal standard (emodin, 1.58 μg mL$^{-1}$) and 25 μL of phosphoric acid (0.2 M). Curcumin was extracted by ethyl acetate (800 μL) twice by vortexing vigorously for 2 min. The resulting organic phase (1,600 μL) was withdrawn and evaporated under $N_2$ gas. The residue was redissolved in 100 μL of methanol, centrifuged for 10 min (14,000 rpm). The supernatant (20 μL) was subjected to HPLC. The pharmacokinetic parameters were calculated by Drug and Statistics (DAS) VER 1.0 software

## Results and discussion

### Effect of pH on curcumin/HP$\beta$CD

The phase-solubility diagrams of curcumin in different pH buffer solutions are shown in Fig. 2. It could be observed that the solubility of curcurmin increased with increasing concentration of HP$\beta$CD. The solubility of curcumin could be greater increased by HP$\beta$CD than $\beta$CD, because of the improved water solubility of HP$\beta$CD [35, 38]. It could be found that the solubility and the slopes of the linear plots faintly increased with increasing pH values. The plots show linear trend within the HP$\beta$CD concentration range studied and display a typical $A_L$ type diagram, which is consistent with a 1:1 molecular complex formation. The apparent stability constant ($K_S$) was been calculated using their regression lines following the equation:

$$K_S = \text{slope}/[S_0(1 - \text{slope})] \quad (1)$$

where $S_0$ is the solubility of curcumin in the absence of CDs and slope means the corresponding slope of the phase solubility diagram. Yadav et al. [38] found that the phase solubility of curcumin shows $A_L$-type at low HP$\beta$CD concentration. However, the $A_P$-type of phase solubility could be concluded by Másson et al. when HP$\beta$CD concentration was higher. In addition, they have concluded that the apparent stability constant $K_{1:1}$ ($4.42 \times 10^3$ $M^{-1}$) is far greater than $K_{2:1}$ ($4.0 \times 10^1$ $M^{-1}$) [33]. The apparent stability constant has been calculated by Wagner et al., and were reported to be $K_{1:1} \geq K_{2:1}$ using fluorescence measurement [35]. It seems difficult to form 2:1 inclusion complex of curcumin with HP$\beta$CD in buffer solution. Tønnesen et al. [36] has reported that a 1:1 stoichiometry between curcumin and $\beta$-CD derivatives was found by phase-solubility diagrams. However, Yadav et al. [38] also found the complex was formed at 2CD: one guest in the solid state. We propose the following speculative scenario to explain this type of phenomena: (1) curcumin could form 1:1 inclusion complexes at low CD concentration; (2) when CD concentration is high, CD can exert some of their effect by forming non-inclusion complexes and surfactant like molecular aggregates [50]; (3) when CD is extremely high concentration or solid-state, initial formation of a 1:1 complex is followed by addition of a second CD for yielding a 2:1 (CD : curcumin) complex [35].

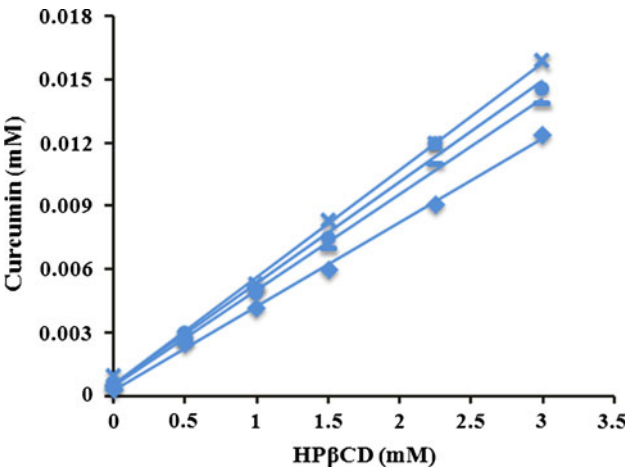



**Fig. 2** Phase solubility study of curcumin with HP$\beta$CD in buffers at pH 3 (*filled diamond*), pH 5 (*horizontal thick line*), pH 6 (*filled circle*), and pH 7 (*cross*)

As reported by Másson et al., ionization of the compounds by increasing the pH did not sufficiently increase solubility [33]. While it does have some influence on the $K_S$ (Table 1). It could be found that the increasing $K_S$ coupled with decreasing pH values. The value of $K_S$ at pH 3.0 was about 1.47 fold higher than that at pH 7.0. With the increasing pH value, the affinity between curcumin and HP$\beta$CD was decreased. As Tang et al. [31] summarized "the optimum pH value for the inclusion complex formation is in the lower pH range, where curcumin is in its acid form, but not its conjugate base, which has higher polarity and therefore is relatively difficult to enter the hydrophobic cavity of $\beta$CD". So, the ionic states of curcumin gradually increase, which may partially offset the desire of curcumin into HP$\beta$CD cavity.

### Effect of temperature on curcumin/HP$\beta$CD

We continue to study the effect of temperatures on complex formation. It can be found that $K_S$ values decrease with increasing temperature (Table 2). Furthermore, some thermodynamic parameters of complex formation could also be obtained by the variable temperature experiments. The values of enthalpy ($\Delta H$) and entropy ($T\Delta S$) changes were calculated following the Van't Hoff equation (2):

$$\ln K_S = -\Delta H/RT + \Delta S/R \quad (2)$$

As shown in Fig. 3, the plot of ln $K_S$ versus $1/T$ shows a linear curve. $\Delta H$ calculated from the slope is $-31.90$ kJ $mol^{-1}$. $T\Delta S$ obtained from the intercept is $-7.39$ kJ $mol^{-1}$. The free energy changes ($\Delta G$) (Table 2) for the complex formation were calculated by the Gibbs equation (Eq. 3):

**Table 1** The apparent stability constants ($K_S$) for curcumin/HP$\beta$CD at different pH calculated from the slope and $S_0$ of phase solubility experiments ($n = 3$)

| pH | $S_0$ ($\times 10^{-7}$ M) | Slope ($\times 10^{-3}$) | $R^2$ | $K_S$ ($\times 10^3$ $M^{-1}$) |
|---|---|---|---|---|
| 3.0 | 3.31 ± 0.38 | 4.23 ± 0.21 | >0.99 | 12.90 ± 0.86 |
| 5.0 | 4.24 ± 0.24 | 4.47 ± 0.06 | >0.99 | 10.60 ± 0.64 |
| 6.0 | 6.89 ± 0.49 | 4.90 ± 0.10 | >0.99 | 7.16 ± 0.41 |
| 7.0 | 9.74 ± 0.41 | 5.07 ± 0.15 | >0.99 | 5.22 ± 0.09 |

**Table 2** The apparent stability constants ($K_S$) and the free energy changes ($\Delta G$) of curcumin/HP$\beta$CD at different temperature ($n = 3$)

| Temperature (K) | $S_0$ ($\times 10^{-7}$ M) | Slope ($\times 10^{-3}$) | $R^2$ | $K_S$ ($\times 10^3$ $M^{-1}$) | $\Delta G$ (kJ $mol^{-1}$) |
|---|---|---|---|---|---|
| 303 | 2.70 ± 0.26 | 3.10 ± 0.17 | >0.99 | 11.62 ± 0.69 | −23.58 |
| 313 | 3.93 ± 0.12 | 3.53 ± 0.06 | >0.99 | 8.58 ± 0.59 | −23.57 |
| 318 | 4.77 ± 0.21 | 3.40 ± 0.10 | >0.99 | 7.19 ± 0.27 | −23.48 |
| 323 | 9.73 ± 0.42 | 5.07 ± 0.15 | >0.99 | 5.22 ± 0.10 | −22.99 |
| 333 | 13.53 ± 1.22 | 5.17 ± 0.23 | >0.99 | 3.85 ± 0.20 | −22.86 |

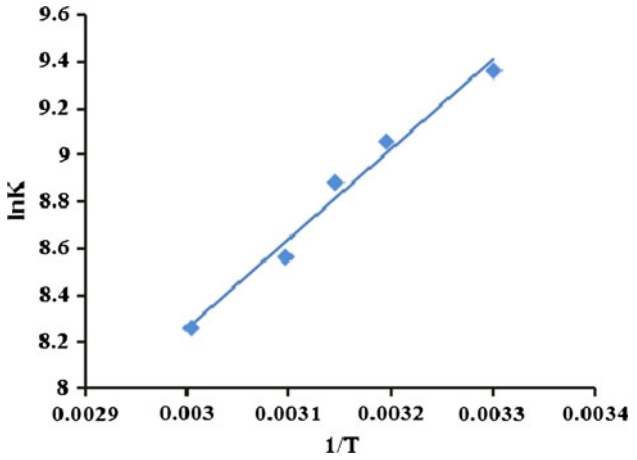



**Fig. 3** Van't Hoff plot of the formation of the inclusion complex between curcumin and HP$\beta$CD

$$\Delta G = -RT \ln K_S \quad (3)$$

The $\Delta H$ is dramatically negative, which implies that the interaction processes between curcumin and HP$\beta$CD are exothermic. It indicates that in the forming complex process, the main driving force is due to 'high energy water' moving out from the cavity of CD, derived from formation of hydrogen bonds and van der Waals interactions. The negative $T\Delta S$ value indicates that during the complexion process, translational and rotational degrees of the curcumin decrease, and a more ordered system environment has been formed [51]. Tang et al. [31] has reported that the $\Delta S$ of curcumin/$\beta$CD was positive (0.014 kJ $mol^{-1}$) using spectrophotometry, while Swaroop et al. [34] found the $\Delta S$ is negative but to a very small extent, based on fluorescence measurements. Compared with $\beta$CD, a more ordered system environment was formed, when curcumin is included into the cavity of HP$\beta$CD. Overall, the values of $\Delta G$ are also negative, which means the complexations are spontaneous processes.

## Effect of pH on hydrolytic stability of curcumin and inclusion complex

The stability experiments were focused on the hydrolysis kinetics of curcumin in different pH buffer solutions in the absence and presence of HP$\beta$CD. The logarithmic remaining percent of curcumin versus hydrolytic time was reasonably linear (Fig. 4), indicating that the degradations followed pseudo first-order kinetics. The observed first-order rate constants ($k$) were obtained by linear regression based on the following equation:

$$\ln([C_t]/[C_0]) = -kt \quad (4)$$

where $t$ is hydrolytic time; $[C_0]$ and $[C_t]$ are the initial and time-dependent concentration of curcumin, respectively. The observed rate constant values were obtained by linear

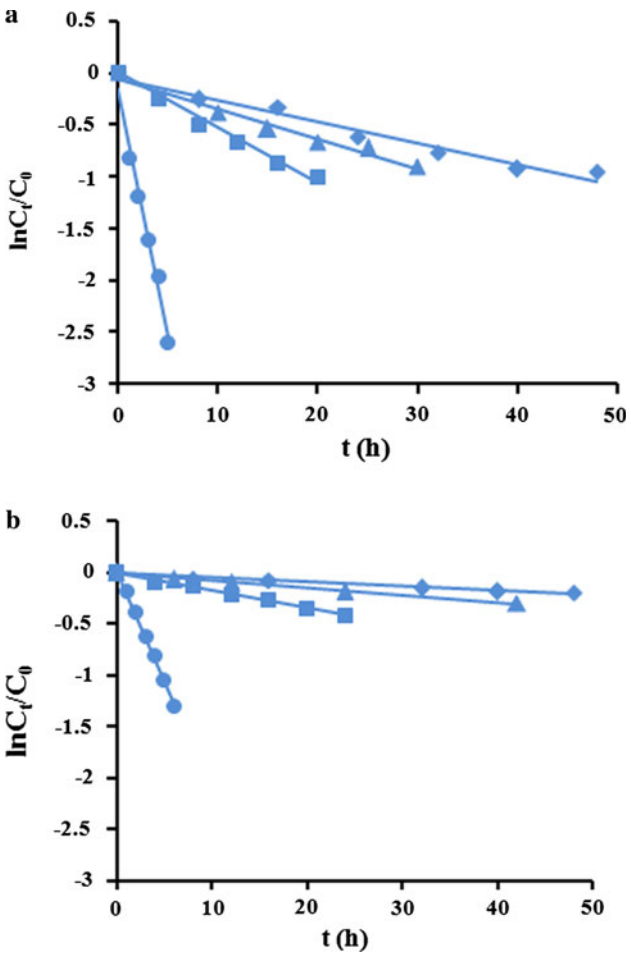



**Fig. 4** First-order plots for the hydrolysis of curcumin (**a**) and the inclusion complex (**b**) in buffer solutions at 50 °C. *Filled diamond* pH 3.0, *filled triangle* pH 5.0, *filled square* pH 6.0, and *filled circle* pH 7.0

**Table 3** Effects of pH of buffer on hydrolytic rate constants for curcumin ($k_0$), the inclusion complex ($k_C$)

| pH | $k_0$ | $k_C$ | $k_0/k_C$ |
|---|---|---|---|
| 3.0 | $2.27 \times 10^{-2} \pm 2.29 \times 10^{-3}$ | $3.96 \times 10^{-3} \pm 7.57 \times 10^{-4}$ | 5.73 |
| 5.0 | $3.18 \times 10^{-2} \pm 8.50 \times 10^{-4}$ | $7.07 \times 10^{-3} \pm 4.04 \times 10^{-4}$ | 4.50 |
| 6.0 | $5.91 \times 10^{-2} \pm 8.08 \times 10^{-4}$ | $1.86 \times 10^{-2} \pm 1.30 \times 10^{-3}$ | 3.18 |
| 7.0 | $5.39 \times 10^{-1} \pm 2.05 \times 10^{-2}$ | $2.07 \times 10^{-1} \pm 5.05 \times 10^{-3}$ | 2.60 |

regression of Eq. 4 ($r > 0.99$) and are showed in Table 3. $k_0$ is the observed first-order rate constant of curcumin in the absence of HP$\beta$CD; $k_C$ is the observed first-order rate constant for the inclusion complexes. As Tønnesen et al. previously described, some degradation could be observed for curcumin in neutral and alkaline solutions [37]. The degradation pathways and products of curcumin had been reported in the previous literature, as shown in Scheme 1 [52, 53]. The hydrolytic degradation is due to attack from the nucleophilic $OH^-$ ion on the carbonyl carbon in the keto-enol moiety. The main hydrolytic products previously proved to be trans-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexena, ferulic acid and feruloyl methane [54]. It should be mentioned that the hydrolytic stability of curcumin is improved in the presence of HP$\beta$CD. When the curcumin combined with CD, the aromatic moiety is included in the CD cavity. It results in a very effective steric hindrance against hydrolysis. Its chemical stability has been less affected by the external environment, such as $OH^-$ ion.

## Effect of temperature on hydrolytic stability of curcumin and inclusion complex

Temperature generally plays a significant role in hydrolysis of drug. Using the above method, the observed rate

**Scheme 1** Structure of curcumin degradation products and possible degradation pathway in solution

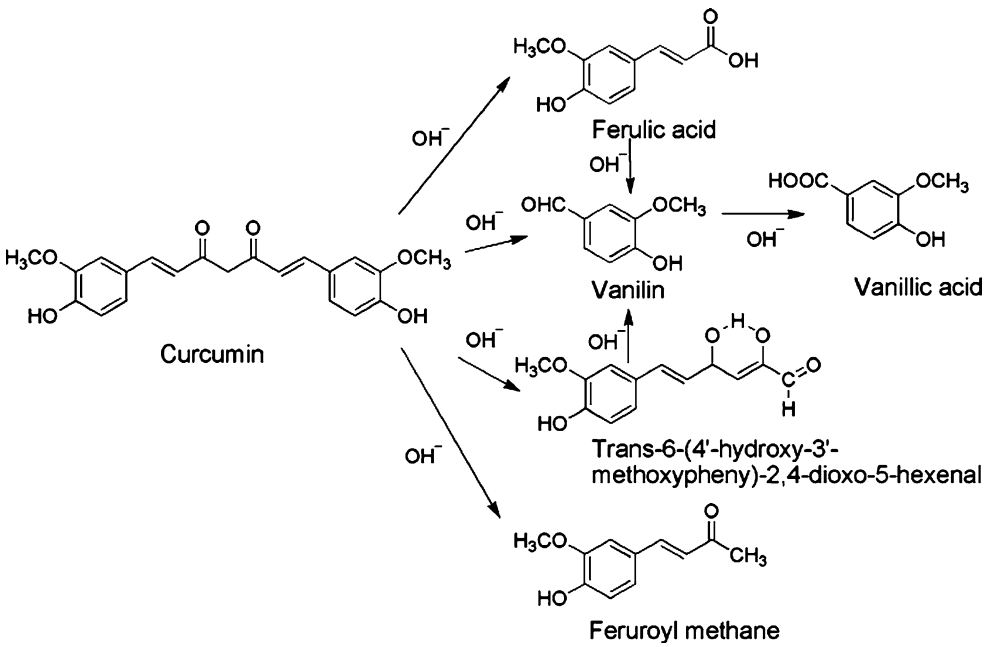



**Table 4** Effects of temperature on hydrolytic rate constants for curcumin ($k_0$), curcumin/HP$\beta$CD ($k_C$)

| Temperature (K) | $k_0$ | $k_C$ | $k_0/k_C$ |
|---|---|---|---|
| 303 | $1.95 \times 10^{-1} \pm 2.25 \times 10^{-3}$ | $3.38 \times 10^{-2} \pm 1.42 \times 10^{-3}$ | 5.75 |
| 313 | $3.35 \times 10^{-1} \pm 2.37 \times 10^{-2}$ | $8.15 \times 10^{-2} \pm 2.47 \times 10^{-3}$ | 4.11 |
| 318 | $4.49 \times 10^{-1} \pm 1.31 \times 10^{-2}$ | $1.39 \times 10^{-1} \pm 7.48 \times 10^{-3}$ | 3.22 |
| 323 | $5.39 \times 10^{-1} \pm 2.05 \times 10^{-2}$ | $2.07 \times 10^{-1} \pm 5.05 \times 10^{-3}$ | 2.60 |
| 333 | $1.49 \pm 5.91 \times 10^{-2}$ | $7.61 \times 10^{-1} \pm 1.64 \times 10^{-2}$ | 1.97 |

constants of curcumin in the absence and present of HP$\beta$CD were obtained and are showed in Table 4. The activation energy for hydrolytic reaction was evaluated by Arrhenius equation (5):

$$\ln k = \ln A - E_a/RT \quad (5)$$

where $A$ represents a preexponential factor; $E_a$ stands for the activation energy; $R$ is the universal gas constant and $T$ is the absolute temperature. As shown in Fig. 5, the linear regression lines can be obtained from the plots of ln $k$ versus $1/T$, and the activation energies ($E_a$) could be calculated from the Eq. 5. The $E_a$ of the inclusion complex (85.78 kJ mol$^{-1}$) is larger than that of curcumin (51.85 kJ mol$^{-1}$). It indicates that HP$\beta$CD could increase the reaction barrier of curcumin degradation and leads to retardation of the degradation process of curcumin.

## Effect of binding strength of curcumin/HP$\beta$CD on the hydrolytic stability

Based on the above results, we compare $k_0$ and $k_C$ so as to estimate the improvement of hydrolytic stability by HP$\beta$CD. The increase in the ratio of degradation rate ($k_0/k_C$) can be ascribed to the improved stabilities of curcumin by HP$\beta$CD. It could be found result is interesting that the values of $k_0/k_C$ decreased with increasing pH values (Table 3). It is also worth noting that the value of $k_0/k_C$ increased with decreasing the temperature (Table 4). To better demonstrate binding strength effects on hydrolysis, the ratio of degradation rate ($k_0/k_C$) of curcumin was plotted against the $K_S$ values. There was a linear relationship between $k_0/k_C$ and $K_S$ values (Fig. 6).

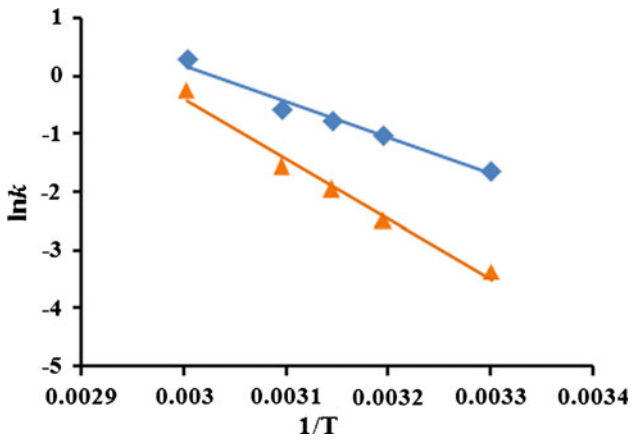



**Fig. 5** Influence of the temperature on the rate constant of the curcumin (*filled diamond*) and curcumin/HP$\beta$CD (*filled triangle*)

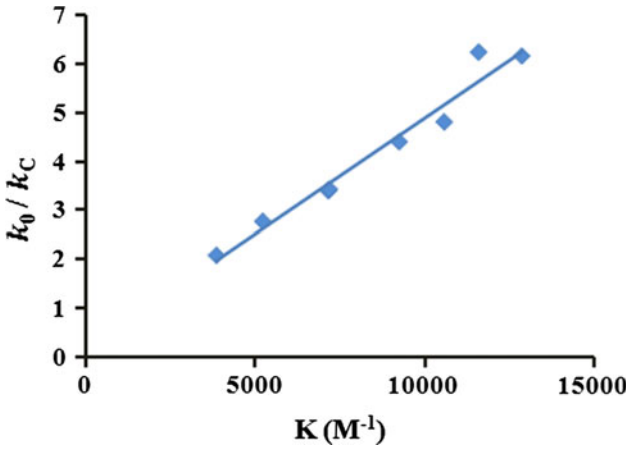



**Fig. 6** Variation of the $k_0/k_C$ with the apparent stability constants ($K_S$) for curcumin/HP$\beta$CD in aqueous solution in the dark

## Pharmacokinetics of curcumin after oral administration of a suspension and inclusion complex

The HPLC method for analysis of curcumin in plasma has been validated. A good linear relationship was obtained for curcumin with concentration ranging from 2.5 to 250 ng mL$^{-1}$. The R.S.D. of curcumin for inter-day and intra-day precision and accuracy at low, medium and high concentration was blow 8.67%. The results of extraction recovery were all above 87% for three concentrations. The plasma samples of curcumin concentration higher than 250 ng mL$^{-1}$ were diluted for assay.

The mean plasma concentration–time curves of curcumin after oral administration of curcumin in 10% gum arabic solution (pH 7.0) and the complexes in pH 3.0, 5.0, and 7.0 buffer solutions are shown in Fig. 7. The plasma concentration–time curve of curcumin displaying two peaks was present in all groups, which suggested the possible presence of an enterohepatic circulation [55]. The pharmacokinetic parameters are listed in Table 5. The plasma levels of curcumin after administration of inclusion complex were clearly faster and higher than those achieved with an equal curcumin dose given alone. The value of the area under the plasma concentration time curve (AUC) for the complex (866.70 ± 156.76 ng mL$^{-1}$ h, pH 7.0) was about three times greater than that of curcumin alone (296.01 ± 284.87 ng mL$^{-1}$ h). As shown in Scheme 2, as the lipophilic drug, curcumin is difficult to across the unstirred layer. Most of curcumin is excreted or degraded via the intestinal tract [56]. In fact, the CD has been regarded as drug carrier which is able to deliver the drug to biological membrane in an efficient way [57]. Consequently, the inclusion complex could efficiently reach intestinal absorption site and significantly increase the bioavailability of curcumin. In particular, the $C_{max}$ after administration of the complex (pH 7.0) was observed at 1.42 h; on the other hand,

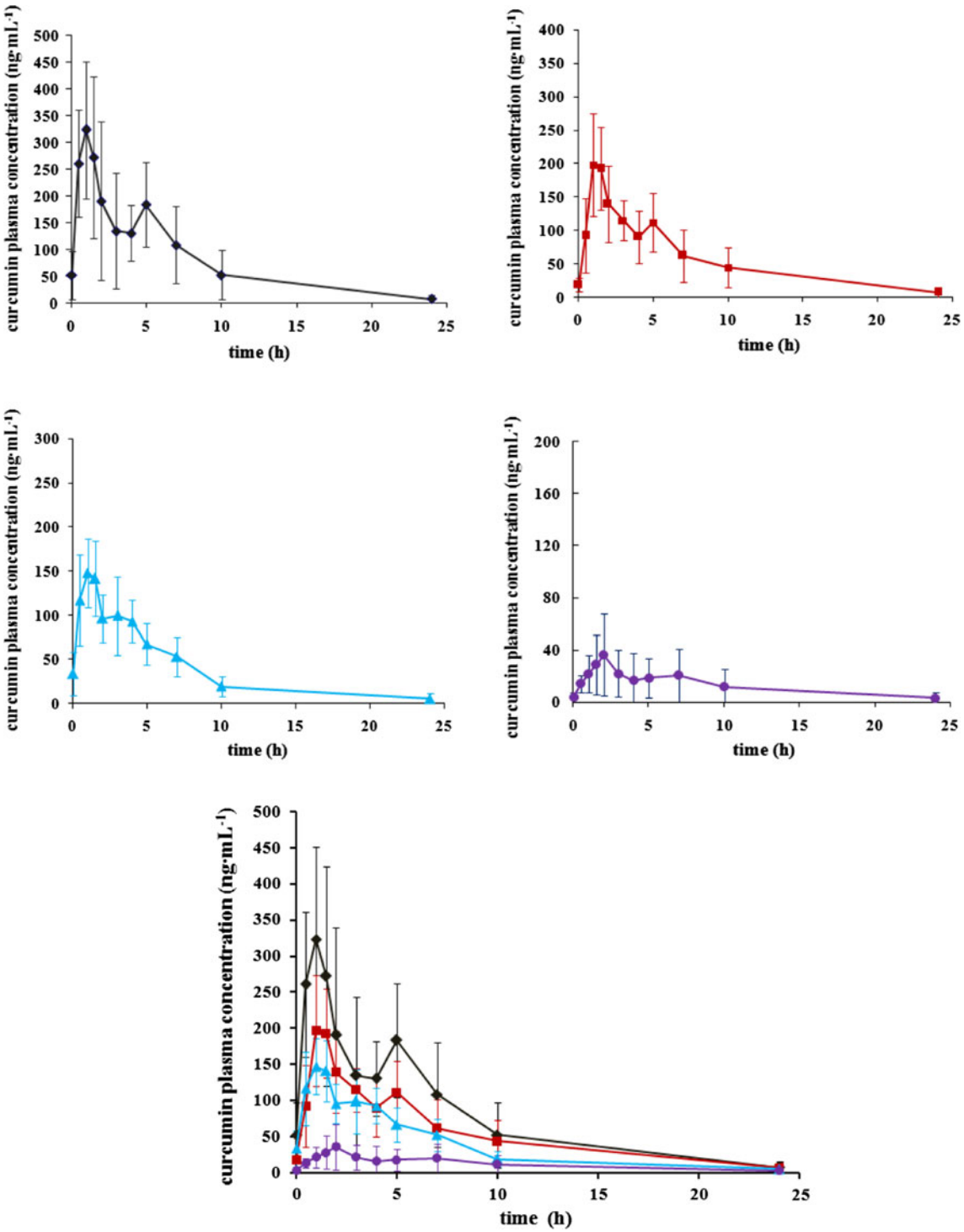



**Fig. 7** Mean plasma concentration/time curve for curcumin after single oral administration of curcumin suspension (*filled circle*) and the curcumin/HP$\beta$CD in buffer of pH 3.0 (*filled diamond*), pH 5.0 (*filled square*) and pH 7.0 (*filled triangle*) in rats ($n = 6$). Error bars represent SD

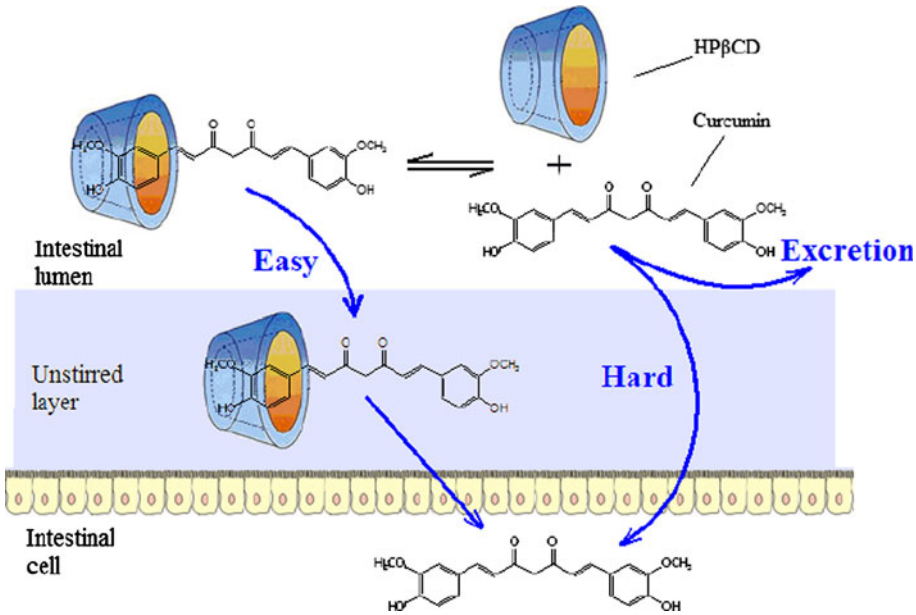
the complex (pH 3.0) resulted in the rapid appearance of curcumin in plasma, attaining the $C_{max}$ after 1.08 h. In addition, the value of $C_{max}$ for the complex is 370.10 ± 118.84 ng $mL^{-1}$ (pH 3.0), 235.98 ± 47.00 ng $mL^{-1}$ (pH 5.0) and 178.11 ± 25.41 ng $mL^{-1}$ (pH 7.0) respectively. A tendency could be observed that the area under the plasma concentration time curve (AUC) of the complex was increased with decreasing pH value. Actually, the complex in acidic buffer

**Table 5** Pharmacokinetic parameters of curcumin after oral administration of curcumin suspension and curcumin/HP$\beta$CD inclusion complex (all equivalent to curcumin 500 mg $kg^{-1}$) to rats

| | Curcumin suspension | Curcumin/HP$\beta$CD | | |
|---|---|---|---|---|
| | | pH 3.0 | pH 5.0 | pH 7.0 |
| $T_{max}$ (h) | 1.50 ± 0.55 | 1.08 ± 0.38 | 1.25 ± 0.27 | 1.42 ± 0.86 |
| $C_{max}$ (ng $mL^{-1}$) | 41.19 ± 27.89 | 370.10 ± 118.84 | 235.98 ± 47.00 | 178.11 ± 25.41 |
| $AUC_{(0-24)}$ (ng $mL^{-1}$ h) | 296.01 ± 284.87 | 1752.76 ± 930.27 | 1305.97 ± 444.03 | 866.70 ± 156.76 |
| $AUC_{(0-\infty)}$ (ng $mL^{-1}$ h) | 403.02 ± 300.43 | 1963.54 ± 872.33 | 1393.49 ± 460.23 | 972.41 ± 191.65 |

Each value represents the mean ± SD for six rats

**Scheme 2** The intestinal absorption of curcumin and the curcumin/HP$\beta$CD




solution has an efficient binding formation. The oral absorption of curcumin has been significantly increased by the strong binding with HP$\beta$CD.

## Conclusions

In this paper, external factor has a significant influence on the interaction of curcumin/HP$\beta$CD. Through complexation with HP$\beta$CD, the solubility of curcumin in neutral aqueous solution was improved significantly. Curcumin has higher affinity for HP$\beta$CD, which could improve the stability of curcumin more effectively. Additionally, pharmacokinetic studies of curcumin/HP$\beta$CD in rats indicated that the complex prepared in acidic solution had higher bioavailability compared to the complex prepared in neutral aqueous solution, which suggested that pH play a significant role in therapeutic efficacy. Through our research, being a nontoxic natural product, curcumin could be useful in clinical application with the help of HP$\beta$CD.

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