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ORIGINAL ARTICLE

Preparation, in-vitro release and antioxidant potential of formulation of apigenin with hydroxypropyl-β-cyclodextrin modified microemulsion

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Abstract This work was carried out to exploit the feasibility of microemulsion combining apigenin/hydroxypropyl-β-cyclodextrin (API/HP-β-CD) complex as the carrier for improving the solubility of API, a bioactive flavonoid with various pharmacological activities. The API/HP-β-CD complex in solid state was prepared by solvent-freeze-drying method and characterized by FT-IR, PXRD and ^{1}H NMR. To further increase the solubility of API, the complex of HP-β-CD with food-grade cosurfactant-free microemulsion was constructed. The aqueous solubility of API significantly increases in the HP-β-CD/Microemulsion complex, via solubilizing dominantly into the "palisade" layer, minor outer phase and inner core. The HP-β-CD modified microemulsion improves the cumulative percentage of API released. Moreover, API loaded in microemulsions with HP-β-CD had a higher antioxidant activity than that without HP-β-CD.

Keywords Apigenin · Hydroxypropyl-β-Cyclodextrin · Microemulsion · Antioxidant activity · In vitro release

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Introduction

Apigenin (5,7-dihydroxy-2-(4-hydroxyphenyl)chromen-4-one, API), one of the most common flavonoids, is found in a variety of fruits and vegetables [1]. API has a wide range of pharmacological applications, including antiviral [2], antioxidant [3], anti-inflammatory [4], anticancer [5] and so on. A large amount of studies on cancer prevention have demonstrated that API acts through numerous mechanisms, including the induction of apoptosis [6], the inhibition of invasive growth involving blocking pathway [7], the down-regulation of cell cycle progression [8] and the activation of caspases involved in late process of cell apoptosis [9]. Particularly attractive, API has the potential to be developed into cancer-preventative agents because of its preferential induction of apoptosis in tumor cells rather than normal cells [10].

However, the aqueous solubility of API in tested phosphate buffers was very poor with maximum solubility of 2.16 µg/mL at pH 7.5 [11]. The poor solubility of API would result in a slow dissolution and may create delivery problems such as unsatisfactory cutaneous permeability, erratic absorption and low oral bioavailability [11], leading to a limited clinical use and therapeutic value. Moreover, studies suggest that API is rapidly metabolized in rats and Caco-2 cells, an established model of human intestinal epithelium [12]. Therefore, by improving water solubility and avoiding extensive presystemic metabolism, alternative route and delivery system of drug administration would be effective strategies to enhance the bioavailability of API.

Hydroxypropyl-β-cyclodextrin (HP-β-CD), an alternative to β-cyclodextrin with high solubility and less toxicity, has been studied as an excipient to overcome the solubility related problems of insoluble drugs [13]. Enhancement of

solubility and antioxidant activity of API by complexation with HP-β-CD has been studied [14–16]. Although HP-β-CD has been shown to be well tolerated in humans, there is some risk such as soft stools and diarrhoea with higher oral doses [17]. Therefore, it is necessary to search another efficient carrier to form a combined delivery system with HP-β-CD for API.

To microemulsions as delivery vehicles for the purpose of enhancing the efficacy of new and existing drugs has been paid more and more attention [18, 19]. Several studies have suggested that microemulsions can significantly improve the bioavailability of drugs by increasing their solubility due to the incorporation by lipophilic and/or hydrophilic phase [20]. However, high surfactants concentration is required to reduce the surface tension between oil and water phase in microemulsion system, hence leading to increased toxicity [21]. Various approaches have been explored to expand microemulsion region and thereby reduce the surfactant content necessary. For instance, Wu et al. [22] combined use of phospholipid complex and self-emulsifying microemulsions (Tween 80/glycerol/ethyl oleate) for improving the oral absorption of baicalin. Inclusion complex of piroxicam with β-cyclodextrin and microemulsion, in vitro drug release and in vivo topical anti-inflammatory effect have been studied [23, 24]. To our best knowledge, no reports were demonstrated to investigate the solubility, in vitro release and antioxidant activity of API in the combined HP-β-CD/Microemulsion system.

In the present study, an API/HP-β-CD inclusion complex in solid state was prepared by a solvent-freeze-drying method, and characterized by FT-IR, PXRD and ^{1}H NMR spectroscopy. Then, stability constants of the complex (K) and thermodynamic parameters of the complexation process were investigated by phase solubility studies. In addition, we developed a microemulsion based on the food ingredients Tween 80 and isoamyl acetate. The phase behaviors of Tween 80/isoamyl acetate/water system were studied with the presence and absence of HP-β-CD in comparison. Lastly, we exploited the feasibility of combining HP-β-CD and microemulsion complex as the carrier for improving the solubility, in vitro release and autoxidation activity of API. Some interesting results were obtained.

Materials and methods

Materials

Apigenin (API, ≥92 %) was purchased from Nanjing Zelang Medical Technology Co., Ltd. China. Apigenin (standards, 98 %) were purchased from Sinopharm Chemical Reagent Co., Ltd. China. Hydroxypropyl-β-cyclodextrin (HP-β-CD, MW ∼ 1454 with DS of 5.5) was purchased from Shandong Binzhou Zhiyuan Bio-Technology Co., Ltd. China. Tween 80 was purchased from Shanghai Zhanyun Chemical Co., Ltd. China. Pyrogallic acid, tris(hydroxymethyl)aminomethane, HCl, ethanol, isoamyl acetate, $Na_2HPO_4 \cdot 12H_2O$ and $NaH_2PO_4 \cdot 2H_2O$ was purchased from Sinopharm Chemical Reagent Co., Ltd. China. All reagents and solvents, which are analytic grade, were used without further purification. Deionized water was used in all samples after doubly distilled.

Preparation of API/HP-β-CD complex

The solvent-freeze-drying method described by Ventura et al. [25] was used to prepare API/HP-β-CD complex with a few modifications. Briefly, HP-β-CD (1.2 g) was solubilized in 30 mL of water/ethanol solution (40:60 v/v) at room temperature. An excess amount of solid API was added into the solution and stirred at 50 °C for 1 h. 0.45 μm Millipore filter was chosen to filter the obtained suspension, and the filtrate was freeze-dried by a SCIENTZ-10N freeze-dryer (Scientz Biotechnology, Ningbo, China).

Apigenin quantification

In order to determine the API content, a standard concentration curve was prepared as demonstrated [26] with a few modifications. Briefly, a certain amount of API standards was weighed accurately and solubilized with water/ethanol solution (1:1 v/v) to obtain the API-stock-solution (46 mg/L). The UV–Vis spectrum of API diluted solution was recorded in the range from 200 to 400 nm by a UV–Vis spectrophotometer (Thermo Scientific, Genesys 10S, USA). The maximum absorption peak could be seen at the wavelength of 343 nm (online appendix Fig. A.1a), which was chosen as the detection wavelength.

A series of API concentration were precisely prepared by diluting the API-stock-solution. Absorbance was measured at the wavelength of 340 nm. Standard concentration curve was prepared by plotting absorbance data against concentration of standard solution (online appendix Fig. A.2). The regression equation was addressed below:

$$\text{Abs} = 19.374 C_{API} + 0.005 \quad (R^2 = 0.9996) \tag{1}$$

where Abs is the absorbance of API, C_{API} is the concentration of API (mmol/L).

Characterization of API/HP-β-CD complex

The UV–Vis spectrum of the API, HP-β-CD and API/HP-β-CD complex were recorded in the range from 200 to

400 nm by a UV–Vis spectrophotometer (Thermo Scientific, Genesys 10S, USA).

The X-ray powder diffractograms of API, HP-β-CD, physical mixture and API/HP-β-CD complex were obtained using an X-ray powder diffractometer (D8 Advance, Bruker, Germany), and the samples were investigated in the 2θ range of 5°–60° using Cu-Kα radiation.

The FT-IR spectra of API, HP-β-CD, physical mixture and API/HP-β-CD complex were obtained using a Fourier transform infrared spectrometer (ALPHA, Bruker, Germany). The spectral range was 400–4000 cm^{-1} with a resolution of 2 cm^{-1}. All of the samples were made to perform the measurements in the solid state.

^{1}H NMR spectra were recorded on Bruker Ascend 400 MHz NMR Spectrometer (Switzerland) using a 5 mm probe at room temperature. Acquisition parameters consisted of spectral width 8012.82 Hz and number of scans of 16. Relaxation delay and acquisition time were 1 and 4.09 s respectively. API and API/HP-β-CD complex were dissolved in DMSO-d$_6$ (Aldrich). HP-β-CD and API/HP-β-CD complex were dissolved in D$_2$O (Aldrich). Tetramethylsilane (TMS) as the internal standard was used to tune chemical shifts reported in ppm.

Phase solubility studies of API/HP-β-CD complex in solution

Solubility phase diagrams of API/HP-β-CD system, plotting the API concentration (C_{API}) against the HP-β-CD concentration ($C_{HP-β-CD}$), were obtained by the Higuchi and Connors method [27]. Briefly, excess amounts of API were added to PBS (pH 7.0), poured into flasks, containing various concentrations of HP-β-CD (0–170 mmol/L) and stirred continuously until reaching to dissolution equilibrium at various temperatures (30, 37 and 45 °C). Time for equilibrium of the dissolution and complexation of API was investigated (online appendix Fig. A.3), and an equilibration time of 48 h was used in the phase solubility studies. The suspensions were centrifuged twice through a high speed centrifuge (MiniSpin, Eppendorf, Germany) at 6000 rpm for 10 min and API concentration was determined by UV–Vis spectroscopy analysis.

The following dynamic equilibrium was constructed among API, HP-β-CD and API/HP-β-CD inclusion complex in solution.

$$\text{API} + \text{HP} - \beta - \text{CD} \overset{K}{\rightleftharpoons} \text{Complex} \tag{2}$$

K was regarded as the apparent stability constant of API/HP-β-CD complex and could be calculated through the following expression.

$$K = \frac{\alpha}{S_0 \times (1 - \alpha)} \tag{3}$$

where α represents the slope of the phase-solubility curve and S_0 represents the apparent solubility of API without HP-β-CD.

The van't Hoff plot [25], plotting log K against the reciprocal of the absolute temperature (T), could be used to evaluate the thermodynamic parameters of the complexation.

$$\log K = -\frac{\Delta H}{2.303R} \cdot \frac{1}{T} + \frac{\Delta S}{2.303R} \tag{4}$$

where the change in enthalpy (ΔH) and the change in entropy (ΔS) were determined from the slope and intercept of the straight line, respectively.

Studies of API/HP-β-CD-Microemulsion system

Construction of phase diagrams

In order to obtain the concentration range of components for the existing region of microemulsions, ternary phase diagrams were constructed using a titration method [28]. Firstly, a series of mixtures of surfactant (Tween 80) and oil phase (isoamyl acetate) were prepared at different mass ratios in a tube with cover. To obtain a homogeneous solution, the mixture could be heated to 50–60 °C. When each of the mixed solution cooled to room temperature, individual drops of water phase (H$_2$O or 0.15 mol/L HP-β-CD aqueous solutions) were then added. After each addition, the resulting mixture was thoroughly mixed and equilibrated at 25 °C in a thermostatic water bath. The transition from transparency to turbidity was regarded as the phase boundary.

To examine the microstructures and their structural changes in microemulsion systems, the electrical conductivity of each microemulsion was measured at 25 °C using a model DDSJ-308A digital conductivity meter equipped with a DJS-1C platinum black electrode (Shanghai INESA Scientific Instrument Co., Ltd., Shanghai, China).

Formulation of HP-β-CD-Microemulsion encapsulated apigenin

The pseudo-ternary phase diagram of microemulsion with HP-β-CD was constructed where the microemulsion region was identified by an isotropic, single phase and fluid formulation. The mixture, prepared by adding appropriate amounts of isoamyl acetate and Tween 80, was kept at 50 °C and well mixed using a vortex mixer. HP-β-CD aqueous solution ($C_{HP-β-CD} = 0.15$ mol/L) was then added to the mixture in a thermostatic chamber at 25 ± 0.1 °C until a transparent and isotropic microemulsion was obtained.

An excess of API was added to the obtained microemulsion sample, and the dispersion was constantly stirred for 24 h in a thermostatic chamber at 25 ± 0.1 °C. After this, the amorphous material was centrifuged at 10,000 rpm for 15 min using a high speed centrifuge, and the supernatants were obtained for further studies.

In vitro release of HP-β-CD-Microemulsion encapsulated apigenin

In vitro drug release studies were performed by using the dialysis method [29] with a few modifications. PBS containing 40 % (v/v) ethanol as release medium was prepared to simulate human intestinal juice. 2 mL of apigenin-saturated microemulsions with or without HP-β-CD were placed in a dialysis tube (molecular weight cut off of 3500). The dialysis tube was suspended in 80 mL of the release medium with a stirring speed of 100 rpm using magneton at 37 ± 0.1 °C. At the predetermined time intervals, 3 mL of release medium was withdrawn, and the same volume of fresh medium was added to maintain the constant volume. The amount of released apigenin was determined at the wavelength of 340 nm by UV–Vis spectroscopy analysis.

Study on antioxidant activity

Pyrogallic acid autoxidation method was used to determine the antioxidant activity of scavenging superoxide anion radical $O_2\bullet^-$ for API. Briefly, 4.9 mL of Tris–HCl buffer solution (pH = 7.8) and 5 mL of double distilled deionized water were mixed homogeneously in a tube of 25 mL. Pyrogallic acid aqueous solution (60 mmol/L, 0.1 mL) contained 10 mmol/L HCl was pipetted quickly into above mixed solution. The absorbance of obtained homogeneous solution was determined every 10 s at the wavelength of 319 nm by UV–Vis spectroscopy analysis. The total measurement time is 10 min.

The double distilled deionized water (2 mL) was replaced with the same volume of samples tested and mixed with Tris–HCl buffer solution. After adding pyrogallic acid aqueous solution, 1 mL mixed solution was pipetted into 4 mL of 0.1 mol/L HCl solution every 100 s. The total measurement time is also 10 min. The absorbance of the obtained solutions were determined at the wavelength of 319 nm by UV–Vis spectroscopy analysis. All measurements were carried out in triplicates and the data were expressed as the mean value ± SD.

The results were expressed as percentage $O_2\bullet^-$ elimination calculated according to the following equation:

$$I(\%) = \frac{(k_0 - 5 \times k_i)}{k_0} \times 100 \tag{5}$$

where, I is radical-scavenging activity, k_0 is the rate of pyrogallic acid autoxidation, k_i is the slope of the antioxidant activity curves of samples tested.

Results and discussion

Characterization of API/HP-β-CD complex

The potential effect of HP-β-CD was investigated by comparing the UV–Vis spectra of API, HP-β-CD and API/HP-β-CD complex. As illustrated in Fig. A.1 (online appendix), a maximum absorption peak could be found at the wavelength of 340 nm in the UV–Vis spectrum of API (a), while there was none for HP-β-CD (c). Furthermore, the absorption peak of API/HP-β-CD complex shift to higher wavelength compared with the spectra of API (a). Similar red shift of the absorption peak was also recorded in the barbigerone/HP-β-CD complex by Qiu et al. [30]. Such a situation might be partly attributed to the shielding of chromophore groups in guest molecule, which also confirms the complex formation between API and HP-β-CD occurred in aqueous solution.

The powder X-ray diffraction (PXRD) patterns of API, HP-β-CD, API/HP-β-CD inclusion complex and physical mixture were shown in Fig. 1A. Free API existed as crystalline form, consistent to several sharp peaks at diffraction angels (2θ) of 7.2, 10.15, 11.35, 14.35, 15.15 and 16.05 in the PXRD pattern (Fig. 1A (a)). On the contrary, API/HP-β-CD inclusion complex (Fig. 1A (c)) was extremely similar to HP-β-CD (Fig. 1A (b)), existed in amorphous state lacking crystalline peaks. Qiu et al. [30] made a similar observation of guest molecule losing its prominent crystalline peaks in the complexation between barbigerone and HP-β-CD. The result indicated the formation of some new complex compounds in amorphous state. However, in the PXRD of physical mixture (Fig. 1A (d)), partly API crystalline peaks were covered, different from the results discussed in the work reported by Qiu et al. The results maybe indicate the occurrence of non-inclusion interactions (e.g. adhesion) between host and guest molecules when kneaded in mortar.

To further confirm the formation of inclusion complex, the FT-IR spectra (Fig. 1B) of API, HP-β-CD, API/HP-β-CD inclusion complex and the physical mixture were compared on peak shape, position and intensity. The FT-IR spectrum of API/HP-β-CD inclusion complex (Fig. 1B (c)) was very similar to that of HP-β-CD (Fig. 1B (b)). No API prominent absorption band was visible except that the C–O stretching (1244 cm^{-1}) and the aromatic conjugated carbonyl group (C = O) at 1650 cm^{-1}. Similar observation of covering partly guest molecule absorption bands attributed
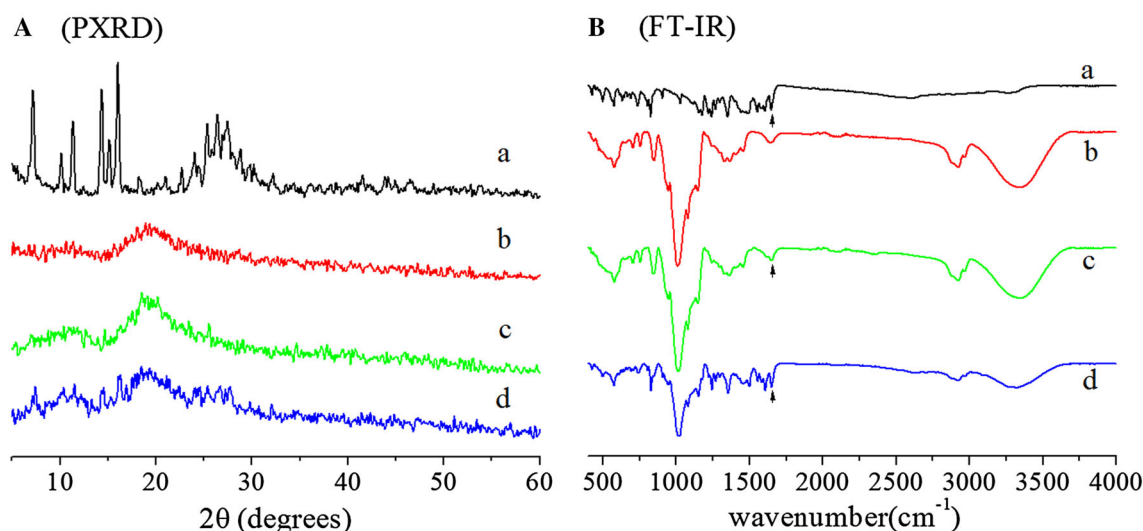
A (PXRD)

B (FT-IR)

Fig. 1 The powder X-ray diffraction patterns (**A**) and the FT-IR spectra (**B**): *a* API, *b* HP-β-CD, *c* API/HP-β-CD inclusion complex, *d* API/HP-β-CD physical mixture

to the complexation between alpinetin and HP-β-CD was reported by Ma et al. [31]. For the physical mixture (Fig. 1B (d)), simple superposition of the individual spectra of API and HP-β-CD was obtained. The majority of characteristic absorption peaks of API (1355, 1558 and 1607 cm^{-1}) retained with slight shift, indicating that API still kept original structure without bonding with HP-β-CD in the physical mixture.

In order to explore the possible inclusion mode of the API-HP-β-CD, we compared the ^{1}H NMR spectra of API, HP-β-CD and API/HP-β-CD complex (Fig. 2). As illustrated in Fig. 2A and B, most of the signals of API protons appeared at 6.1–13.0 ppm, which were distinct from the HP-β-CD protons (3.0–6.0). The majority signals of API protons were emerged in the spectra of API/HP-β-CD complex, consistent with the formation of API/HP-β-CD complex. Furthermore, we compared the ^{1}H chemical shifts (δ) of API in the presence and absence of HP-β-CD. The HP-β-CD-induced variations (0.010–0.012 ppm) observed in H-6 and H-8 protons, which were assigned to the protons from A ring in API, were more significant than that in other protons (≤0.006 ppm) from B and C ring of API. This result indicated that the phenolic hydroxy group (A ring) may be included within the cavity of HP-β-CD.

For the internal protons of the HP-β-CD cavity, which were H-3 and H-5, were evaluated for changes in the partial enlarged drawing of ^{1}H NMR spectra (Fig. 2C, D). H-5 proton peak of HP-β-CD split into two sub-peaks causing by the inclusion complexation. In contrast, inclusion complexation with API had a negligible effect on the δ value of the H-3 proton of HP-β-CD. These results suggested that the A ring of API has entered the HP-β-CD cavity from the narrow side to form an inclusion complex.

Similar inclusion mode that the hydrophobic group of guest molecule penetrated inside the cyclodextrins cavity from the narrow side was also concluded in previous publication [32].

Phase solubility studies of API/HP-β-CD complex in solution

Solubility phase diagrams of the API/HP-β-CD system in phosphate buffers (pH = 7.0) were determined at various temperatures (30, 37 and 45 °C). Linear phase-solubility diagrams (A_L-type systems, Fig. 3) were observed for API/HP-β-CD system, showing the presence in solution of a complex with 1:1 stoichiometry at low HP-β-CD concentrations in all ranges of temperatures studied. This is consistent with the conclusion presented by Pápay Z. et al. [15].

An enhancement of *K* value was observed at the increase of the temperature, showing that the binding process between API and HP-β-CD is endothermal in the ranges of temperature studied. The van't Hoff plot, plotting log *K* against 1/*T*, was showed in the inset of Fig. 3. Thermodynamic parameters of the complexation could be calculated by it.

An unfavorable change in enthalpy ($\Delta H = 29.192$ kJ/mol) and a large favorable change in entropy [$\Delta S = 155.50$ J/(K mol)] accompanying the complexation were obtained. In this case, classical hydrophobic interactions can be regarded as driving forces for complexation [25]. Apolar binding was also considered as a complexation characterized by unfavorable ΔH and a large favorable ΔS [33]. The transfer of API from aqueous medium to the cavity of HP-β-CD could result in the aforementioned ΔH and ΔS, because it requires
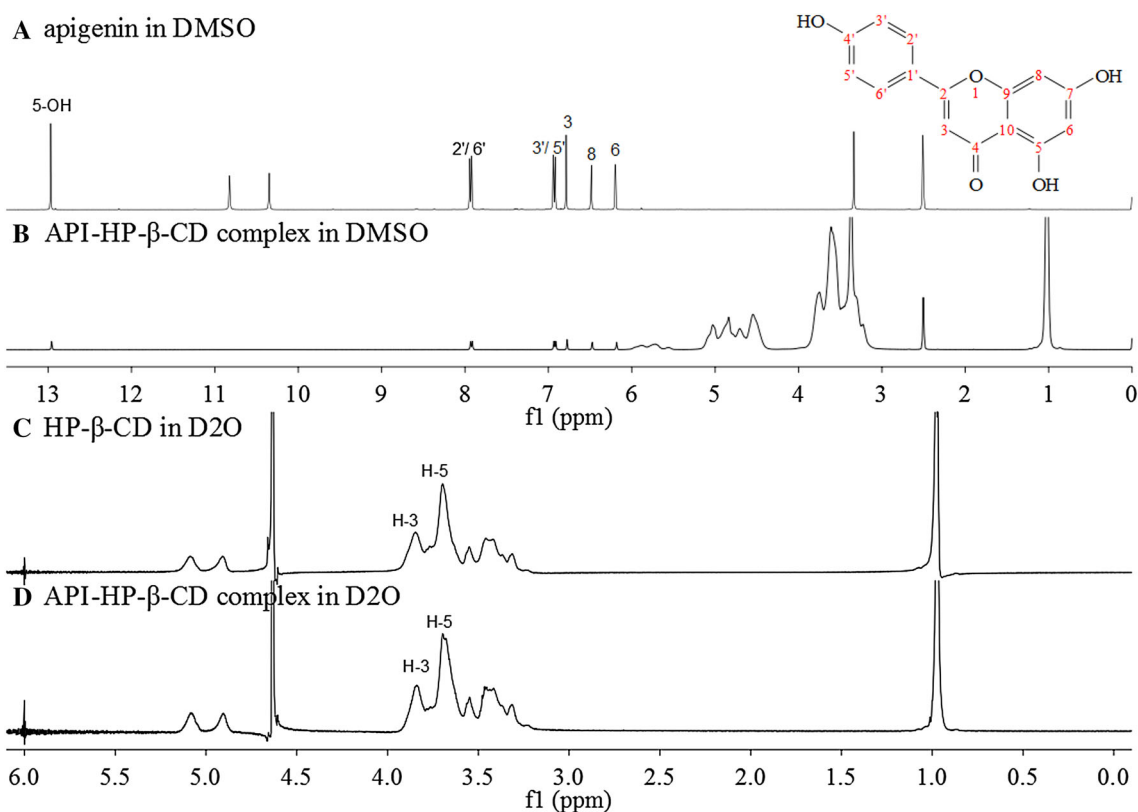
Fig. 2 ^{1}H NMR spectra of **A** API, **B** API/HP-β-CD complex in DMSO-d$_6$; partial enlarged drawing of **C** HP-β-CD, **D** API/HP-β-CD complex in D$_2$O with region 6.0–0 ppm
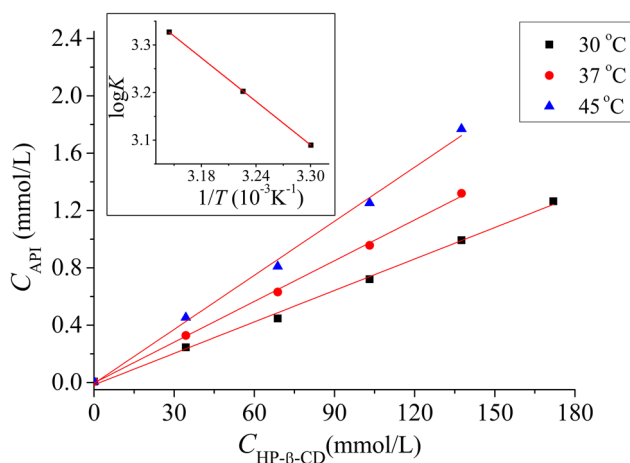

Fig. 3 Solubility phase diagrams of the API/HP-β-CD system at various temperatures. (*Filled square*) 30 °C, (*filled circle*) 37 °C, (*filled triangle*) 45 °C; The *inset* is the van't Hoff plots on the basis of the dependence of the stability constant (*K*) on temperature

Fig. 4 The partly phase diagram for Tween 80/isoamyl acetate/water phase at 25 °C

API/HP-β-CD/Microemulsion system

Phase behavior

The partly phase diagram for Tween 80/isoamyl acetate/water in 25 °C was showed in Fig. 4. An isotropic, transparent, thermodynamically stable microemulsion single phase was obtained, which was marked with the dot line in

breaking down the structure formed by water molecules around API. Therefore, the formation of the 1:1 inclusion complex is favored by an entropic contribution rather than enthalpic one.
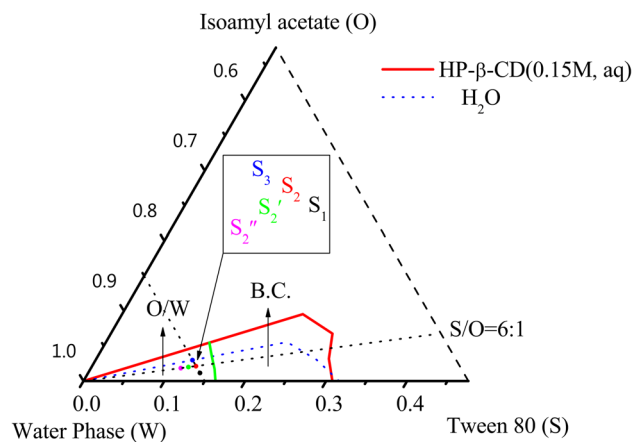
the phase diagram. By replacing the water phase with the 0.15 mol/L HP-β-CD aqueous solution, an increased microemulsion are marked with the solid line shifted to lower surfactant concentration. This might be due to the decreased oil/water interfacial tension resulted from the introduction of HP-β-CD.

In order to examine the microstructures and their structural changes in microemulsion systems, the variations of electrical conductivity (κ) as a function of the water phase mass fraction (wt%) along dilution lines at different S/O values were determined in this study (online appendix Fig. A.4). As can be seen, with the increase of water phase content, the curves may be divided into two parts by the solid lines: (1) the nonlinear increase of κ indicates that a bicontinuous (B.C.) microstructure is formed; (2) the decrease of κ corresponds to the appearance of water-continuous microemulsion, that is, an O/W microemulsion is formed at high water content [28].

Then, three points S_1, S_2 and S_3, with 85 wt% constant water content, were selected to prepare microemulsion formulas, where the surfactant/oil mass ratio is 12:1, 6:1 and 4:1, respectively. In addition, another three formulas (S_2, S_2' and S_2'') along the water phase dilution line at the 6:1 surfactant/oil mass ratio were prepared. The mass percent (wt%) of water phase were 85, 86 and 87 % for S_2, S_2' and S_2'', respectively.

Solubility of apigenin in HP-β-CD/Microemulsion complex

The solubility of saturated solutions of API in HP-β-CD, Microemulsion and HP-β-CD/Microemulsion complex was investigated by UV–Vis spectroscopy analysis. As shown in Fig. 5A, the absorbance at λ_{max} increased significantly in the order of API/HP-β-CD, API/Microemulsion and API/HP-β-CD/Microemulsion. The result revealed that a

synergistic effect on the aqueous solubility of API was obtained in the HP-β-CD complex with microemulsion.

The solubility of API in various formulations of HP-β-CD/Microemulsion was further investigated by UV–Vis spectroscopy analysis. As shown in Fig. 5B, the absorbance at λ_{max} gets significantly decreased in the order of $S_2 < S_2' < S_2''$. However, as for sample S_1, S_2 and S_3, the absorbance increased slightly one by one. These results reflected the effect of different composition of microemulsion on the solubility of API.

The API solubility decreased significantly with the increase of the water phase content at the constant surfactant/oil (S/O) values. This reflected API mainly solubilized in the O/W microemulsion droplets. It is suggested that API, with an aromatic part in its structure, shows high affinity towards hydrophobic environment of the amphiphilic molecules [34]. In addition, at the constant water phase content, the API solubility increases slightly with the reduction of surfactant/oil (S/O) values. It might be because the reduction of S/O ratio makes the micelle swollen, resulting in solubilizing more API. This high solubilization effect in Tween 80 has been attributed to the ability of Tween 80 to form either a drug-surfactant complex or hydrogen bonds with the drug in an investigation of Tween 80 microemulsion as the carrier of an anti-inflammatory drug Ibuprofen [35]. Therefore, a trend that API solubilized into the hydrophobic chains of Tween 80 molecules was confirmed. This is consistent with the finding that the solubility of API in Tween 80 (26.59 ± 1.16 mg/mL [36]) is far higher than in the oil phase isoamyl acetate (191.57 ± 1.14 mg/L) tested in preliminary experiment.

Based upon the results of the UV–Vis spectroscopy analysis, the API/HP-β-CD/Microemulsion complex is further schemed in Fig. 6. Tween 80 formed the interfacial
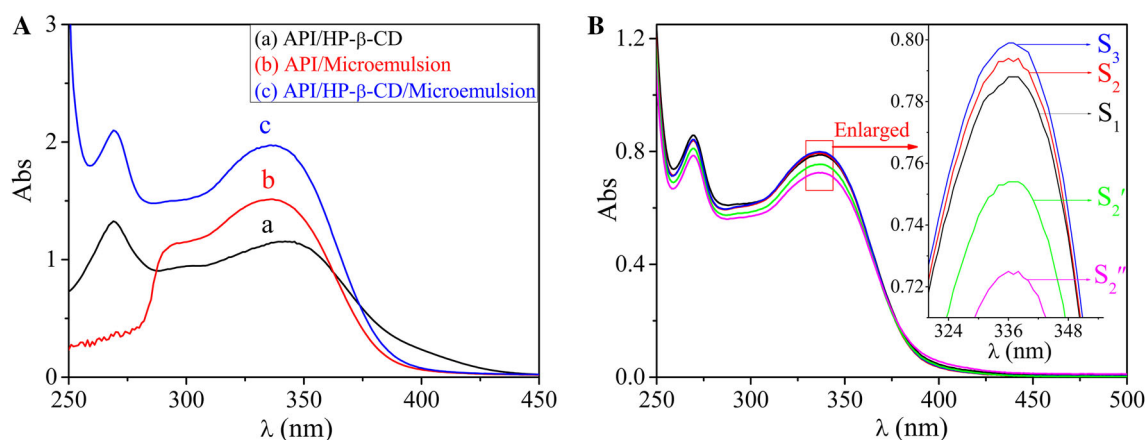

Fig. 5 **A** The UV–Vis spectrum of saturated solutions of API; **B** The UV–Vis spectrum of various formulations of HP-β-CD/Microemulsion encapsulated API

film of the complex, isoamyl acetate the inner phase, and HP-β-CD aqueous solution the outer phase. API mainly solubilized in the "palisade" layer consisted of the hydrophobic chains of Tween 80 molecules, followed in the inner core and the outer aqueous phase. The results exploited the feasibility of using combined HP-β-CD/Microemulsion complex as the carrier for improving the solubility of API.

In vitro release studies

In vitro drug release profiles of API from samples S_1 and S_2 were plotted in Fig. 7. Microemulsion S'_1 without HP-β-CD was also studied as control. As we can see from samples S_1 and S_2, the API releasing rates were at a constant speed and fitted by a zero-order kinetic process at initial stage (0–15 h). However, for samples S'_1, the result
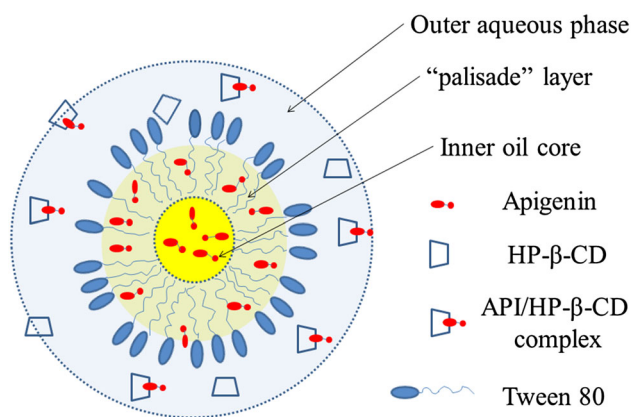

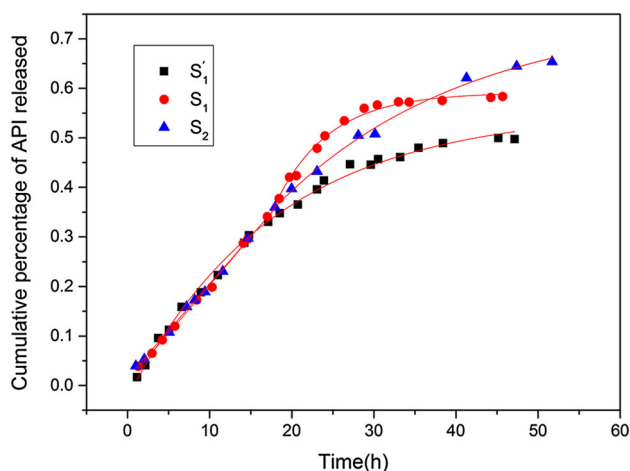Fig. 6 The possible position of apigenin solubilized in HP-β-CD/Microemulsion system

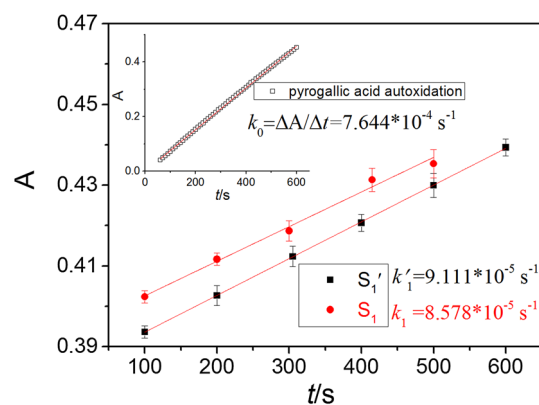Fig. 7 In vitro drug release profiles of API from samples S'_1, S_1 and S_2 at 25 °C

Fig. 8 The superoxide anion radical $O_2\bullet^-$ scavenging activity of samples S_1 and S'_1

in vitro release conformed to first-order kinetic process at the whole range of measurement. This may be attributed to the release of API loading outer HP-β-CD aqueous phase at initial stage. At the same time API in the inner core diffuse to the outer phase to maintain the constant releasing rates. Compared with S'_1, sample S_1 has a higher cumulative percentage of API released, indicated that HP-β-CD could enhance drug delivery through biological membranes. Similar result was reported previously [37].

Superoxide anion radical scavenging activity

API could reduce the rate of pyrogallic acid autoxidation by scavenging superoxide anion radical $O_2\bullet^-$. So the inhibitory rates for pyrogallic acid autoxidation reflect the antioxidant activity of API. The superoxide anion radical $O_2\bullet^-$ scavenging activity of samples S_1 and S'_1 were determined and shown in Fig. 8. The inset showed a curve of pyrogallic acid autoxidation with a rate of k_0 (7.644×10^{-4} s^{-1}). The rates of pyrogallic acid oxidation became slow when adding API-loaded microemulsions with or without HP-β-CD. Superoxide anion radical scavenging rates for S_1 and S'_1 were calculated to 43.9 and 40.4 %. This indicated that API loaded in microemulsions with HP-β-CD had a higher antioxidant activity than that without HP-β-CD.

Conclusion

It is concluded that the formation of API/HP-β-CD inclusion complex is induced by hydrophobic interactions and apolar binding. The API/HP-β-CD inclusion complex forms in solution with the 1:1 stoichiometry, which is favored by an entropic contribution rather than enthalpic one. The aqueous solubility of API significantly increases

in the complex of HP-β-CD with Tween 80 based O/W microemulsions, through solubilizing in the palisade layer, the inner oil core, and outer water phase. The HP-β-CD modified microemulsion could improves the cumulative percentage of API released. Moreover, API loaded in microemulsions with HP-β-CD had a higher antioxidant activity than that without HP-β-CD. The observations suggest that microemulsion combined with an inclusion complex is an effective approach to improve bioavailability of API.

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