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ORIGINAL ARTICLE

Preparation and characterization of the inclusion complex of baicalein with γ-cyclodextrin: an antioxidant ability study

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Abstract The formation of the complex of Baicalein with γ-cyclodextrin (γ-CD) was studied by UV–Vis absorption spectroscopy, fluorescence spectra and nuclear magnetic resonance spectroscopy (NMR) in solution. The solid inclusion complex of Baicalein with γ-CD was synthesized by the co-precipitation method. The characterization of the solid inclusion complexes have been proved by infrared spectra. The formation constant (K) of complex was determined by fluorescence method. The results suggested that in different pH solutions, γ-CD has different inclusive capacity to different forms of Baicalein. γ-CD was most suitable for inclusion in neutral media. In addition, the experimental resulted confirmed the existence of 1:1 inclusion complex of Baicalein with γ-CD. Kinetic studies of DPPH• with Baicalein and γ-CD complex were done. The results obtained indicated that the Baicalein/γ-CD complex was the most reactive form. Special configuration of complex has been proposed on NMR technique.

Keywords Baicalein · Cyclodextrin · Absorption spectroscopy · Fluorescence · NMR · DPPH•

Introduction

Investigations of molecular recognition have attracted much attention in supramolecular chemistry involving natural and artificial host–guest systems [1]. The inclusion process of pharmaceutical molecules with CDs usually results in a modulation of the physicochemical and pharmaceutical properties of guest molecules, such as increased solubility, improved chemical stability and bioavailability, reduced toxicity controlled-rate release and so on [2–4]. Therefore, it would be of great importance to comprehensively understand the inclusion behavior of molecules of pharmaceutical interests with CDs. Recently, various hydrophilic, hydrophobic and ionic cyclodextrin derivatives have been utilized to extend the physicochemical properties and inclusion capacity of natural cyclodextrin [5, 6]. γ-cyclodextrin (γ-CD) is a water-soluble derivative of cyclodextrin, which has been widely studied as a complexion agent for many pharmaceuticals because of it larger inner cavity. The ability of cyclodextrin to form inclusion complexes is highly affected by size, shape, hydrophobicity and the form of the guest's molecular.

Herbal medicines have a long history in medical practice and health care especially in some Asian and African countries. Now, herbal medicines have expanded globally and gained considerable attention because of low toxicity and good therapeutical performance. *Scutellaria baicalensis Georgi* is one commonly used herbal medicine in China and other East Asian countries. Baicalein (Ba, Fig. 1) is one of the main active components, which has a variety of interesting activities such as antibacterial [7], anti-HIV activity [8], attenuating oxidative stress [9–11], inhibiting the growth of several types of cells [12–14] inducing cell death in human hepatocellular carcinoma cell [15] and in human promyelocytic leukemia HL-60 cells [16] and so on. However, one disadvantage of this compound is its low water solubility, lead to limited use in pharmaceutical field.

When the fluorescent guests are included in the CD cavity, the non-radioactive decay processes of

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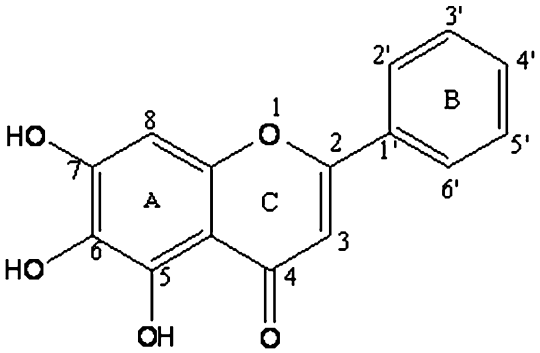

Fig. 1 The chemical structure of Baicalein

luminophores are significantly attenuated and hence fluorescence emission increased [17–19]. Due to its high sensitivity, selectivity and instrumental simplicity, fluorescence method has been used to investigate the phenomena of inclusion complexes and determine the association constants of complexes [20–22]. High resolution nuclear magnetic resonance (NMR) is also a powerful tool for studying CD complexes [23] that can provide not only quantitative information, but also detailed information on geometry of the complex.

The complex of Ba with β-CD and HP-β-CD have been studied in our another paper, and effect was not perfect due to the lesser cavity. Such, the present work was designated to study the complex of Ba with γ-CD by different methods and to determine the effect of the complex process on their antioxidant capacity.

Experimental

Apparatus and materials

UV-757CRT spectrophotometer (Shanghai Precision & Scientific Instrument Co. LTD); Fluorescence measurements were performed by F-2500 FL spectrofluorometer (Hitachi) using 1 cm quartz cell and both the slits were set at 20 nm with the excitation wavelength at 270 nm. All the NMR date was obtained on Bruker Avance DRX 300 MHz NMR spectrometer. IR spectra were obtained with Perkin Elmer FT-1730 IR spectroscopy using KBr pelleting. The range of spectra was from 400 to 4,000 cm^{-1}.

A stock solution of 1.0×10^{-4} mol/L Ba (provided by Dr. Zhang and was purified by recrystallization) was prepared by dissolving and diluting its crystals in water. γ-CD and DPPH• were purchased from Sigma-Aldrich, Inc., St. Louis, MO. All other reagents were of analytical-reagent grade and were used without purification. Doubly distilled water was used throughout. Phosphate buffer solution (0.2 mol/L) was used to control the pH-value of the media. All experiments were carried out at room temperature (20 ± 1 °C).

Experimental procedure

A 1 ml aliquot of the stock solution of Ba was transferred into a 10 mL volumetric burette, and then an appropriate amount of 1.0×10^{-2} mol/L γ-CD was added. The solution was diluted to a final volume of 10 mL with distilled water. 2 mL of 0.2 mol/L phosphate buffer solution was used to control the pH value of the media. The final mixture solution was dissolved thoroughly under ultrasonic for 30 min, and then equilibrated for 30 min at 20 ± 1 °C. The working solution was transferred into a 1×1 cm^2 quartz cell to record absorption and fluorescence spectra. All measurements of absorption and fluorescence were made against a blank solution treated in the same way but without Ba in a 1.0 cm quartz cell.

NMR measurements

1×10^{-4} mol/L Ba and 1×10^{-4} mol/L γ-CD solution with a volume ratio of 1:1 were mixed thoroughly. With D_2O as solvent, ^1H NMR spectra was obtained at 300.13 MHz with 10 μs as 90° pulse width. All experiments were performed at 20 ± 1 °C.

Determination of antioxidant activity by the scavenging of the stable radical DPPH•

The antioxidant activity was measured, wherein the bleaching rate of a stable free radical, DPPH• is monitored at a characteristic wavelength in the presence of the sample. In its radical form, DPPH• absorbs at 517–520 nm, but upon reduction by an antioxidant or a radical species its absorption decreases.

A volume of 2 ml of 1.0×10^{-5}M DPPH• was used. Furthermore, DPPH* is insoluble in aqueous solution and the scavenging study was performed in mixture of ethanol–water (20:80).

The reaction was started by addition of 1 mL of Ba (1.0×10^{-5} M) and Ba/γ-CD complex samples, which correspond to the 3 mM γ-CD concentration. The bleaching of DPPH• was followed at 520 nm.

The decrease in absorbance at 520 nm was measured against a blank of ethanol–water (20:80) 1 mL and 2 mL 1.0×10^{-5} M DPPH• to estimate the radical scavenging capacity of each antioxidant sample. The results were expressed as percentage DPPH• elimination calculated according to the following equation [24]:

$$AU = [1 - A_s/A_0] \times 100, \quad (1)$$

where AU is radical-scavenging activity, A_s is absorbance of sample and A_0 absorbance of blank sample.

Preparation of solid complexes of Ba with γ-CD

Accurately weighting 0.1297 g γ-CD was placed into a 50 mL conical flask and 10 mL distilled water was added, stirred, then 0.0135 g Ba was put into a 50 mL beaker and 10 mL of distilled water was added and put over electromagnetic stirrer to stir until it was dissolved, then slowly poured γ-CD solution into stirred Ba solution, continually stirred for 12 h at room temperature. The reaction mixture was put into refrigerator for 24 h. precipitation was filtrated by G4 sand filtering funnel, and washed with distilled water. After drying in oven at 60 °C, white powdered products were obtained. This is inclusion complex of Ba with γ-CD.

Results and discussion

UV spectroscopy

Figure 2 shows the absorption spectra of Ba in the absence and presence of γ-CD at room temperature. The absorption of Ba varied significantly with the addition of γ-CD. Ba alone in water exhibited two absorption peaks at 272 and 319 nm, respectively. The increase in γ-CD concentration from 1 to 8 mmol/L resulted in an increase in the absorption of Ba. Simultaneously, as the γ-CD increase a weak blue shift of absorption peak at 272 nm was observed. These might be partly attributed to the change of chromophore groups in Ba molecular due to the complex formation between Ba and γ-CD through hydrophobic interaction, and suggested that the likely formation of an inclusion complex between Ba and γ-CD.

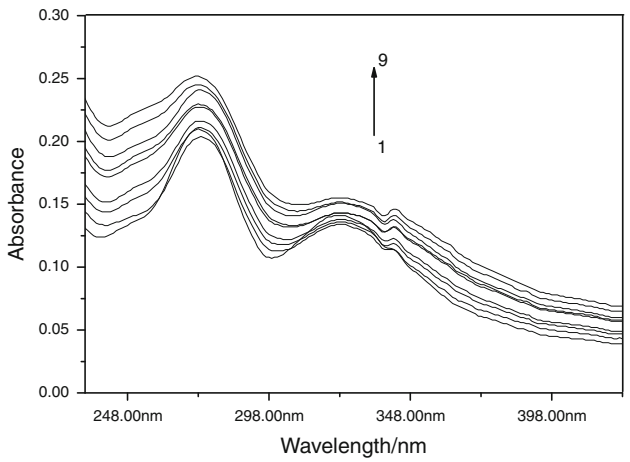

Fig. 2 The absorption spectra of 1.0×10^{-5} mol/L Baicalein in the presence of γ-CD. The concentration of γ-CD (M): 0–8.0×10^{-3}

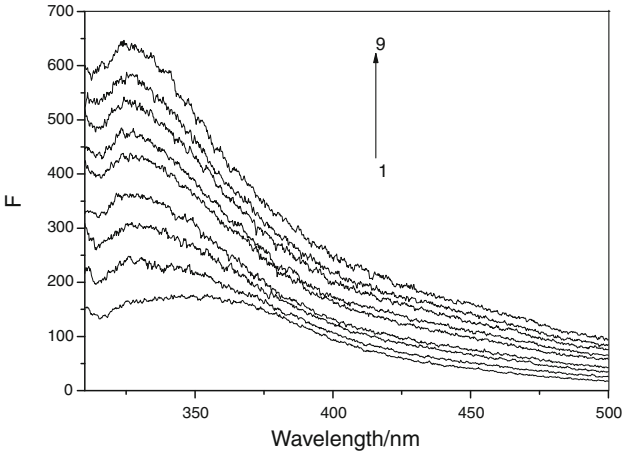

Fig. 3 The fluorescence emission spectra of 1.0×10^{-5} mol/L Baicalein in γ-CD solution. The concentration of γ-CD (M): 0–8.0×10^{-3}

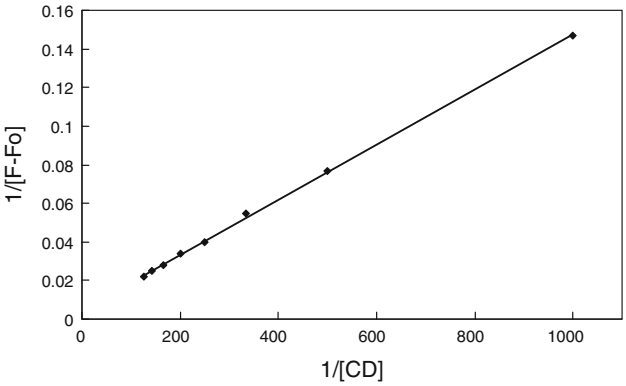

Fig. 4 the double reciprocal plot of $1/(F - F_0)$ versus 1/[CD]

Table 1 The constants of inclusion complex between Ba with γ-CD

PH	3.00	5.00	6.50	9.00
$K_{(Ba/\gamma\text{-CD})}$	933	750	1,067	500
R^2	0.9962	0.9991	0.9947	0.9995

Fluorescence study

Figure 3 showed that adding γ-CD to Ba solution resulted in a significant enhancement of the fluorescence signal. The excitation wavelength was at 270 nm, the maximum emission wavelength at 363 nm. With the increasing of γ-CD, the emission wavelength appeared lower wavelength shift. These suggested that the inclusion complex was likely formed between Ba and γ-CD. The γ-CD cavity provided an apolar environment for the Ba molecule and

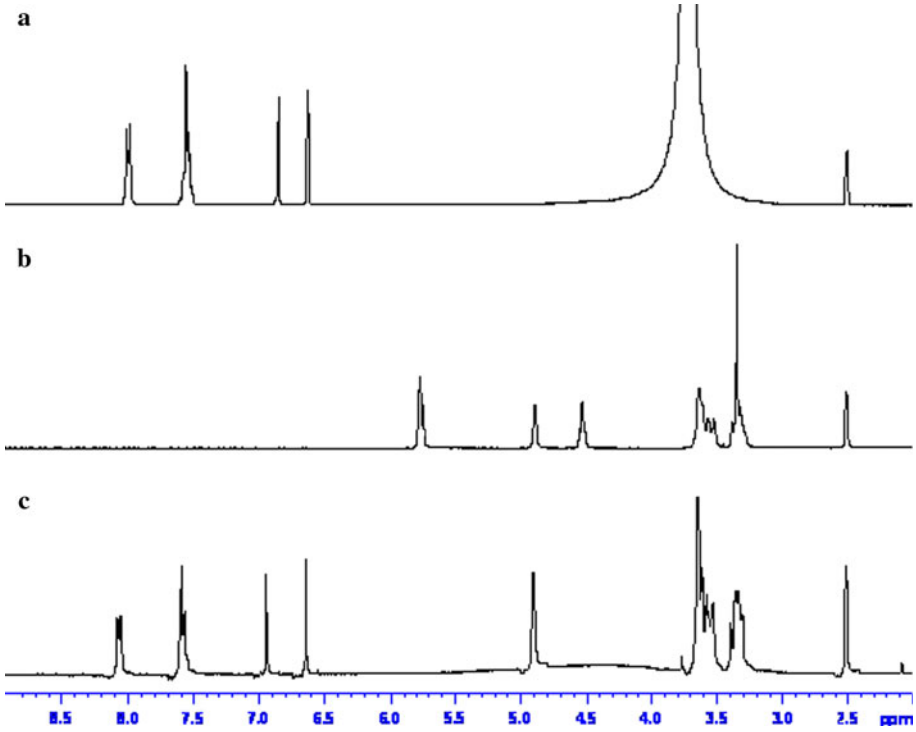

Fig. 5 The ^{1}H NMR spectra of inclusion complexes. **a** Baicalein, **b** γ-CD, **c** Baicalein/γ-CD

Table 2 ^{1}H-NMR chemical shifts Baicalein and Baicalein/γ-CD in D_2O

Baicalein (H)	Baicalein (δ0)	Baicalein/γ-CD (δ1)	$\Delta\delta$1
H-8	6.627	6.641	0.014
H-3	6.847	6.939	0.092
H-3′4′5′	7.560	7.598	0.038
H-2′6′	8.012	8.080	0.068

Table 3 ^{1}H-NMR chemical shifts γ-CD and the inclusion complex in D_2O

γ-CD (H)	γ-CD(δ0)	Ba/γ-CD(δ1)	$\Delta\delta$1
H-2	3.413	3.374	0.039
H-3	3.728	3.653	0.075
H-4	3.376	3.312	0.064
H-5	3.633	3.571	0.062
H-6	3.633	3.625	0.008

the motion of the Ba molecule in the cavity was largely confined. Thus, the enhanced rigidity of the Ba molecule resulted in an increase of its fluorescence quantum yield.

The inclusion formation constant (K) is a measure of the complexing power of γ-CD. The formation constant and ratio of the complex were obtained from fluorescence data using the modified Benesi-Hildebrand equation [25]

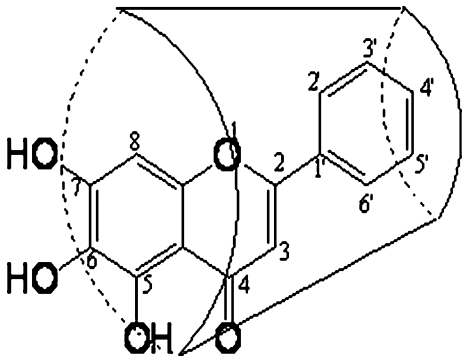

Fig. 6 The structure of inclusion complex between Ba and γ-CD

$$1/(F - F_0) = 1/([CDs]\ K\alpha) + 1/\alpha \quad (2)$$

where, F and F_0 represent the fluorescence intensity of Ba in the presence and absence of γ-CD, respectively; K is a forming constant; α is a constant.

Figure 4 shows the double reciprocal plots of $1/(F - F_0)$ versus 1/[CD]. The good linear relationship obtained when 1/(F − F0) was plotted against 1/[CD] supports the existence of a 1:1 complex.

Besides, we have done the fluorescence spectra of Ba in different pH (including pH 3.00, pH 5.00, pH 6.50 and pH 9.00) in the presence of γ-CD, the result was the same. The excitation wavelengths were 270 nm, the emission wavelength shifted to lower wavelength at different pH. Simultaneously, the increasing concentration of γ-CD resulted in the enhancement of fluorescence signals. The

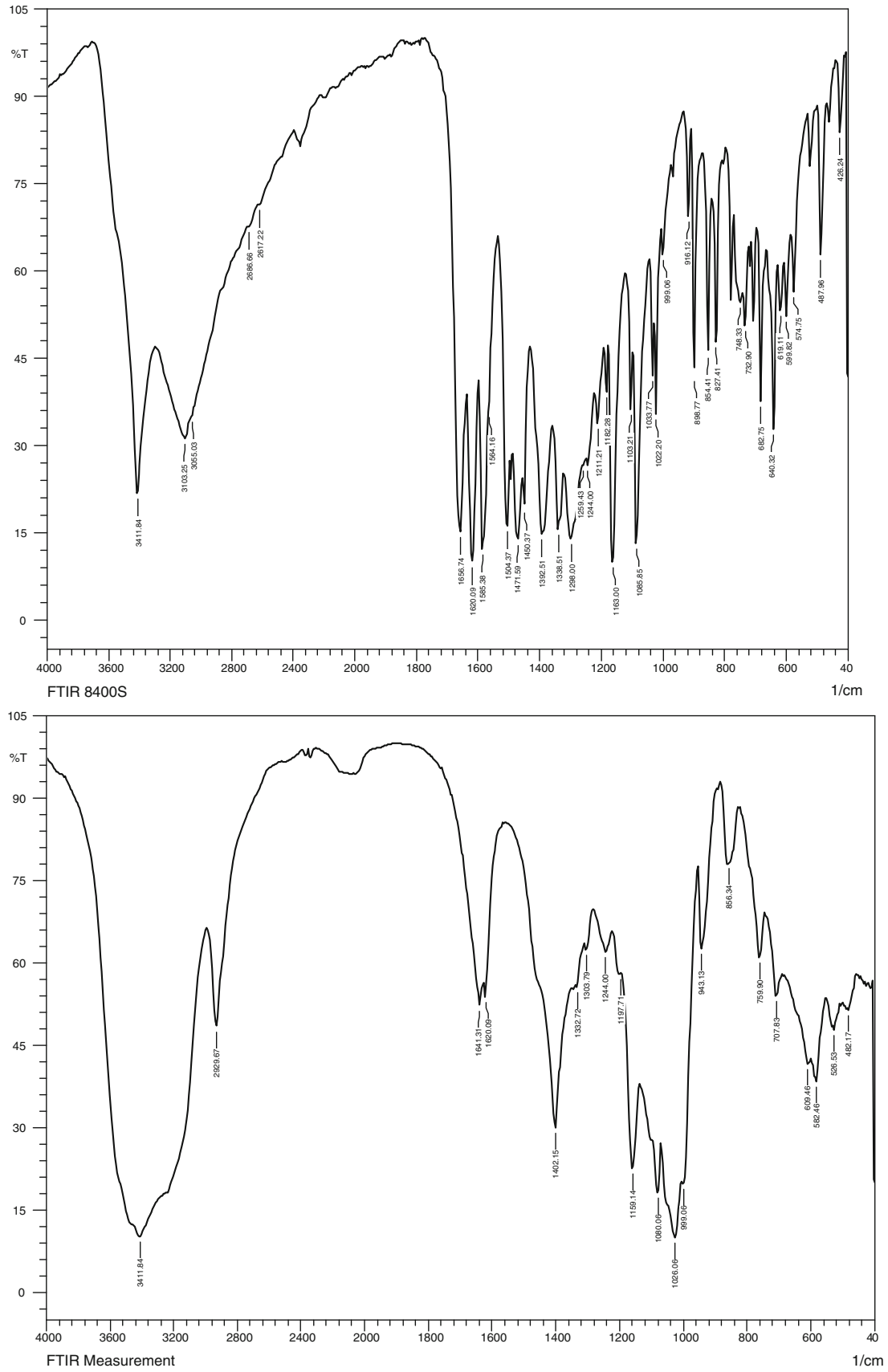

Fig. 7 IR spectra of Ba, γ-CD and Ba/γ-CD form up to down

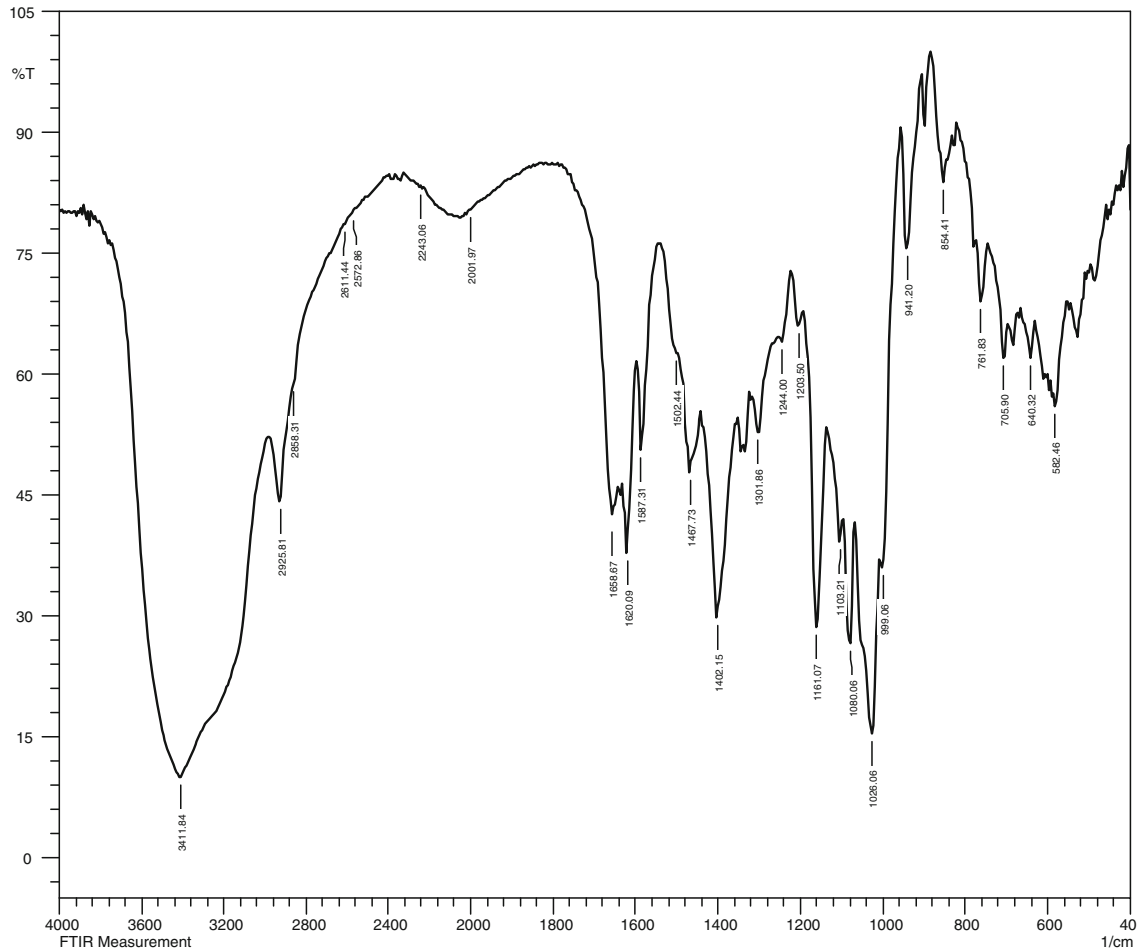

Fig. 7 continued

inclusion formation constants in different pH vales were shown in Table 1. From the table it showed that γ-CD has different inclusive capacity in different pH solutions, γ-CD was most suitable for inclusion in neutral media.

NMR measurements

To ascertain the structure of the inclusion complexes between Ba and γ-CD, ^{1}H-NMR spectroscopy studies of free drug and inclusion complex were therefore undertaken. Figure 5 illustrated the ^{1}H-NMR spectroscopy Ba and γ-CD before and after forming the inclusion complex. The difference in hydrogen chemical shift values between Ba in the free and complexed state were presented in Table 2. Table 3 showed the hydrogen chemical shift change values of γ-CD after forming the complex.

It can be seen from the figure that the H-8, H-3, H-3′, H-4′, H-5′ and H-2′, H-6′ of Ba exhibited larger chemical shifts, namely, the A, B and C ring of Ba were all entered into the cavity of γ-CD, because of the diminished freedom of rotation caused by the penetration of Ba molecule into the γ-CD cavity. And the seam time, the H-3 of γ-CD experienced larger chemical shift than H-5, which illustrated that the molecular of Ba entered into the cavity of γ-CD from the large port.

From all the above, the mechanism of complex between Ba and γ-CD were shown as follow (Fig. 6).

Scavenging study of DPPH• by free or complexed-Ba

DPPH• is a stable free radical generating a deep violet solution in organic solvents. Its progressive discoloration when in the presence of Ba indicated that it is acting as an antioxidant. Furthermore, since the mechanism of DPPH• reduction is known, the amount remaining of both reagents may be determined.

The rate of the DPPH•-scavenging reaction was measured by monitoring the decrease in absorbance at 520 nm due to DPPH•. The conclusion showed the consumption of DPPH• which indicates that the complex Ba/γ-CD was more effective than free Ba, with the γ-CD complex (56.67%) > free Ba (33.33%). The scavenging ability was

measured as a relative scavenging in presence of free or complex Ba. Also theses results indicated that the complexes formed maintained the Ba antioxidant activity.

The antioxidant activity of phenolic compounds depends on the position and degree of hydroxylation, as well as the nature of radicals of the ring structure. Anti-oxidative activity is intensified by the presence of a second hydroxy group, through the formation of an intramolecular hydrogen bond [26]. It might be that that the –OH positions of Ba molecules is close enough to secondary –OH groups of γ-CD to form hydrogen bonds and contribute to antioxidant activity [27]. Therefore, the formation of an "intramolecular" hydrogen bond of the inclusion complex is possible and consequently an increase of antioxidant capacity is expected.

IR spectra studies

Compared IR spectra of Ba, γ-CD and complex of γ-CD with Ba, just as Fig. 7 showed that absorption intensity of C–O group and phenyl ring gave rise to changes, the absorption intensity of C–O and phenyl ring in inclusion complex were weaker than in Ba, and have a right shift, and so can be deduced that C–O and phenyl ring in Ba were included into cavity of γ-CD.

Conclusion

The present study has demonstrated the inclusion complex interaction between Baicalein with γ-CD by absorption spectroscopy, fluorescence spectra, IR spectra and NMR spectroscopy. The major factors affecting molecular recognition is size matching, between γ-CD and guest, and the hydrophobic property of the guest molecule. And the activity of eliminating free radical DPPH• was γ-CD inclusion complex > free Baicalein. In addition, the fluorescence spectroscopy data showed that γ-CD was most suitable for inclusion in neutral media, the formation of 1:1 stoichiometric complex of Baicalein with γ-CD over the concentration range evaluated. Moreover, the study demonstrated that γ-CD severed as drugs carrier system in a dosage-controlled manner. A mechanism was set up to expound the structure of the inclusion complexes.

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