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Original Research

Synthesis, characterize and antioxidating activity of the inclusion complex of Wogonin with β-cyclodextrin and hydroxypropyl-cyclodextrin

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Abstract

The aim of the study was to synthesize the complexes of Wogonin with β-CD and HP-β-CD by refluxing method, and the experimental resulted confirmed the forming of Wogonin complexes with CDs by nuclear magnetic resonance spectroscopy, infrared spectra and differential scanning calorimetry at 60 ℃ refluxing for 6h. Kinetic studies of DPPH• with Wogonin and CDs complexes were done. The results obtained indicated that the Wogonin/HP-β-CD complex was the most reactive form.

INTRODUCTION

Wogonin (Fig.1), a flavonoid present in the root of Scutellaria baicalensis Georgi, has attracted considerable attention because of the activities, such as anti-respiratory syncytial virus [1], anti-tumor effects [2] and anti-hepatitis B virus [3]. However, in spite of the wide spectrum of pharmacological properties, its use in pharmaceutical field is limited because of its poor solubility.

Investigations of molecular recognition have attracted much attention in supramolecular chemistry involving natural and artificial host-guest systems [4]. Cyclodextrin complex has been successfully used to improve the solubility, chemical stability and bioavailability of a number of poorly soluble compounds [5-7]. Recently, various hydrophilic, hydrophobic and ionic cyclodextrin derivatives have been utilized to extend the physicochemical properties and inclusion capacity of natural cyclodextrin [8, 9].

The inclusion of Wogonin with Cyclodextrins, including β-CD and HP-β-CD in solution had been reported in another research [10]. The inclusion of Wogonin with biologic-molecular for example human gammaglobulin [11], human serum albumin [12] and

DNA [13] had been researched by many groups. Such, in this paper, we synthesized and characterized the inclusion of Wogonin with β-CD and HP-β-CD in detail, and determined the effect of the complexation process on their antioxidant capacity.

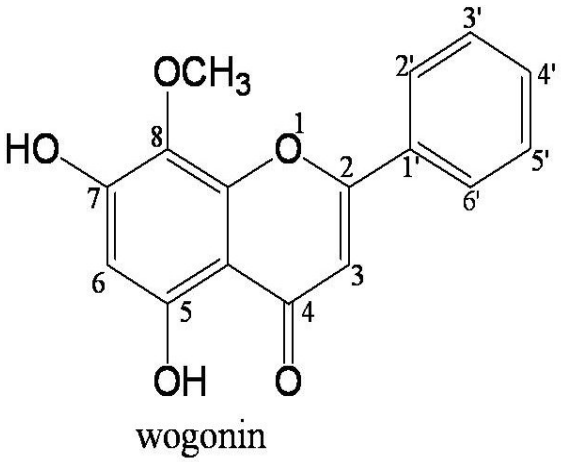

Figure.1. The chemical structure of Wogonin.

MATERIALS AND METHOD

Apparatus and Materials

UV-7504PC spectrophotometer (Shanghai Precision £ Scientific Instrument Co. LTD); The NMR date was obtained on Bruker Avance DRX 300MHZ NMR spectrometer. IR spectra were obtained with FT-1730 IR spectroscopy using KBr pelleting. The range of spectra was from 400 to 4000 cm^{-1}. DSC analyses were carried out in the temperature range from 30 to 500℃ in a stream of nitrogen atmosphere on DSC-60 thermal analyzer (Shimadzu). During experiments, aluminium crucibles were used. Sample weighs were 5 mg. The heating rate was 10℃/min and the flow rate of nitrogen atmosphere was 20 ml/min.

Wogonin (provided by Dr. Zhang and was purified by recrystallization) was prepared by dissolving and diluting its crystals in water. CDs 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. Stock solutions were stored at 4 ℃ and used no more than 4 days after preparation.

Synthesis of Complexes

An alcohol solution (10 mL) of Wogonin (0.1 mmol) was added to a aqueous solution (10 mL) of Cyclodextrins (0.1 mmol), and the mixture was refluxed with stirring for 6 hours, and then allowed to cool to room temperature and filtered by G4 sand filtering funnel. The solid was washed with water and ethanol, and then vacuum drying for 30 min at 40 ℃.

NMR measurements

All the concentrations of Wogonin and CDs solution were 1.0×10^{-4} mol/L and Wogonin solution is diluted with CDs solutions, respectively, at the volume ratio of 1:1. ^{1}H NMR of Wogonin solution as well as its inclusion complexes solutions was also performed to get further evidence. D_2O was as solvent.

Antioxidant activity

Determination of antioxidant activity by the scavenging of the stable radical DPPH•. A volume of 2 ml of 1.0×10^{-5}M DPPH• was used in mixture of ethanol–water (20:80). The reaction was started by addition of 1ml of Wogonin (1.0×10^{-5}M), Wogonin/β-CD, and Wogonin/HP-β-CD complex samples. The bleaching of DPPH• was followed at 520 nm.

The results were expressed as percentage DPPH· elimination calculated according to the following equation [14]:

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

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

RESULTS

Synthesis of the Complexes

The Wogonin complexes with CDs were synthesized by aqueous solution of CDs and the alcohol solution of Wogonin in 1:1molar ratio. The mixture was refluxed with stirring for 6 hours at 60 ℃, and during refluxing, brown subsidences were observed. The subsidences were filtered and vacuum drying for 30 min at 40 ℃.

NMR

To ascertain the structure of the inclusion complexes between Wogonin and CDs, ^{1}H-NMR spectroscopy studies of free drug and inclusion complexes were therefore undertaken. Fig.2 illustrated the change of hydrogen atom of Wogonin and CDs before and after forming the inclusion complexes. Table 1 shows the change of hydrogen atom of Wogonin and CDs before and after forming the inclusion complexes.

It can be seen from the figures that the H-3 and H-2', H-6' of Wogonin exhibited larger chemical shifts change after forming inclusion with β-CD, namely, the H-3 and H-2' , H-6' of Wogonin were all entered into

the cavity of β-CD, while H-6 and H-3', 4' 5' were not into the β-CD cavity. But in the inclusion with HP-β-CD, H-6, H-3 and H-2', H-6' and H-3', 4', 5' of Wogonin exhibited larger chemical shifts change, which indicated that the A, B and C rings of Wogonin were all entered into the cavity of HP-β-CD.

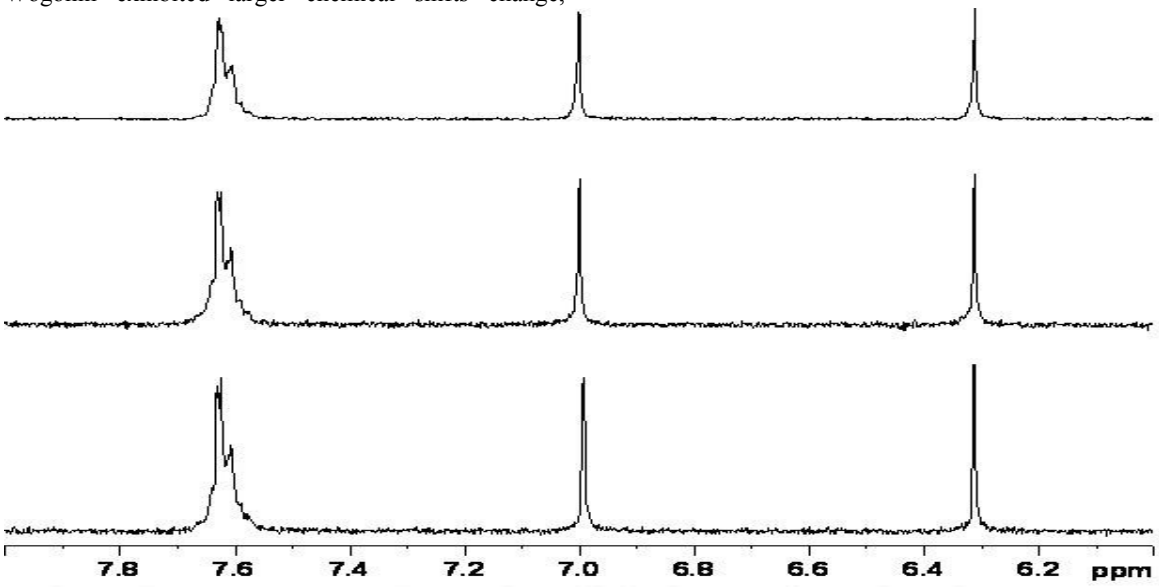

Figure.2. ^{1}H NMR spectra of Wogonin and inclusion complexes: the order were Wogonin, Wogonin/β-CD and Wogonin/HP-β-CD from the below to the up.

Table.1. ^{1}H NMR chemical shifts corresponding to Wogonin in the absence and presence of CDs in D_2O.

Wogonin (H)	Wogonin (δ0)	Wogonin/β-D(δ1)	Δδ1	Wogonin/HP-β-D(δ2)	Δδ2
H-6	6.313	6.312	0.001	6.311	0.002
H-3	7.002	6.999	0.003	6.990	0.010
H-3'4'5'	7.629	7.628	0.001	7.626	0.003
H-2'6'	8.085	8.066	0.019	8.063	0.021

Scavenging study of DPPH•

The rate of the DPPH•-scavenging reaction was measured by monitoring the decrease in absorbance at 520 nm due to DPPH•, which indicates that the complexed Wogonin/CDs were more effective than free Wogonin [10]. The scavenging ability was measured as a relative scavenging in presence of free or complex Wogonin. 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 [15]. It might be that that the –OH positions of Wogonin molecules is close enough to secondary –OH groups of CDs to form hydrogen bonds and contribute to antioxidant activity [16]. The intensive ability is also related with enhanced solubility of Wogonin forming the inclusion.

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 Wogonin, HP-β-CD and complex of HP-β-CD with Wogonin, just as Fig. 3 showed the absorption around 3200 cm^{-1}-3500 cm^{-1} due to phenolic hydroxyl in the free Wogonin shows weak spectral change which HP-β-CD, just the intensity stronger in the Wogonin complex, indicating the hydroxyl groups of Wogonin were outside the cavity of HP-β-CD. The 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 stranger than in free Wogonin, and have a slight shift (from 1660cm^{-1}-1579cm^{-1} to1660cm^{-1} -1581cm^{-1}, from 1508 cm^{-1}-1448 cm^{-1} to 1506 cm^{-1} -1558 cm^{-1}, respectively), and so can be deduced that C-O and phenyl ring in Wogonin were included into cavity of HP-β-CD. And the same as β-CD (the figure was not been given).

Differential scanning calorimetry studies

The DSC curves of Wogonin, HP-β-CD and inclusion complex were shown in Fig. 4. It can be shown that DSC curves of inclusion complex with the DSC curves of Wogonin and HP-β-CD was different. This proved that the new inclusion complex was formed. And the same phenomena was observed as β-CD (the figure was not been given).

DISCUSSION

The present study has demonstrated the inclusion complex of Wogonin with β-CD and HP-β-CD were synthesized by refluxing method, and characterized by infrared spectra (IR), differential scanning calorimetry (DSC) and nuclear magnetic resonance spectroscopy (NMR). The major factors of affecting guest/host binding are size matching between CD and guest, and the hydrophobicity of the guest molecule. The activity of eliminating free radical DPPH• were inclusion complexes free Wogonin. Moreover, the present study demonstrated that CDs served as drugs carrier system in a dosage-controlled manner and can increase biology activity of guest molecular.

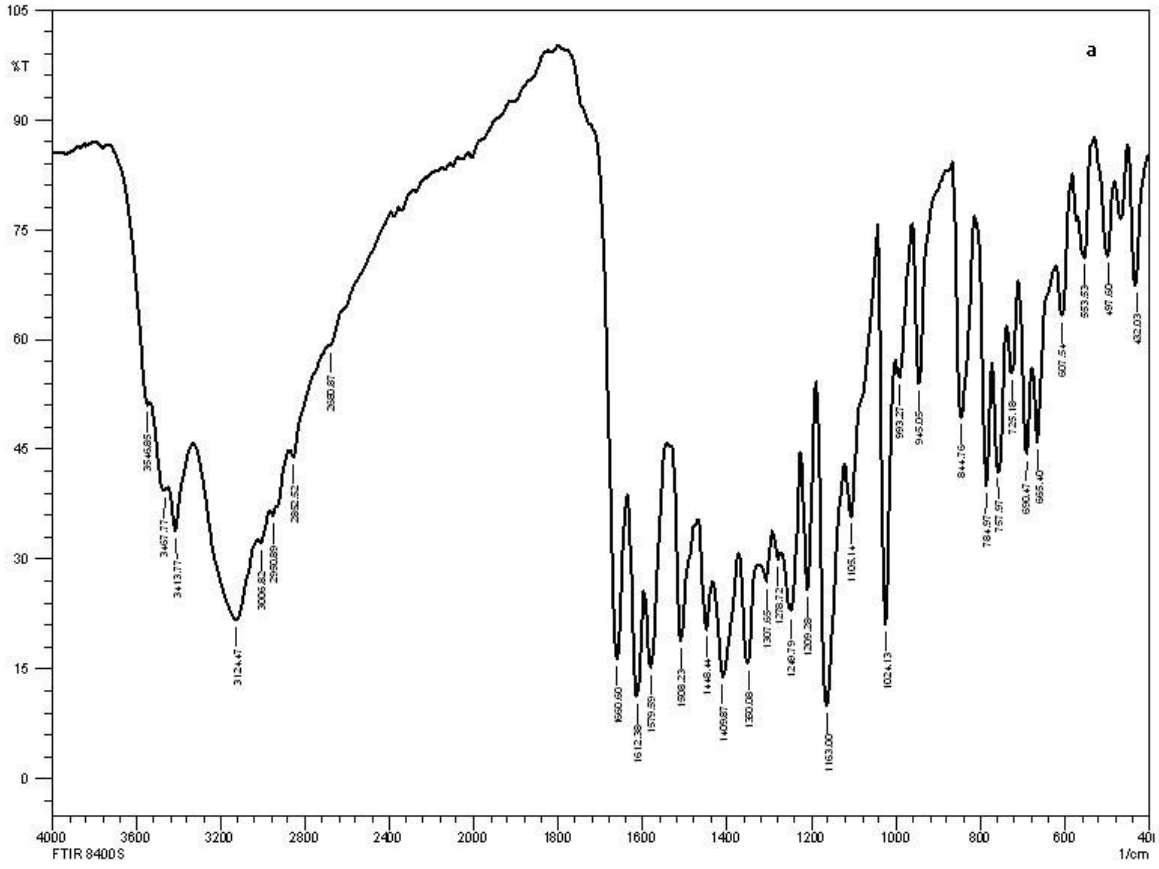

Figure.3. (a) IR spectra of Wogonin

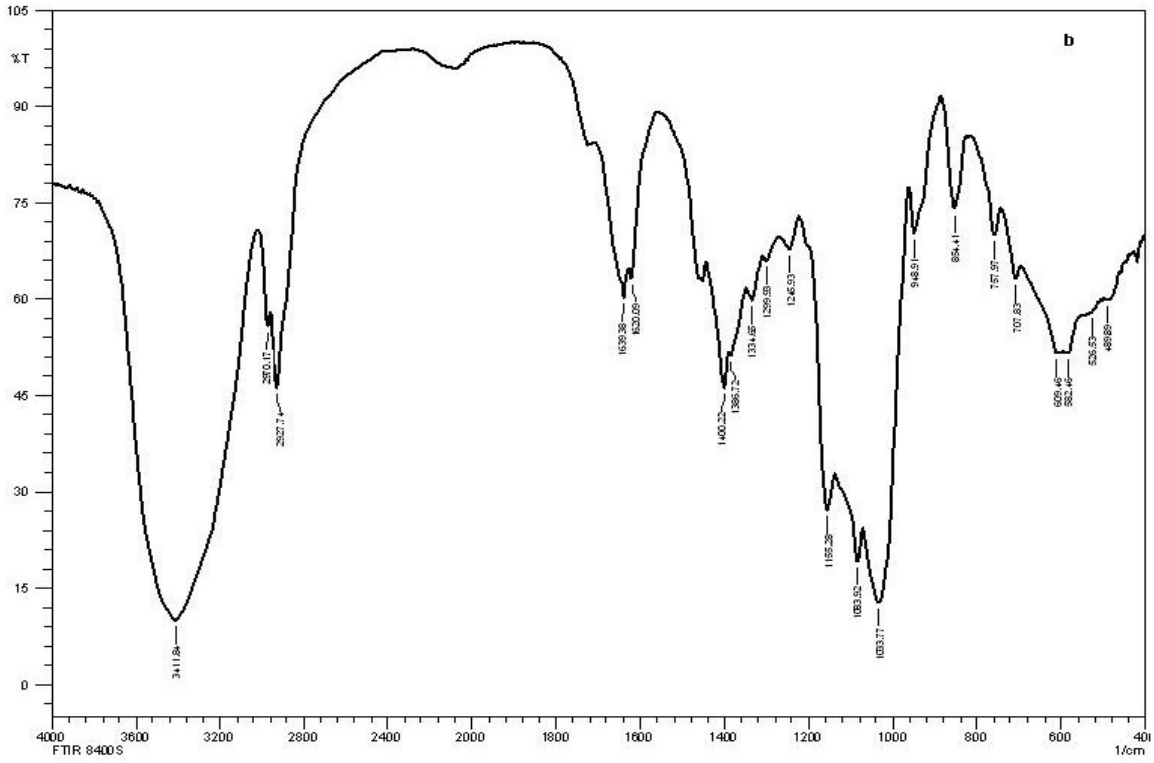

Figure.3. (b) IR spectra of HP-β-CD.

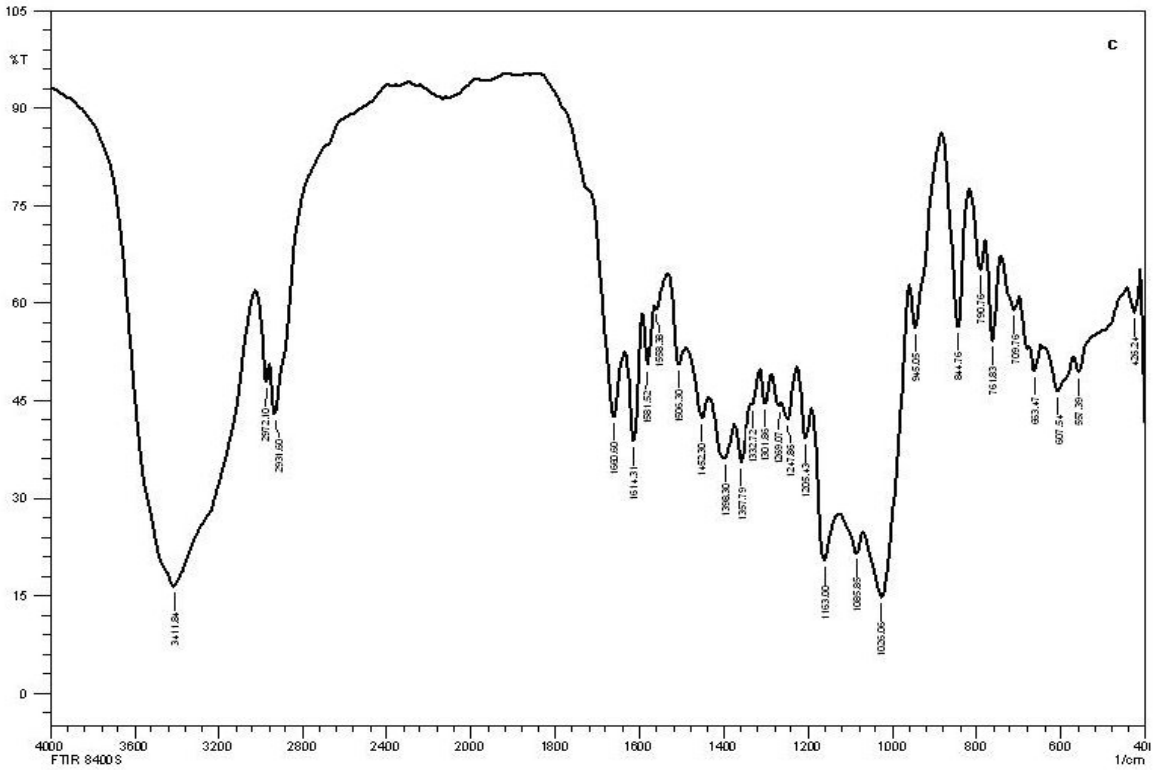

Figure.3. (c) IR spectra of Wogonin/HP-β-CD inclusion complex.

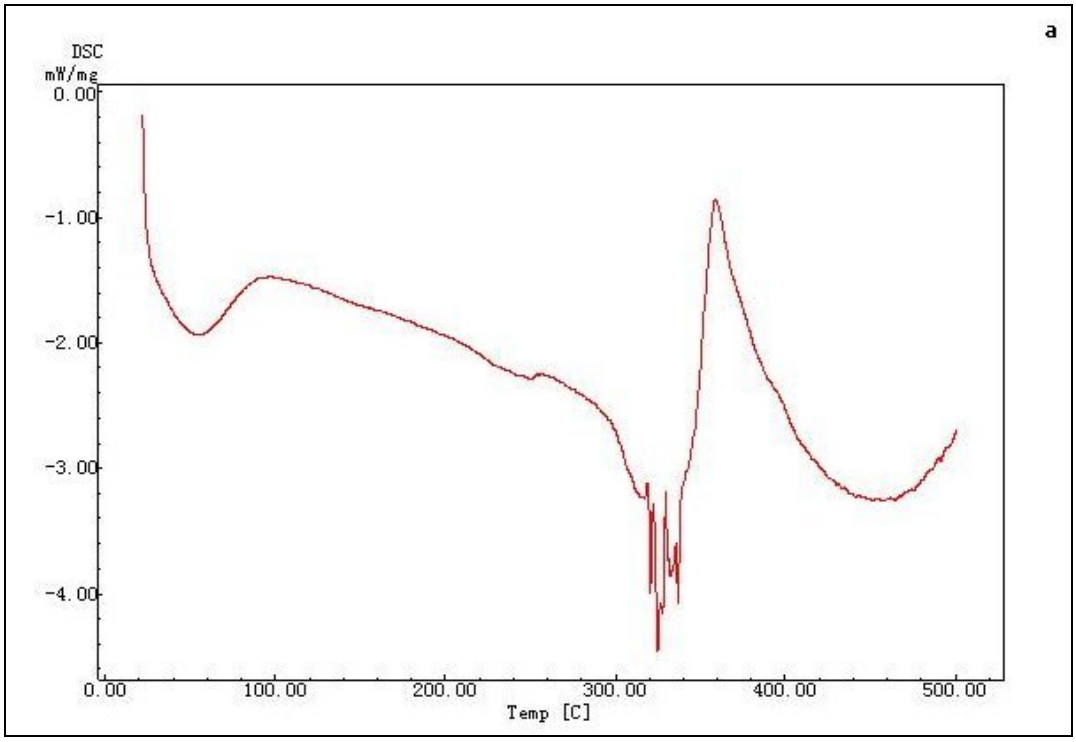

Figure.4. (a) Thermal spectra of HP-β-CD.

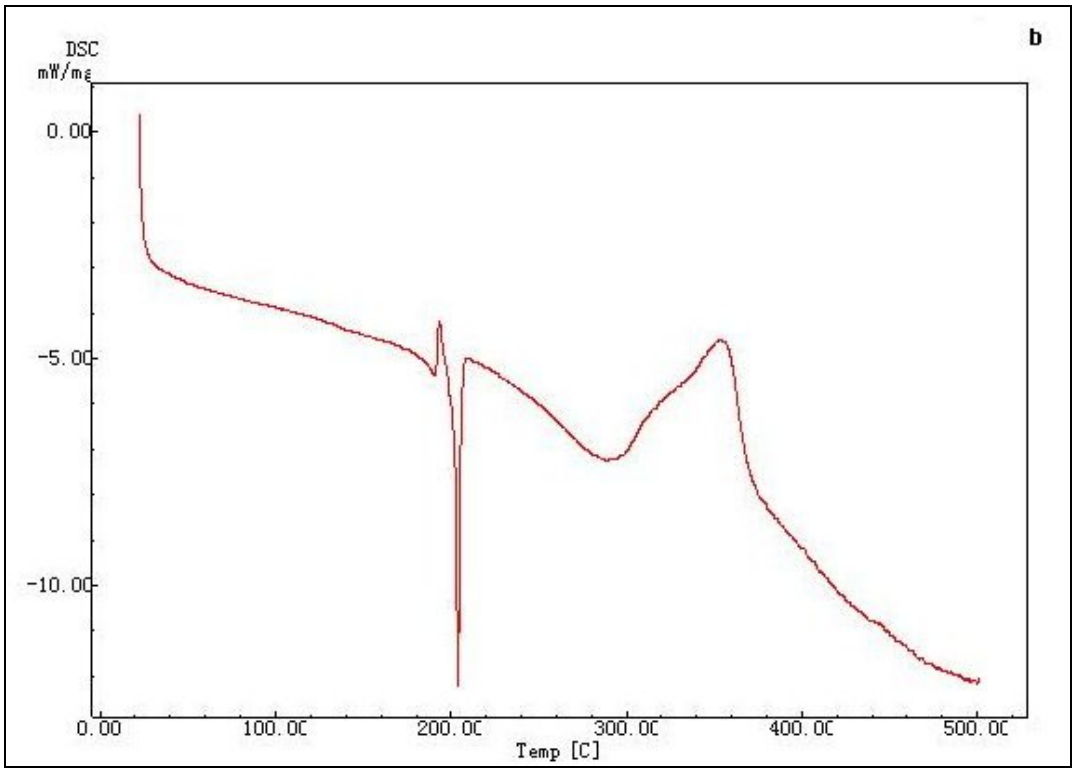

Figure.4. (b) Thermal spectra of Wogonin.

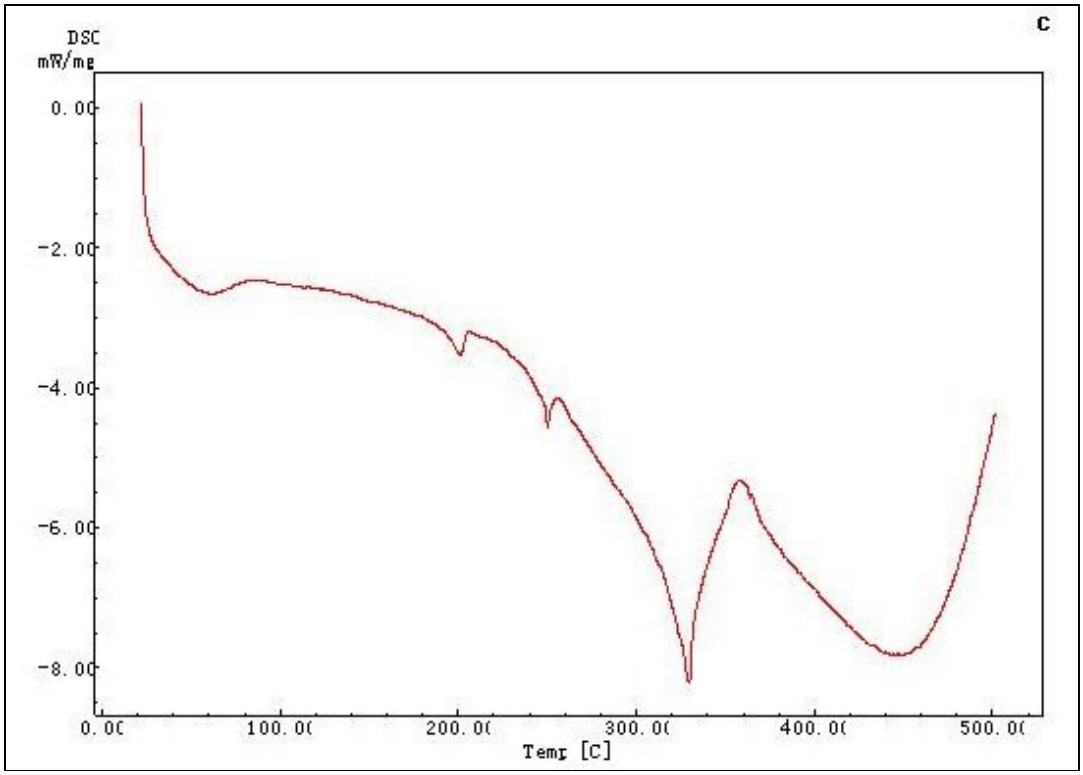

Figure.4. (c) Wogonin/HP-β-CD inclusion complex.

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