

Original Research Article

Preparation and Characterization of a Complex of Paeonol and Hydroxypropyl- β -cyclodextrin

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Abstract

Purpose: To improve the solubility of paeonol in water by complexing with hydroxypropyl- β -cyclodextrin (HP- β -CD) and also to characterize the physicochemical properties of the complex.

Methods: The complex of paeonol and HP- β -CD was prepared by freeze-drying method. Its physicochemical properties were studied by phase-solubility method, ultraviolet-visible spectrometry (UV), infrared spectrometry (IR), scanning electron microscopy (SEM), x-ray diffractometry (XRD) and thermogravimetric/differential scanning calorimetry (TG/DSC).

Results: The phase-solubility results showed that paeonol formed a 1:1 stoichiometric complex with HP- β -CD while UV and IR spectra suggested that HP- β -CD and paeonol in the complex interacted by a non-covalent bond. SEM and XRD indicate that the heat stability of paeonol was significantly improved by complexing with HP- β -CD.

Conclusion: Paeonol can be efficiently complexed with HP- β -CD to form a complex by freeze-drying method. The aqueous solubility and heat stability of paeonol is significantly improved by complexation HP- β -CD.

Keywords: Paeonol, Hydroxypropyl- β -cyclodextrin, Complexation, Aqueous solubility, Heat stability

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INTRODUCTION

Paeonol (Figure 1) is a natural phenolic compound found in many traditional Chinese medicines, such as *Paeonia suffruticosa*, *Arisaema erubescens*, *Dioscorea japonica* [1-3]. It exhibits anti-inflammatory, antibacterial and antitumor activities, and has good prospects in functional food and medicine [4-6]. However, the poor aqueous solubility and heat stability of paeonol limit its wide application. Interestingly, these problems can be resolved by complexing with cyclodextrins (CDs), which have hydrophilic outer surfaces and a hydrophobic cavity at the center [7]. The most common CD is β -CD

containing seven glucopyranose units. Tsai et al reported the complex of paeonol and β -CD [8]. Hydroxypropyl- β -cyclodextrin (HP- β -CD) is a hydroxyalkyl β -cyclodextrin derivative, which is widely used in the field of food and medicine encapsulation for its higher water solubility and safety [9,10].

To the best of our knowledge, there is no report on the complex of paeonol and HP- β -CD. The objective of this study was to prepare the complex of paeonol and HP- β -CD by freeze-drying method, and characterize its physicochemical properties by different analytical techniques, including phase-solubility method, ultraviolet-visible spectrometry (UV), infrared

spectrometry (IR), scanning electron microscopy (SEM), x-ray diffractometry (XRD) and thermogravimetric/differential scanning calorimetry (TG/DSC).

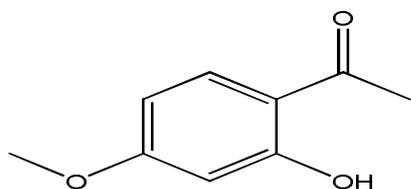


Figure 1: Chemical structure of paeonol

EXPERIMENTAL

Materials and chemicals

Paeonol (purity, 98 %) was obtained from Shaanxi Huike Botanical Development Co., Ltd (Xi'an, China) while HP- β -CD (purity, 99 %, MW 1375) was purchased from Seebio Biotech, Inc. (Shanghai, China). Other chemicals used were of analytical grade.

Phase-solubility method

The phase solubility study was conducted according to a previous method [11]. An excess amount of paeonol (50 mg) was added to each 5 mL sample of HP- β -CD aqueous solution with the concentrations ranging from 0 to 10 mM. The test tubes containing the reagents were shaken in a water bath for 72 h at 30 °C. Then, the suspensions were filtered through 0.45 μ m membrane filters to remove undissolved paeonol. The concentration of paeonol in the filtrates were determined by measuring their absorbance with a TU-1810PC UV spectrophotometer (Purkinje, China) at 275 nm and comparing it with a standard curve of pure paeonol.

Preparation of the complex of paeonol and HP- β -CD

Paeonol (0.166 g) and HP- β -CD (1.375 g) were dissolved in 25 mL of distilled water, stirred for 24 h and then filtered through a 0.45 μ m membrane filter. The filtrate was lyophilized and collected as the complex of paeonol and HP- β -CD.

Preparation of the physical mixture of paeonol and HP- β -CD

Paeonol (0.166 g) and HP- β -CD (1.375 g) were mixed and stirred in a small beaker at room temperature. This was used as the physical mixture of paeonol and HP- β -CD.

Ultraviolet-visible spectrometry (UV)

UV analysis was performed on a TU-1810PC UV spectrophotometer (Purkinje, China). Each sample was dissolved in water and scanned from 220 to 400 nm to obtain the UV spectra.

Infrared spectrometry (IR)

IR analysis was performed on a TENSOR 27 infrared spectrophotometer (Bruker, Germany) by using the KBr method. IR spectra of the samples between 4000 and 400 cm^{-1} were collected and recorded by the OPUS software (Bruker, Germany).

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was performed with a Quanta 200 environmental scanning electron microscope (FEI, USA). The samples were evenly distributed on SEM specimen stubs with double adhesive tape. The micrographs were obtained with an accelerating potential of 10 kV under low vacuum.

X-ray diffractometry (XRD)

XRD analysis was performed on a D8 Advance X-ray diffractometer (Bruker, Germany). The powders were packed tightly in a rectangular aluminum cell and exposed to the X-ray beam. The scanning region of the diffraction angle, 2θ , was 5- 80°.

Thermogravimetric/differential scanning calorimetry measurement (TG/DSC)

The TG/DSC analysis was conducted for each sample with a Q600 TG/DSC system (TA Instruments, USA) according to a previous method [12]. And the operation conditions were as following: dynamic atmosphere of nitrogen (99.999 %) at 100 mL/min and heating rate of 20 °C /min from 50 to 500 °C, with sample mass of about 10 mg.

RESULTS

Phase-solubility

The phase-solubility diagram of the complex of paeonol and HP- β -CD is shown in Figure 2. The phase-solubility diagram displayed a typical AL type diagram, which suggested that the aqueous solubility of paeonol increased with the increase of HP- β -CD concentration. The result indicated a 1:1 stoichiometry of the complex between paeonol and HP- β -CD.

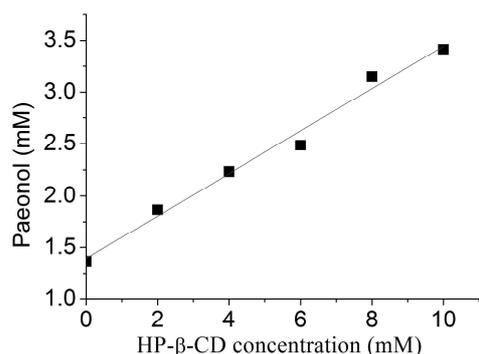


Figure 2: Phase-solubility diagram of the complex of paeonol and HP-β-CD

UV spectra

The UV spectra of HP-β-CD, paeonol, their physical mixture and the complex are shown in Figure 3. There was no UV absorbance of HP-β-CD in the range of 220 to 400 nm. There was also no difference between the UV spectra of the physical mixture and the complex. The characteristic absorption peaks of paeonol, the physical mixture and the complex were still present at 228, 275 and 311 nm.

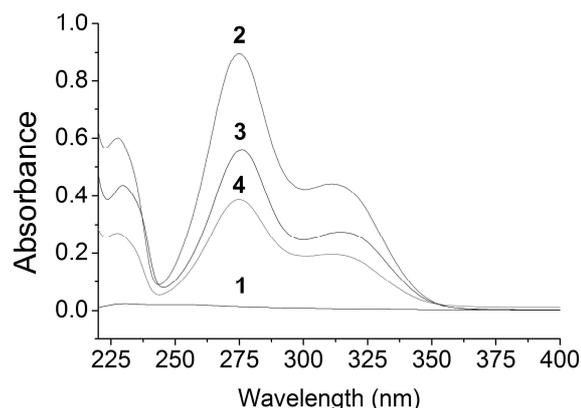


Figure 3: UV spectra of (1) HP-β-CD, (2) paeonol, (3) their physical mixture and (4) complex

IR spectra

The IR spectra of HP-β-CD, paeonol, their physical mixture and the complex are shown in Figure 4. The IR spectra of the complex and the mixture of paeonol and HP-β-CD were similar to that of HP-β-CD for the low content of paeonol in the system. There was no significant difference between the physical mixture and the complex. However, some small characteristic absorption peaks of paeonol between 500 and 1500 cm^{-1} were masked by that of HP-β-CD in the spectrum of the complex.

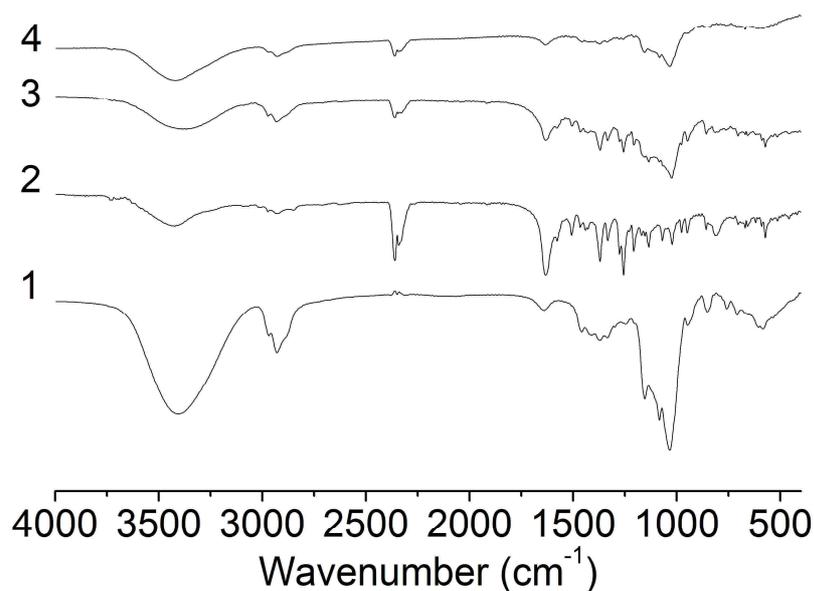


Figure 4: IR spectra of (1) HP-β-CD, (2) paeonol, (3) their physical mixture and (4) complex

SEM

The scanning electron micrographs of HP- β -CD, paeonol, their physical mixture and the complex were shown in Figure 5. Paeonol existed in the crystals while HP- β -CD appeared as amorphous spheres. For their physical mixture, both the characteristic crystals of paeonol and the amorphous spheres of HP- β -CD could be found. But their complex appeared as irregular size pieces.

XRD

The diffractograms of HP- β -CD, paeonol, their physical mixture and the complex are shown in Figure 6. The diffractogram of paeonol displayed sharp crystalline peaks. In contrast, HP- β -CD was amorphous as it lacked crystalline peaks. Furthermore, compared with the diffractogram of

the physical mixture, the crystalline peaks had disappeared in the diffractogram of the complex.

Thermal characteristics

The TG/DSC thermograms of HP- β -CD, paeonol and their complex are shown in Figures 7 - 9, respectively. Both TG curves of paeonol and HP- β -CD mainly showed one step, which meant that both the samples were pure compounds. For HP- β -CD, it decomposed at about 310 °C and ended at about 400 °C. Paeonol showed an endothermic peak at about 50 °C, which coincided with its melting point and then decomposed. For their complex, the endothermic peak at about 75 °C attributed to the loss of water like HP- β -CD. And the disappearance of the melting peak of paeonol could be found.

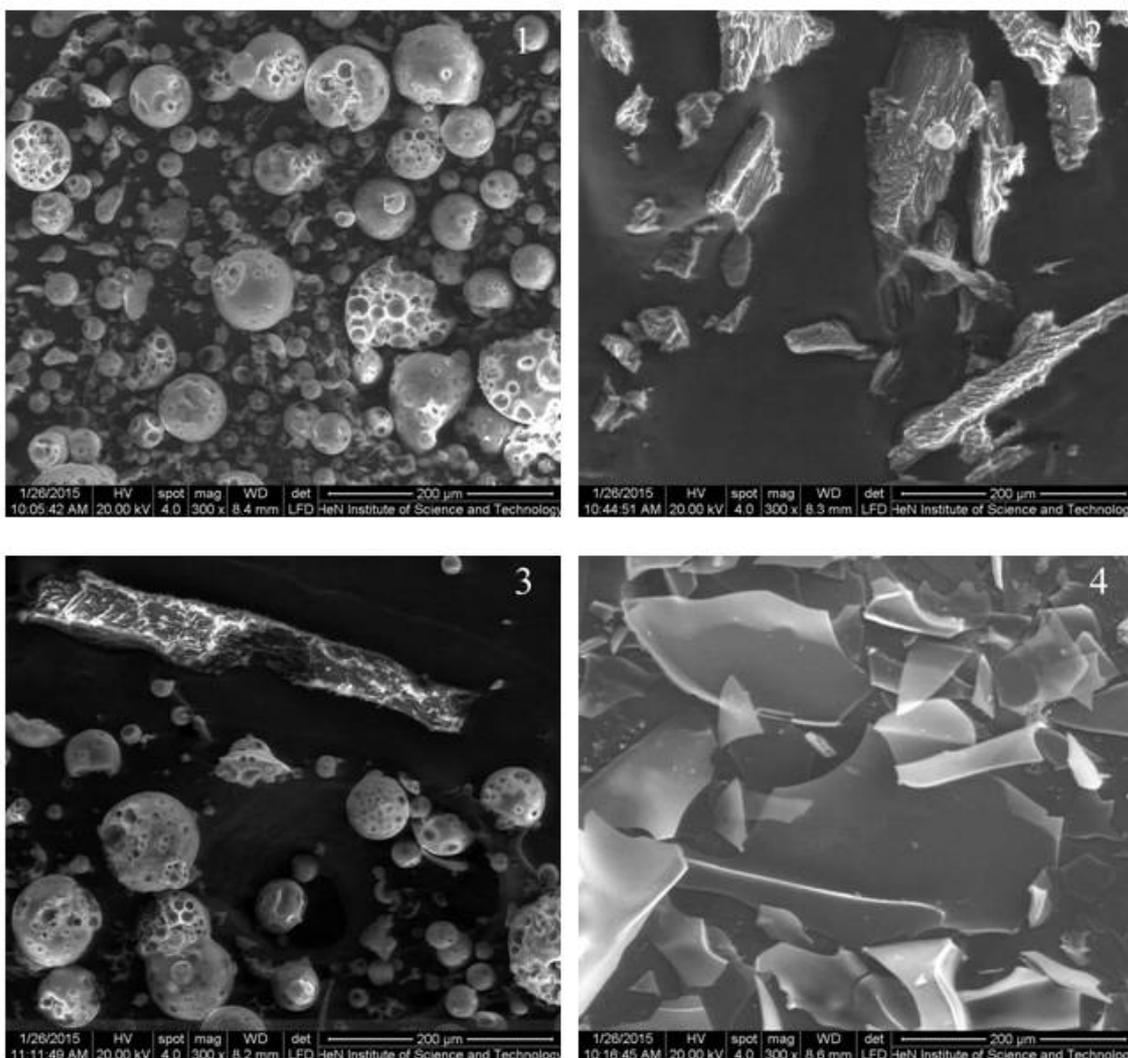


Figure 5: Scanning electron micrographs of (1) HP- β -CD, (2) paeonol, (3) their physical mixture and (4) complex

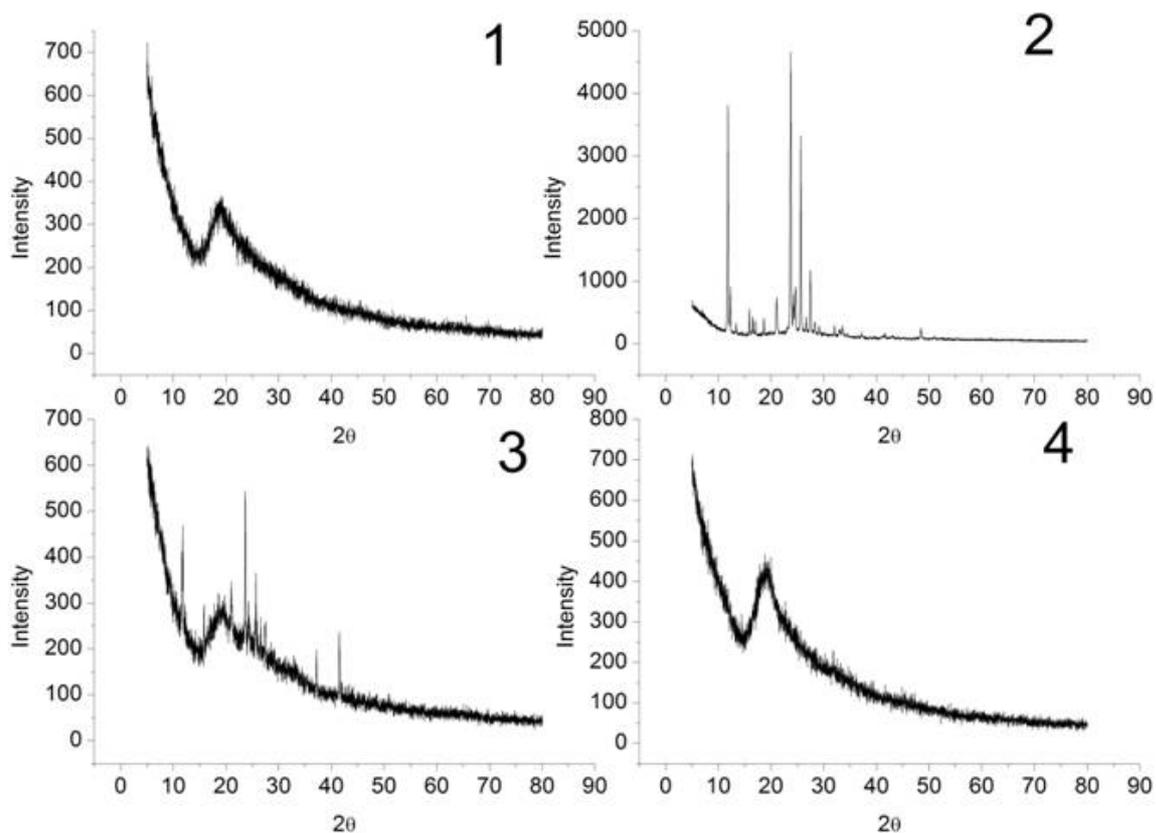


Figure 6: X-ray diffractograms of (1) HP-β-CD, (2) paeonol, (3) their physical mixture and (4) complex

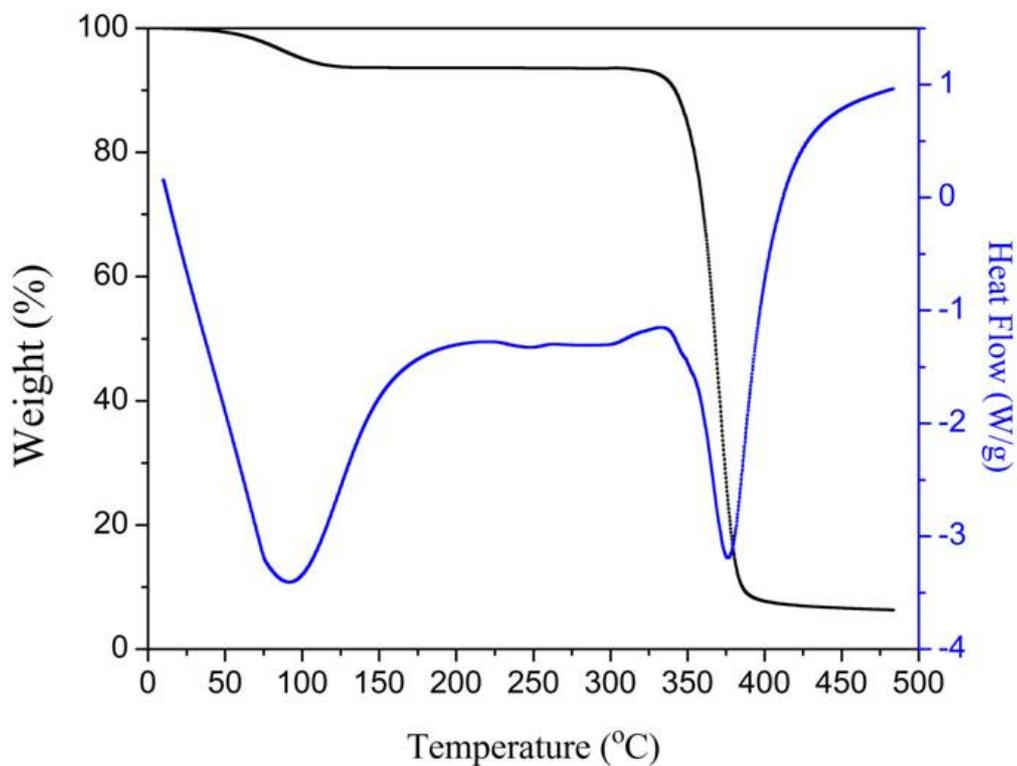


Figure 7: TG/DSC thermograms of HP-β-CD

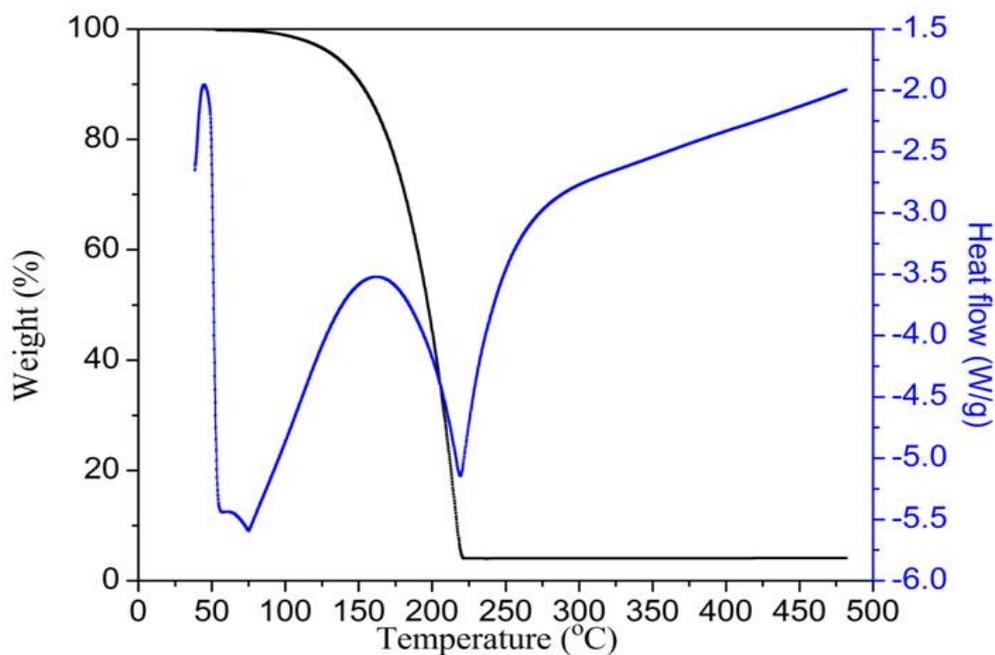


Figure 8: TG/DSC thermograms of paeonol

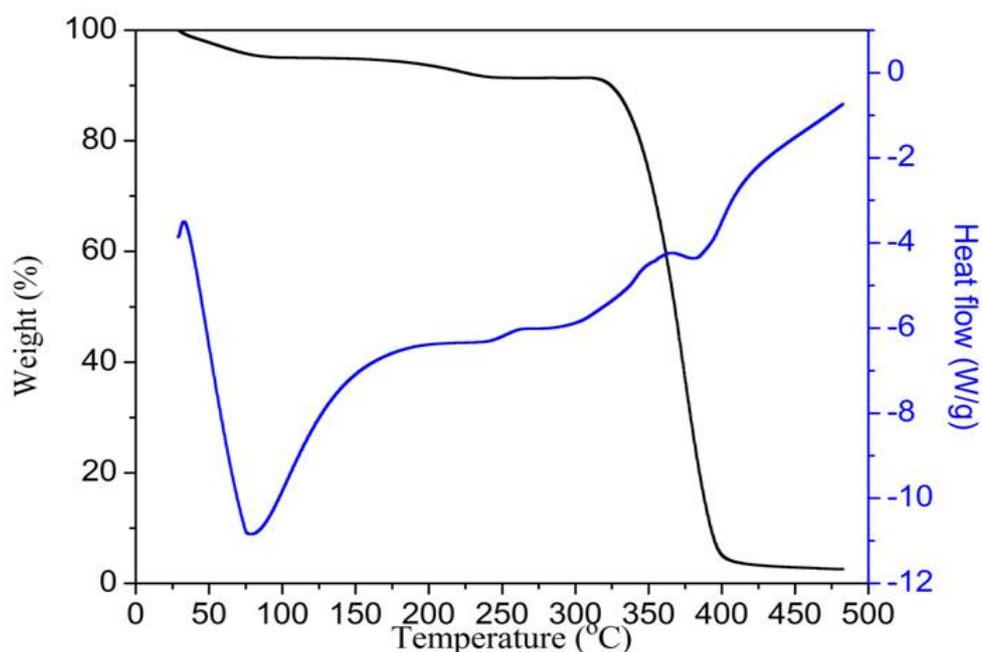


Figure 9: TG/DSC thermograms of the complex

DISCUSSION

In recent years, HP- β -CD has gained appreciable acceptance among various types of CDs that have been successfully used to improve solubility and stability of many natural products. Many methods such as coprecipitation, spray drying, kneading, freeze-drying, ball-milling, can be used to prepare the complexes of CDs. For high efficiency and low loss of bioactivity, the freeze-drying method was

selected to prepare the complex of paeonol/HP- β -CD in this study.

The phase-solubility study showed that the aqueous solubility of paeonol could be significantly improved by forming a 1:1 molecular complex with HP- β -CD. Based on the results of UV and IR, HP- β -CD and paeonol in the complex interacted by non-covalent bond. SEM showed that the complex appeared as irregular particles, in which the original morphology of both HP- β -CD and paeonol disappeared. It could be inferred

that when the solutions of HP- β -CD and paeonol were mixed, they formed a close association, probably in the form of the inclusion complex, in which paeonol no longer existed in the crystal state. XRD also proved that paeonol in the complex was molecularly dispersed in HP- β -CD matrix. For TG/DSC, HP- β -CD exhibited two weight loss steps at 50-100 °C and 310 - 420 °C. The former was associated with the evaporation of water while the latter was due to the decomposition of HP- β -CD. Paeonol exhibited a single weight loss step at 50 - 100 °C for its decomposition. In contrast, the TG/DSC thermogram of the complex showed only the features of HP- β -CD, while the characteristic peak of paeonol totally disappeared, suggesting that paeonol was dispersed inside HP- β -CD, probably in the form of an inclusion complex. And as a result, the heat stability of paeonol was significantly improved.

CONCLUSION

The results of this study clearly demonstrate that paeonol can be suitably complexed with HP- β -CD to form an inclusion complex by freeze-drying method. The aqueous solubility and heat stability of paeonol in the resulting complex is significantly enhanced. HP- β -CD complexation approach is a promising strategy to improve the application of paeonol in functional food and medicine.

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