Structural Characterization of Inclusion Complex of Hesperidin Methyl Chalcone and Hydroxypropyl-β-cyclodextrin

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Summary: Hesperidin methyl chalcone (HMC) was a semisynthetic derivative of hesperidin, which owned antiviral and antimicrobial activities. Owing these properties, it can be applied in pharmaceutical industry. However low stability had become a barrier to its application. In order to overcome this problem, an inclusion complex of hesperidin methyl chalcone and hydroxypropyl-β-cyclodextrin (HP-β-CD) were prepared by freeze-drying, using some analytical techniques to characterize the inclusion complex, including ultraviolet-visible spectroscopy (UV), Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), X-ray diffractometry (XRD) and differential scanning calorimetry (DSC). The results of these analytical techniques indicated that the hesperidin methyl chalcone has been dispersed completely in the HP-β-CD without a new compound formed, but entrapped inside the cavity of HP-β-CD.

Keywords: Hesperidin methyl chalcone; Hydroxypropyl-β-cyclodextrin; Inclusion complex; Physicochemical property

Introduction

Flavonoids, a large family of secondary plant metabolites with the structure of phenyl-benzo-γ-pyran ($C_6C_3C_6$) skeleton, were natrually polyphenols [1-3]. They were widely existing in plants, especially in fruits, vegetables, tea and legumes [4]. Many flavonoids had exhibited pharmacological properties, such as anti-inflammatory, antioxidant, antitumoral, antiviral, antimicrobial, anti-allergic and antidiarrheal activities [5-11]. Hesperidin (HES), 3,5,7-trihydroxy-4-methoxy-flavanone-7-rhamnoglucoside, was a bioflavanoid mainly present in oranges and lemons [12]. Owing to the structure of 3 hydroxyl groups, HES had a greater antioxidant activity and ability to activate cellular antioxidant preventing enzymes than other flavanones [13]. But similar to many other flavonoids, HES was insoluble in water and not available in the small intestine [14, 15]. It was reported that methylation of flavonoids improves their metabolic stablity and intestinal absorption [16]. The methylation of hesperidin using methyl sulphate in an alkaline medium forms hesperidin methyl chalcone (HMC), which was a hydrosoluble product and show higher metabolic resistance [15, 17].

As was known to us, HMC owned vitamin P properties used to treat capillary and venous diseases, and it was also added to many oral medications [17]. It had been reported that HMC has the capable of reducing inflammation and inflammatory pain which was a clue to its applicability in inflammatory diseases and treating ultraviolet B irradiation-induced skin inflammation and oxidative stress [16, 18]. However, poor stability had been a barrier to the clinical use of HMC.

Cyclodextrins (CDs) were a class of cyclic oligosaccharides composed of (α-1,4) - linked-glucopyranose units. Due to a hydrophilic outer surface and an inner hydrophobic cavity, CDs were extensively used as host molecules producing inclusion complexes in aqueous solutions [19, 20]. Compared with the native compounds, inclusion complexs have higher stability, solubility and bioavailability [21]. As a consequence, inclusion complexs were generally applied in many realm, such as supramolecular chemistry, pharmacology and food science [22]. Among CDs, the α-, β-, and γ-cyclodextrin, consisting of 6, 7 and 8 glucopyranose units respectively, were the most common formulation vehicles [23]. Hydroxypropyl-β- cyclodextrin (HP-β-CD), a derivative of β-cyclodextrin, was the most popular for its higher bioavailability, more solubility and

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lower toxicity and hemolytic activity [19, 24, 25]. Moreover, HP-β-CD was able to be well absorbed by people whenever in the form of oral or intravenous injection [24]. Therefore, in order to improve the stability of hesperidin methyl chalcone, we prepared the inclusion complex of hesperidin methyl chalcone with HP-β-CD.

In this study, the inclusion complex of hesperidin methyl chalcone with HP-β-CD was formed for the first time. The physicochemical properties of samples were tested and characterized by ultraviolet-visible spectroscopy (UV), Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), X-ray diffractometry (XRD) and differential scanning calorimetry (DSC).

Experimental

Materials and Chemicals

Hesperidin methyl chalcone(>98%) was obtained from Sigma Chemicals Co. (USA). Hydroxypropyl-β-cyclodextrin (MW 1460) was obtained from Sigma Chemicals Co. (USA). All other chemicals and solvents were of analytical grade.

Preparation of the Inclusion Complex of Hesperidin methyl chalcone and HP-β-CD

Hesperidin methyl chalcone and HP-β-CD was dissolved in 25 mL of distilled water with a 1:1 molar ratio in order to produce a inclusion complexe. This mixture was stirred for 24 h at 25 °C and then centrifuged to remove excess material. The supernatant was frozen at -80 °C and then lyophilized. The collected powder is the inclusion complex.

Preparation of the Physical Mixture of Hesperidin methyl chalcone *and HP-β-CD*

The physical mixture of hesperidin methyl chalcone and HP-β-CD were prepared by homogenizing hesperidin methyl chalcone and HP-β-CD with a molar ratio of 1:1 with a spatula.

UV-visible Spectroscopy (UV)

The UV spectra of hesperidin methyl chalcone, HP-β-CD, their physical mixture and inclusion complex were performed by a TU-1810PC spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., Beijing, China). The solvent of Each sample was distilled water. The solutions of samples were scanned respectively with the wavelength range of 200 to 400 nm.

Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectra of hesperidin methyl chalcone, HP-β-CD, their physical mixture and inclusion complex were measured from 4000 and 400 cm^{-1} (Mid infrared region) on a TENSOR 27 infrared spectrophotometer (Bruker, Germany) with a blank KBr disk as background. Samples was mixed with dry potassium bromide (KBr) powder respectively and then compressed into 1 mm disk (2 mg of sample per 200 mg dry KBr).

Scanning Electron Microscopy (SEM)

The surface morphologies of hesperidin methyl chalcone, HP-β-CD, their physical mixture and inclusion complex were observed on a Quanta 200 environmental scanning electron microscope (FEI, USA). About 0.5 mg samples were dispersed into a 5 mm × 5 mm silicon wafer attached by graphite tape to aluminium discs.

X-ray Diffractometry (XRD)

The X-ray Diffractometry patterns of hesperidin methyl chalcone, HP-β-CD, their physical mixture and inclusion complex were recored by a D8 X-ray diffractometer (Bruker, Germany). The scanning regions of the diffraction angle, 2θ, were 10–40˚.

Differential Scanning Calorimetry Measurement (DSC)

DSC of hesperidin methyl chalcone, HP-β-CD, their physical mixture and inclusion complex were recorded at a heating rate of 10 □/min from 25 to 250 □. The samples are about 5 mg(±0.5 mg).

Results and Discussion

UV Analysis

The UV absorption spectra of Hesperidin methyl chalcone, HP-β-CD, their physical mixture and inclusion complex were shown respectively in Fig. 1. The UV absorbance of HP-β-CD didn't

present any absorption peak within 220-400nm. However, the inclusion complex exhibited two absorption peaks at 285 nm and 347 nm, which were similar to the characteristic absorption peak of Hesperidin methyl chalcone.The 285 nm band is from C = O and the 347 nm band corresponds to the chromophore C = C.The result indicated that no new unsaturated bond produced in the inclusion complex.

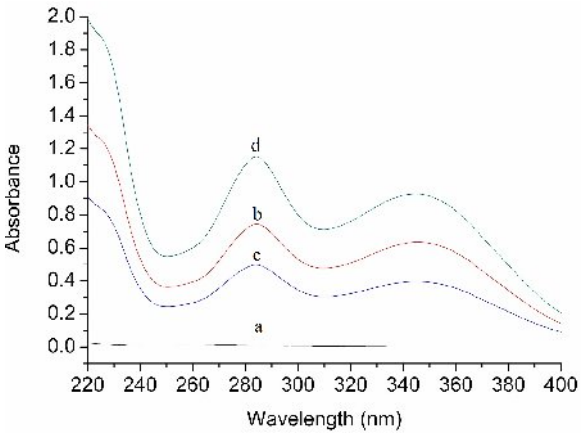

Fig. 1: UV spectra of HP-β-CD (a), HMC(b), their physical mixture (c) and inclusion complex (d)

IR Analysis

The IR spectra of hesperidin methyl chalcone, HP-β-CD, their physical mixture and inclusion complex were presented in Fig. 2. In the FT-IR spectrum of HP-β-CD, the distinct absorption peaks were O-H stretching (3400 cm^{-1}), C-H stretching (2930 cm^{-1}) and C-O stretching (1157, 1084 and 1033 cm^{-1}). The characteristic peaks of hesperidin methyl chalcone were at 3436 cm^{-1}(for the hydroxyl group) and 1517, 1464 and 1421 cm^{-1}(for the aromatic nucleus). The FT-IR spectrum of the physical mixture was in fact a combination of the spectra of hesperidin methyl chalcone and HP-β-CD. As in shown in Fig. 2, the spectrum of inclusion complex was different from that of physical mixture and more similar to that of HP-β-CD. In the spectrum of the complex, a broad band around 3400 cm^{-1} was formed indicating that hesperidin methyl chalcone was involved in the cavity of HP-β-CD.

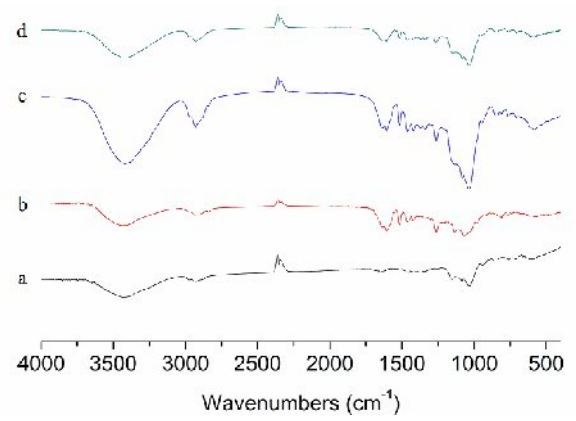

Fig. 2: IR spectra of HP-β-CD (a), HMC(b), their physical mixture (c) and inclusion complex (d)

SEM Analysis

The surface morphology of hesperidin methyl chalcone, HP-β-CD, their physical mixture and complex was assessed by SEM. As shown in Fig. 3, HP-β-CD appeared as amorphous spheres, while the hesperidin methyl chalcone existed in crystalline state. In the physical mixture, the two characteristics, amorphous spheres and crystalline state, were existed separately. However, the inclusion complex appeared in the form of irregular amorphous pieces with the absence of the original morphology of both components, which suggested the formation of inclusion complex.

XRD Analysis

The X-ray diffractometry is an insighting method to study cyclodextrin complexation. The X-ray diffraction patterns of hesperidin methyl chalcone, HP-β-CD, their physical mixture and inclusion complex were shown in Fig. 4. Due to its amorphous structure, HP-β-CD displayed one broad peak in its diffraction pattern. While the diffraction pattern of hesperidin methyl chalcone exhibited some sharp crystalline peaks. As is observed, the X-ray diffraction pattern of inclusion complex was more similar to that of HP-β-CD and obviously differed from that of hesperidin methyl chalcone, which could be considered as a further evidence for the formation of inclusion complex of the two components.

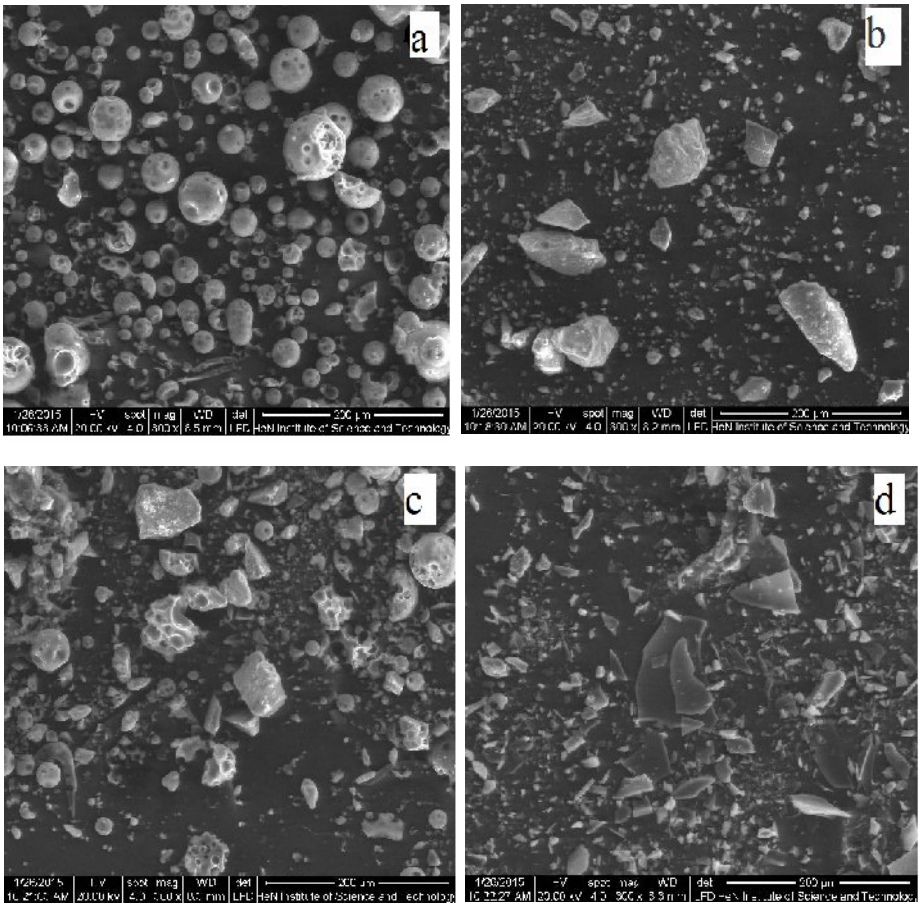

Fig. 3: Scanning electron micrographs of HP-β-CD (a), HMC (b), their physical mixture (c) and inclusion complex (d).

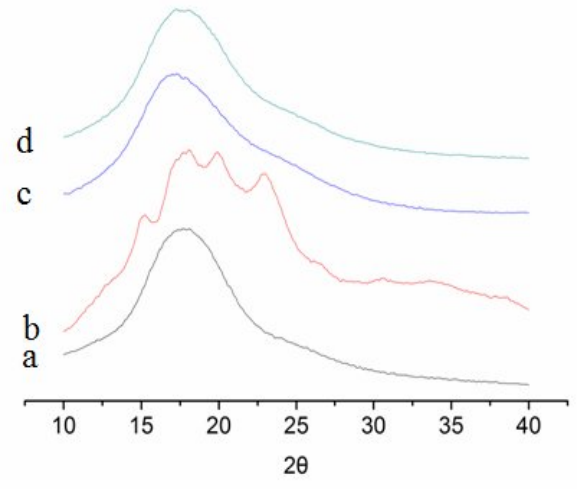

Fig. 4: X-ray diffraction patterns of HP-β-CD (a), HMC (b), their physical mixture (c) and inclusion complex (d).

DSC Analysis

DSC thermograms of hesperidin methyl chalcone, HP-β-CD, their physical mixture and inclusion complex were shown in Fig. 5. The curve of HP-β-CD has a broad endothermic peak at about 90 °C for the loss of residual moisture. DSC curve of hesperidin methyl chalcone exhibited a endothermic peak at around 120 °C and a low-intensity peak at about 213 °C. The peak at 213 ºC was an exothermic peak, which was caused by the decomposition of the sample after melting, not by the transition from crystalline to amorphous phase.The endothermic peak at 220 ºC was a fusion peak, because the fusing point of the HMC is 120 ºC.The DSC thermogram of the inclusion complex has a broad endothermic peak at about 80°C which mainly exhibited the characteristic of HP-β-CD with the typical endothermic peak of hesperidin methyl chalcone disappearance, indicating the formation of inclusion complex.

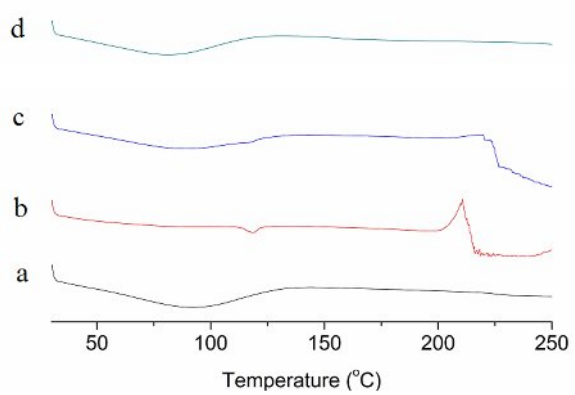

Fig. 5: DSC curves of HP-β-CD (a), HMC (b), their physical mixture (c) and inclusion complex (d)

Conclusions

This study successfully obtained the inclusion complex of hesperidin methyl chalcone and HP-β-CD. The results of UV, IR, SEM, XRD and DSC analysis sufficiently reveal the fact that the inclusion complex has been formed and the stablity of hesperidin methyl chalcone has been improved effectively. As a consequence, the application prospects of hesperidin methyl chalcone in pharmacological field will be extended greatly.

Acknowledgments

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