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Original Research Article

Physiochemical Properties of the Complex of Myricetin and Hydroxypropyl-β-Cyclodextrin

Haijuan Nan*, Hanjun Ma, Ruiting Zhang and Rui Zhan
School of Food Science, Henan Institute of Science and Technology, Xinxiang 453003, China

For correspondence:* ***Email:* *nanhaijuan1@163.com*

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Abstract

Purpose: *To improve the hydrophilic property of myricetin by complexing it with hydroxypropyl-β-cyclodextrin (HP-β-CD)*
Methods: *The complexes of myricetin and HP-β-CD were prepared in both water and ethanol. The physicochemical properties of the complex were investigated by ultraviolet-visible spectrometry (UV), Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), differential scanning calorimetry (DSC) and x-ray diffractometry (XRD).*
Results: *The content of myricetin in the complex prepared in ethanol (3.71 %) was higher than that prepared in water (1.43 %). Characterization studies indicate that myricetin in the complex was molecularly dispersed in HP-β-CD matrix.*
Conclusion: *Ethanol is the better solvent to prepare the complex of myricetin and hydroxypropyl-β-cyclodextrin. Myricetin and HP-β-CD in the complex are combined by non-covalent bond, and thus did not form a new compound.*

Keywords: *Myricetin, Hydroxypropyl-β-cyclodextrin, Complexation, Physicochemical properties*

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INTRODUCTION

Myricetin is a naturally occurring flavonol, a flavonoid found in many grapes, berries, fruits, vegetables, herbs, as well as other plants, which possesses antioxidant, antifungal, antiviral, anticancer activities [1-3]. But the poor solubility and stability of myricetin in water restricted its application as an antioxidant and functional supplement in hydrophilic food. Today, the water solubility of flavonoids was generally improved by glycosidation using enzyme or chemical method.

Glycosidation would reduce the number of the conjugated hydroxyl groups, thus potentially weakening the bioactivities of flavonoids. Furthermore, glycosidation would create a group of new compounds with unknown safety, which must be ascertained before their application in food or medicine is permitted. Hydroxylpropyl-β-cyclodextrin (HP-β-CD), a hydroxyalkyl derivative of β-cyclodextrin, is an alternative to α-, β- and γ-cyclodextrin, with improved water solubility and may be slightly more toxicologically benign. As the first approved CD derivatives by FDA, HP-β-CDs have widely applications in food, agriculture and the pharmaceutical field [4,5].

In this study, the complex of myricetin with HP-β-CD was prepared to improve the hydrophilic property of myricetin. And the physicochemical properties of the obtained complex were also investigated by UV, FT-IR, SEM, XRD and DSC.

EXPERIMENTAL

Materials and chemicals

Myricetin (> 97 %) was obtained from Aladdin (Shanghai, China). (2-hydroxypropyl)-β-cyclodextrin (average MW 1460) was purchased from Sigma (St. Louis, MO). Other chemicals used were of analytical grade unless otherwise stated.

Preparation of the complex of myricetin and HP-β-CD in water

Myricetin (0.318 g, 1 mM) and HP-β-CD (1.460 g, 1 mM) was mixed in 50 mL of distilled water, stirred for 48 h at 40 °C and filtered through a 0.45 μm membrane filter to remove undissolved material. The filtrate was freeze-dried (Alpha 1-2, Christ, Germany) and the resultant powdery material was weighed and collected as complex 1.

Preparation of the complex of myricetin and HP-β-CD in ethanol

Myricetin (0.318 g, 1 mM) and HP-β-CD (1.460 g, 1 mM) was mixed in 50 mL of ethanol, stirred for 24 h at room temperature and filtered through a 0.45 μm membrane filter to remove undissolved material. After ethanol was removed, the residue was dissolved in 50 mL water and filtered. The filtrate was freeze-dried (Alpha 1-2, Christ, Germany) and the resultant powdery material was weighed and collected as complex 2.

Preparation of physical mixture of myricetin and HP-β-CD

Myricetin (0.318 g, 1 mM) and HP-β-CD (1.460 g, 1 mM) was mixed thoroughly in a small beaker at room temperature, respectively. The obtained product was collected as the physical mixture of myricetin and HP-β-CD.

Ultraviolet–visible spectroscopy (UV)

The UV spectra were recorded for myricetin, HP-β-CD, their physical mixture and the complex by using a TU-1810PC UV-visible spectrophotometer (Purkinje, China). Each sample was dissolved with water at the room temperature. The aqueous solutions were scanned, respectively, in the range from 200 to 400 nm to obtain the UV spectra. To determine the amount of myricetin in the complex, 10 mg of the complex was dissolved in ethanol and the absorbance of the solution was measured at 255 nm and compared with a standard curve of pure myricetin.

Fourier transform infrared spectroscopy (FT-IR)

The FT-IR spectra of myricetin, HP-β-CD, their physical mixture and the complex were collected between 4000 and 400 cm^{-1} (Mid infrared region) on a Tensor 27 FTIR Spectrometer (Bruker, Germany) with 256 scans at a resolution of 4 cm^{-1}. The procedure consisted of grinding the sample together with KBr (spectroscopic grade) into a fine powder, placing the powder into the sampling cup, smoothing the powder, and compressing the powder bed into the holder using a compression gauge. The sample was placed in the light path and the spectrum was obtained.

Scanning electron microscopy (SEM)

The surface morphology of the sample was examined by 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 of samples were packed tightly in a rectangular aluminum cell. The samples were exposed to the X-ray beam. The scanning regions of the diffraction angle, 2θ, were 5-80°.

Differential scanning calorimetry (DSC)

DSC analysis was conducted for myricetin, HP-β-CD, their physical mixture and the complex with a Q200 Differential Calorimeter calibrated with indium (TA, USA). The samples were sealed in an aluminum crimp cell, whose lid was not perforated. The sample was heated at a rate of 10 °C/min from 30 to 350 °C in an atmosphere of nitrogen. An empty pan sealed in the same way was used as reference. The data were recorded and processed by Universal Analysis 2000 software (TA, USA).

RESULTS

UV properties

The UV spectra of myricetin, HP-β-CD, their physical mixture and the complex were shown in Figure 1. There was no difference between the

physical mixture and the complex. The characteristic absorption peaks of myricetin were still present at 255 and 379 nm. And the concentrations (X) and the absorbance (Y) of myricetin had good linear relationship. The regression equation is as in Eq 1.

$Y = 50.114X+0.0115$ ($r^2 = 0.9998$) ……. (1)

The contents of myricetin in complex 1 and 2 were determined as 1.43 and 3.71 %, respectively. And the complex 2 was used for the following analysis.

IR

The FT-IR spectra of myricetin, HP-β-CD, their physical mixture and the complex were shown in Figure 2. The IR spectrum of the complex was similar to that of HP-β-CD, some small characteristic absorption peaks of myricetin between 500 and 1500 cm^{-1} were almost masked by that of HP-β-CD. But the spectrum of the physical mixture showed an additive effect of myricetin and HP-β-CD, in which the characteristic absorption peaks of HP-β-CD and HP-β-CD could be still found.

SEM

The surface morphology graphs of myricetin, HP-β-CD, their physical mixture and the complex examined by SEM were shown in Figure 3. HP-β-CD was observed as amorphous cylindrical spheres whereas myricetin existed in needle-like crystal. In the myricetin and HP-β-CD physical mixture, both the characteristic appearance of myricetin and HP-β-CD could be found. In contrast, the complex of myricetin and HP-β-CD appeared in the form of irregular particles in

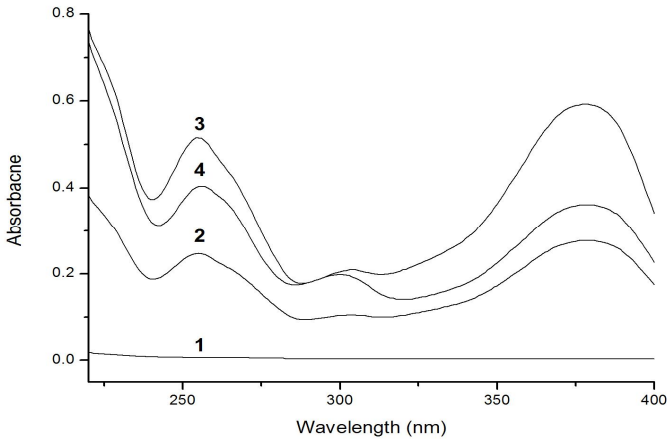

Figure 1: UV spectra of HP-β-CD (1), myricetin (2), their physical mixture (3) and inclusion complex (4)

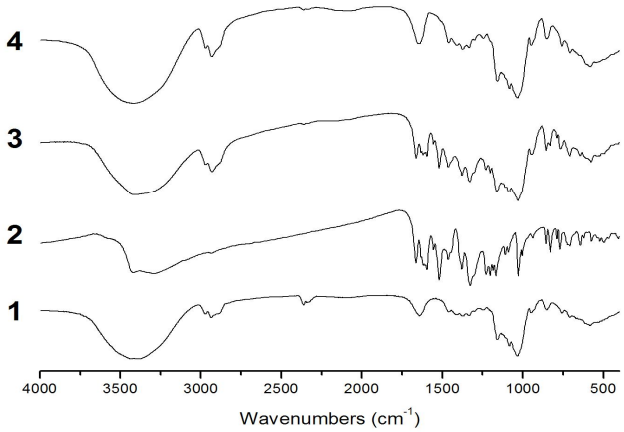

Figure 2: IR spectra of HP-β-CD (1), myricetin (2), their physical mixture (3) and inclusion complex (4)

which the original morphology of both components disappeared and tiny aggregates of amorphous pieces of irregular size were present.

XRD

The powder X-ray diffraction patterns of myricetin, HP-β-CD, their physical mixture and the complex were shown in Figure 4. Myricetin had strong crystallinity peaks while the XRD pattern of HP-β-CD revealed two broad peaks, confirming its amorphous character. In the case of myricetin and HP-β-CD physical mixture, the diffraction pattern was simply the superposition of the two patterns of the crystalline myricetin and the amorphous HP-β-CD. But the XRD pattern of the complex was the same as that of HP-β-CD.

Thermal characteristics

Figure 5 showed the DSC curves of myricetin, HP-β-CD, their physical mixture and the complex. The DSC curve of myricetin showed an endothermal peak with onset temperature at 310 °C, which was attributed to the melting of myricetin. The DSC curve of the physical mixture mainly showed the effect of myricetin and HP-β-CD. But the DSC curve of the complex mainly showed the effect of HP-β-CD, in which the characteristic endothermal peaks of myricetin disappeared.

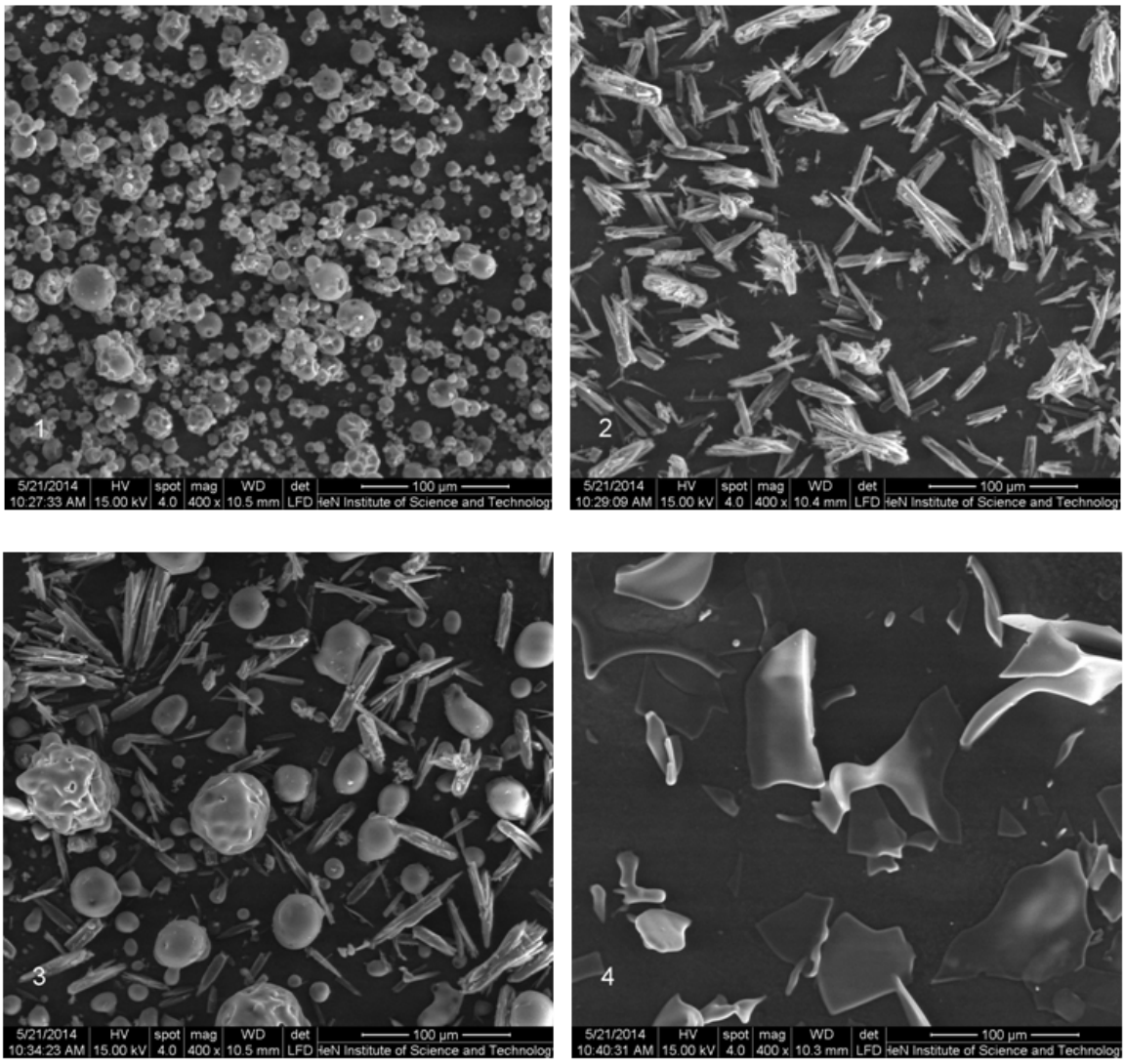

Figure 3: Scanning electron micrographs of HP-β-CD (1), myricetin (2), their physical mixture (3) and inclusion complex (4)

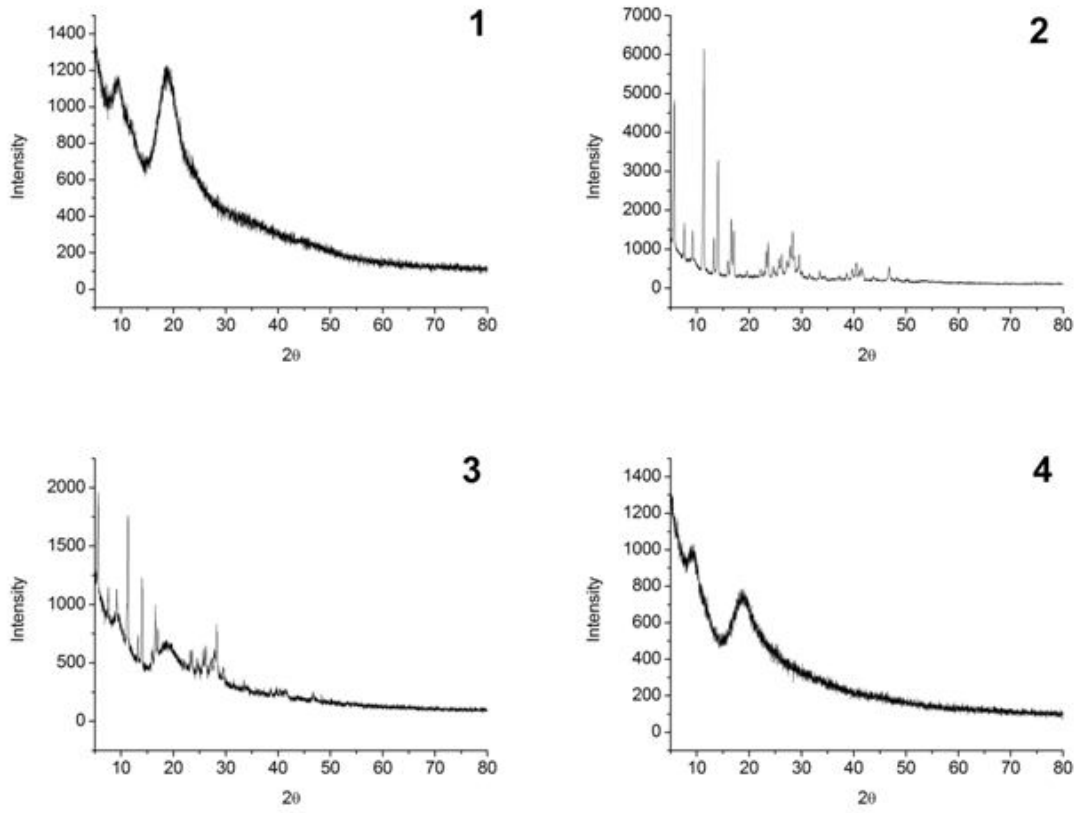

Figure 4: XRD patterns of HP-β-CD (1), myricetin (2), their physical mixture (3) and inclusion complex (4)

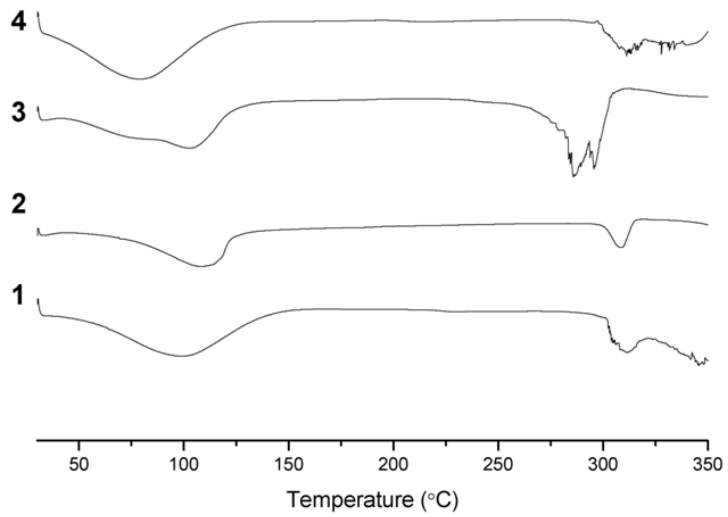

Figure 5: DSC thermograms of HP-β-CD (1), myricetin (2), their physical mixture (3) and inclusion complex (4)

DISCUSSION

In this study, the complexes of myricetin and HP-β-CD were prepared to improve the hydrophilic property of myricetin in water and ethanol, respectively. It was found that the content of myricetin (3.71 %) in the complex prepared in ethanol was higher than that prepared in water (1.43 %), which suggested that ethanol is the optimal solvent to prepare the complex of HP-β-CD.

Based on UV and IR spectra, no new peaks were observed in the mixture and complex, which suggested that myricetin and HP-β-CD in the complex were combined by non-covalent bond, and some weak physical interactions between myricetin and HP-β-CD could take place during

the formation of the complex. The difference in the electron micrographs of the physical mixture and complex suggested that when the powders of myricetin and HP-β-CD were simply mixed together, they formed no close association and continued to exist in their original individual forms, whereas when the solutions of the two compounds were freeze-dried, they formed a close association, probably in the form of inclusion complex, in which myricetin no longer exist in the crystal state.

In this study, the sharp peaks of the XRD pattern of the physical mixture indicated the retention of the crystalline structure of myricetin in the physical mixture. However, the complex of myricetin with HP-β-CD gave twp large, broad background under the crystalline peaks, which was similar to that of the amorphous HP-β-CD and did not exhibit the characteristic peaks of myricetin, indicating the formation of a significant amount of amorphous material.

The DSC thermogram of the complex exhibited mainly the features of HP-β-CD while the characteristic endothermic peaks of myricetin disappeared entirely, suggesting that an association structure was formed between the two molecules. According to an earlier report [6], it was found that dihydromyricetin was completely dispersed in lecithin and that there were some interactions, possibly a combination of hydrogen bonds and van der Waals force.

CONCLUSION

By forming complex with HP-β-CD, the hydrophilic property of myricetin is significantly enhanced. The complex is combined via a non-covalent-bond, and no new compound is formed. Myricetin in the complex is molecularly dispersed in HP-β-CD matrix.

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