

Original Research Article

Physicochemical Properties of Diosmetin and Lecithin Complex

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Abstract

Purpose: To improve the hydrophobicity of diosmetin, the complex of diosmetin and lecithin was prepared and its physicochemical properties were characterized in this study.

Methods: The complex of diosmetin and lecithin was prepared by solvent method. The physicochemical properties of the complex were investigated by ultraviolet-visible spectrometry (UV/Vis), infrared spectrometry (IR), scanning electron microscopy (SEM), differential scanning calorimetry (DSC) and x-ray diffractometry (XRD).

Results: Based on UV and IR analysis, there was no significant difference between the physical mixture and the complex while SEM showed that diosmetin in the complex did not present a crystal appearance but rather was dispersed in lecithin. DSC thermograms of the complex mainly showed that the presence of lecithin caused the characteristic endothermic peaks of diosmetin to disappear while x-diffractograms showed that the crystalline peaks of diosmetin was absent in the complex.

Conclusion: The complex is held together by non-covalent-bond, and thus a new compound was not formed. Diosmetin in the complex is molecularly dispersed in the lecithin matrix.

Keywords: Diosmetin, Lecithin, Complex, Physicochemical, Non-covalent bond

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INTRODUCTION

Diosmetin (3',5,7-trihydroxy-4'-methoxyflavone) is a bioflavonoid found in spearmint, oregano, and many other plants. Like all bioflavonoids, diosmetin helps maintain normal capillary permeability and fragility [1-3]. But for its poor solubility in oil, the application of diosmetin in functional food and medicine is restricted. The low hydrophobicity of diosmetin could result in its poor permeation across the intestinal epithelial cells and reduce the gastrointestinal tract absorption, which could decrease its oral bioavailability. Lecithin is a group of yellow-brownish fatty substances occurring in animal and plant tissues, and in egg yolk [4]. It has been used as a matrix to improve the lipophilic

property and targeted delivery of bioactive compounds [5,6]. It is expected that diosmetin combined with lecithin might result in the improvement of its lipophilic property. In this study, the complex of diosmetin with lecithin was prepared for the first time, and the physicochemical properties of the complex investigated.

EXPERIMENTAL

Materials

Diosmetin (98 %) was obtained from Shaanxi Huike Botanical Development Co, Ltd, (Xi'an, China). Lecithin from soya bean was a product of

Sangon (Shanghai, China). Other chemical were of analytical grade.

Preparation of diosmetin-lecithin complex

Diosmetin (100 mg) and lecithin (200 mg) were dissolved in 100 ml of tetrahydrofuran and stirred for 2 h. After tetrahydrofuran was removed under vacuum, the residue was collected and pulverized. The resultant light yellow powder was collected as diosmetin-lecithin complex.

Preparation of physical mixture of diosmetin and lecithin

Diosmetin (200 mg) and lecithin (400 mg) were mixed and stirred in a small beaker at room temperature. The product obtained was collected as the physical mixture of diosmetin and lecithin.

Ultraviolet-visible (UV) and infrared spectroscopy (IR)

UV spectra were recorded for lecithin, diosmetin, their physical mixture and complex using a model TU1810 scanning UV spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., Beijing, China). Each sample was dissolved in methanol. The absorbance of each solution was scanned in the wavelength range of 220 - 500 nm to obtain the UV spectra. IR spectra of lecithin, diosmetin, their physical mixture and complex were collected between 4000 and 400 cm^{-1} on a TENSOR 27 infrared spectrophotometer (Bruker, Germany) with 256 scans at a resolution of 4 cm^{-1} by the KBr method. The data were recorded and processed by the OPUS software (Bruker, Germany) supplied with the instrument.

Scanning electron microscopy (SEM)

Analysis with scanning electron micrographs (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.

Differential scanning calorimetry (DSC)

The samples sealed in the aluminum crimp cell were heated at the speed of 10 $^{\circ}\text{C}/\text{min}$ from 30 to 300 $^{\circ}\text{C}$ in an atmosphere of nitrogen (50 ml/min using a DSC equipment (Q200, TA, USA). The data were recorded and processed by Universal Analysis 2000 software (TA, USA).

X-ray diffractometry (XRD)

Monochromatic Cu K α radiation (wavelength = 1.54056 A°) was produced by a D8 Advance X-ray diffractometer (Bruker, Germany). The powdery samples were packed tightly in a rectangular aluminum cell prior to exposure to the X-ray beam. The scanning regions of the diffraction angle, 2θ , were 5 - 70 $^{\circ}$ and radiation was detected with a proportional detector.

RESULTS

UV and IR characteristics

The UV spectra of lecithin, diosmetin, their physical mixture and the complex are shown in Figure 1. There was no difference between the physical mixture and the complex. The characteristic absorption peaks of diosmetin were still present at 253, 269, 344 nm. The infrared spectra of lecithin, diosmetin, their physical mixture and the complex are shown in Figure 2. There was no significant difference between the physical mixture and the complex. The spectra of the physical mixture and the complex showed an additive effect of diosmetin and lecithin. In IR analysis, no new peaks were observed in the mixture and complex.

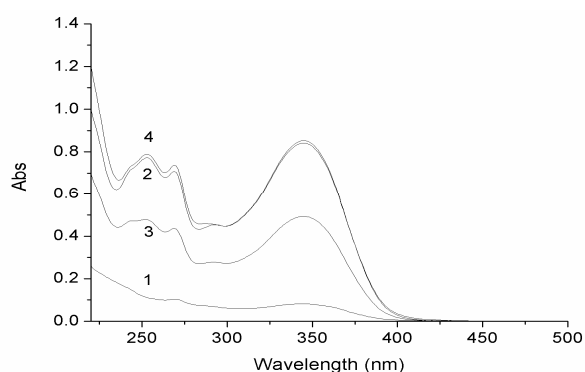


Figure 1: UV spectra of lecithin (1), diosmetin (2), their physical mixture (3) and complex (4)

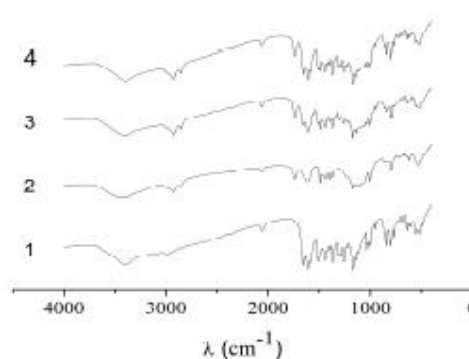


Figure 2: IR spectra of lecithin (1), diosmetin (2), their physical mixture (3) and complex (4)

SEM analysis

The surface morphology of the complex as examined by SEM is shown in Figure 3. It could be found that diosmetin did not present in the appearance of crystal but dispersed in lecithin.

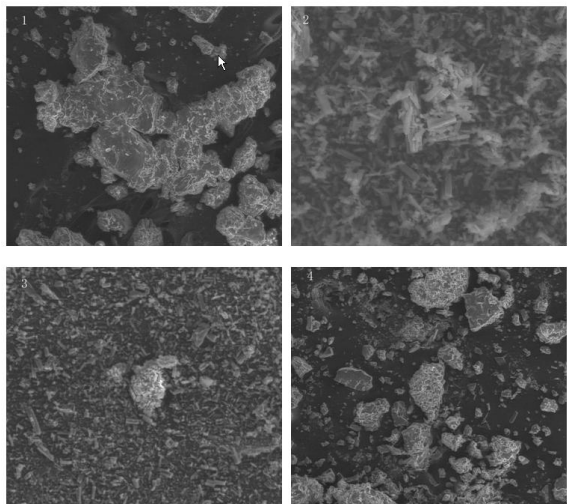


Figure 3: Scanning electron micrographs of lecithin (1), diosmetin (2), their physical mixture (3) and complex (4)

Thermal characteristics

Figure 4 shows the DSC thermograms of lecithin, diosmetin, their physical mixture and the complex. Diosmetin thermogram showed an endothermic peak with onset temperature at about 256 °C, which was attributed to the melting of diosmetin. The thermogram of the physical mixture mainly showed the effect of diosmetin and lecithin. But the thermogram of the complex mainly showed the effect of lecithin, in which the characteristic endothermic peaks of diosmetin disappeared.

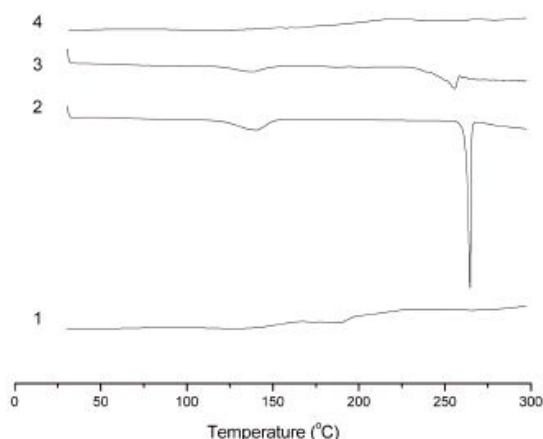


Figure 4: DSC curves of lecithin (1), diosmetin (2), their physical mixture (3) and complex (4)

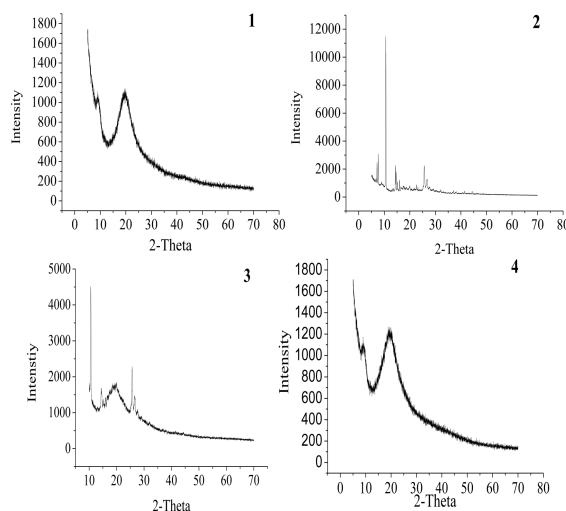


Figure 5: X-ray diffraction patterns of lecithin (1), diosmetin (2), their physical mixture (3) and complex (4)

XRD analysis

The powder X-ray diffraction patterns of diosmetin, lecithin, their physical mixture and the complex are shown in Figure 5. The powder diffraction pattern of diosmetin displayed sharp crystalline peaks, which is the characteristic of a crystalline organic molecule [7]. In contrast, lecithin showed amorphous property lacking crystalline peaks. Compared with that of the physical mixture, the crystalline peaks disappeared in the complex. This suggests that diosmetin in the lecithin matrix was molecularly dispersed. However, some crystalline drug signals were still detectable in the physical mixtures of lecithin and diosmetin.

DISCUSSION

Diosmetin-lecithin complex has been prepared using different ratios of lecithin to diosmetin, such as 1/10, 1/2, 1/1, and 2/1. The results showed that when the ratio of lecithin to diosmetin was lower than 1, the stability of the complexes was low. In order to get optimal combinations using the lowest acceptable quantity of diosmetin, the quantity ratio of lecithin to diosmetin in the complex was determined as 2. The obtained complex was used for the following structural analysis. The complex obtained was easy to dissolve in oil, which means that the lipophilic property of diosmetin was significantly improved by complexing with lecithin. UV and IR data indicate that there was no difference between the physical mixture and the complex, all the maximum absorption peaks of the samples were all at 253, 269, 345 nm, which suggests that weak physical interactions between diosmetin and lecithin were involved in the formation of the complex. SEM indicates that lecithin appeared as

amorphous while diosmetin existed in rectangular crystals. In the scanning electron micrograph of the physical mixture of the two powders, both the characteristic crystals of diosmetin and the amorphous spheres of lecithin were found. In contrast, the complex appeared as irregular shapes in which the original morphology of diosmetin disappeared. These images further demonstrate that when the powders of diosmetin and lecithin were simply mixed together, there was no close association between them and they continued to exist in their original individual forms, whereas when the complex was prepared, they formed a close association, in which diosmetin no longer existed in the crystal state.

The thermogram of the physical mixture of diosmetin and lecithin exhibited combined characteristics of the curves of diosmetin and lecithin, indicating that no close association formed between the two samples when the two powders were simply mixed together. In contrast, the thermogram of the complex exhibited mainly the features of lecithin thermogram while the characteristic endothermic peaks of diosmetin disappeared. According to a previous study [8], it was stated that diosmetin completely dispersed in lecithin and that some interaction occurred, possibly a combination of hydrogen bonds or van der Waals force.

Powder x-ray diffractometry is a useful method for the detection of compound complexation in powder or microcrystalline states. The diffraction pattern of the complex is supposed to be clearly distinct from that of the superposition of each of the components [9]. The formation of the complex between lecithin and diosmetin means that the latter might have lost its crystalline nature and, consequently, the diffraction pattern of the complex would not be a simple superposition of those of the two components [10]. The diffractogram of lecithin showed one broad peaks, consistent with its amorphous character, whereas numerous sharp, intense peaks appeared in the diffractogram of diosmetin, confirming its crystalline nature. The diffractogram of the physical mixture of the powder of diosmetin and lecithin showed essentially a superposition of the patterns of the two compounds, confirming that no association was formed between them and both retained their original physical characteristics. In contrast, the diffractogram of the complex was virtually the same as that of the amorphous lecithin and exhibited none of the characteristic peaks of diosmetin. According to the previous report [4],

this suggested that diosmetin in the lecithin matrix was molecularly dispersed.

CONCLUSION

By forming a complex with lecithin, the lipophilic solubility of diosmetin is significantly enhanced. The physicochemical analysis shows that the complex was formed via non-covalent-bonds with diosmetin in the complex molecularly dispersed in lecithin matrix, and thus a new compound was not formed. The complex obtained can be used in medicine and functional food.

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