Preparation and Characterization of Hesperidin - PEG 6000 Complex

[1] Haiyan Gao*, [2] Yuzhen Chen, [1] Hanjun Ma, [1] Jie Zeng and [1] Guanglei Li
[1]*School of Food Science, Henan Institute of Science and Technology, Xinxiang 453003, China.*
[2]*School of Mathematical Sciences, Henan Institute of Science and Technology, Xinxiang 453003, China.*
gaohaiyan127@163.com*

(Received on 17th January 2014, accepted in revised form 26th May 2014)

Summary: In this study, the preparation and characterization of hesperidin-PEG 6000 solid dispersion were investigated. The ultraviolet-visible spectrometry (UV), infrared spectrometry (IR), X-ray diffractometry (XRD) and Differential scanning calorimetry (DSC) were used to analyze the physicochemical properties of the complex. By forming the complex with polyethylene glycol 6000 (PEG 6000), the solubility of hesperidin in water could be significantly improved. UV spectra and IR spectrum showed that there was no obvious difference between hesperidin and the complex. The XRD pattern of the complex suggested that hesperidin in the matrix was either molecularly dispersed or in amorphous form. DSC analysis implied that hesperidin had been completely dispersed in PEG6000 and there were some interaction between hesperidin and PEG 6000. The results showed that hesperidin in the complex had been completely dispersed in the PEG 6000, not forming a new compound.

Keywords: Hesperidin, Polyethylene glycol, Complex, Characterization.

Introduction

Hesperidin is one of the most abundant natural flavonoids and naturally exists in the rinds of citrus or other plants. Hesperidin can be extracted from the rinds of the citrus species by some solvents [1, 2]. Hesperidin have many biological effects, such as anti-inflammation, antimicrobial properties, anticarcinogenic activities, antioxidant effects [3, 4]. The biological activities of hesperidin cover a broad spectrum, from anticancer and antibacterial activities to inhibition of bone reabsorption and neuroprotection effect [5]. Hesperidin is the main ingredient of several traditional Chinese medicines [6, 7], such as effects on the vascular deseases, inflammation and inhibition of platelet aggregation [8]. It was reported that hesperidin protects acetaminophen-induced hepatic stress [9, 10]. Due to its poor solubility in water, the hesperidin was less used in food industries and pharmaceuticals industries. Some evidences indicated that hesperidin had important roles on health of human beings, but little was known about its mechanism [11]. Polyethylene glycol 6000 (PEG) is used in Clinical Biochemistry as a precipitant [12, 13]. Therefore, the present study aimed to improve dissolution characteristics of hesperidin solid dispersions by adding PEG 6000, and analyze physicochemical properties of the complex of hesperidin - PEG 6000.

Results and Discussion

Solubility

A, B, C were the complex samples of hesperidin and PEG 6000. D, E, F was the mixture samples of hesperidin and PEG 6000. The proportion of hesperidin and PEG 6000 was described in the bracket.

Table-1 showed the solubility of complex and mixture of hesperidin and PEG 6000. The solubility of hesperidin in water is 0.019 mg/mL at 25 °C. The solubility of complex of hesperidin and PEG 6000 increased more than that of hesperidin. The highest was obtained by the treatment C (1:20) and the solubility was up to 0.399 mg/mL. Zerrouk *et al* [14] reported that an increase in carbamazepine-PEG6000 solubility was found, but the solubility was still less than 200 mg/L. Therefore, the solubility of complex of hesperidin and PEG 6000 would have broad prospects in pharmaceuticals industry.

Table-1: Solubility of complex and mixture of hesperidin and PEG 6000 (mg/mL, 25 °C).

Hesperidin	Complex			Mixture		
	A (1:2)	B (1:10)	C (1:20)	D (1:2)	E (1:10)	F (1:20)
0.019	0.089	0.089	0.399	0.076	0.076	0.019

The solubility of hesperidin and PEG 6000 mixture D (1:2) and E (1:10) slightly increased while the solubility of sample F (1:20) was the same as the hesperidin.

*To whom all correspondence should be addressed.

UV Analysis

Fig. 1 showed the UV spectra of PEG6000, hesperidin and their complex and mixture samples. There was no difference in hesperidin, complex and mixture samples in UV analysis and their characteristic absorption wavelengths were present at 285 nm. PEG6000 had no absorption in the range of 200 - 400 nm.

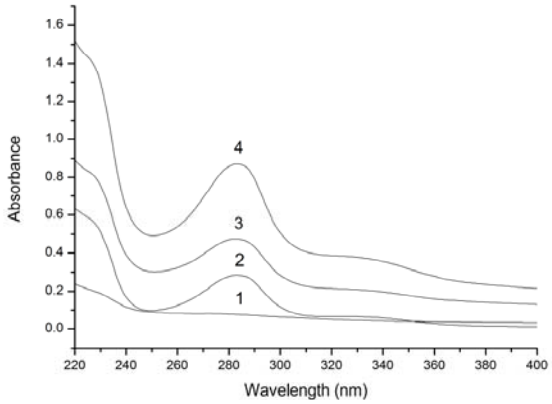


Fig. 1: UV spectra of PEG 6000, hesperidin, complex and mixture.

(1. PEG6000; 2. hesperidin; 3. complex; 4. mixture)

IR analysis

The IR spectra of PEG 6000, hesperidin and their complex and mixture samples were shown in Fig. 2. The IR spectrums of the complex are similar to that of PEG 6000. This might be attributed to the low quantity of hesperidin in the complex samples. And in the IR spectrum of their complex and mixture samples, some small characteristic absorption peaks of hesperidin between 500 and 1500 cm^{-1} were almost masked by PEG 6000. In IR analysis, no new significant peaks were observed in the complex samples compared with that of PEG 6000. These results indicated that there were some weak physical interactions between hesperidin and PEG 6000 during the formation of their complex samples.

Fig. 3 showed the XRD patterns of hesperidin, PEG 6000 and their complex and mixture samples. The XRD pattern of PEG 6000 showed two broad peaks, consistent with its structural character, whereas the XRD peak of hesperidin was not significant. In contrast, the XRD pattern of the complex samples was virtually similar to that of PEG 6000, which suggested that hesperidin had lost its crystalline structure during forming complex samples. So hesperidin was either molecularly dispersed or in amorphous form in the matrix, while the mixture showed the XRD pattern of both PEG 6000 and hesperidin, indicating that the crystalline structure of the hesperidin was not changed when present in a physical mixture [15].

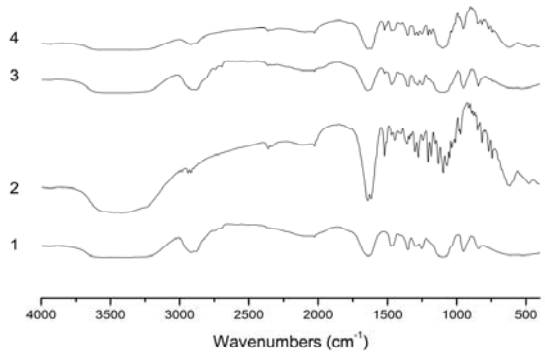
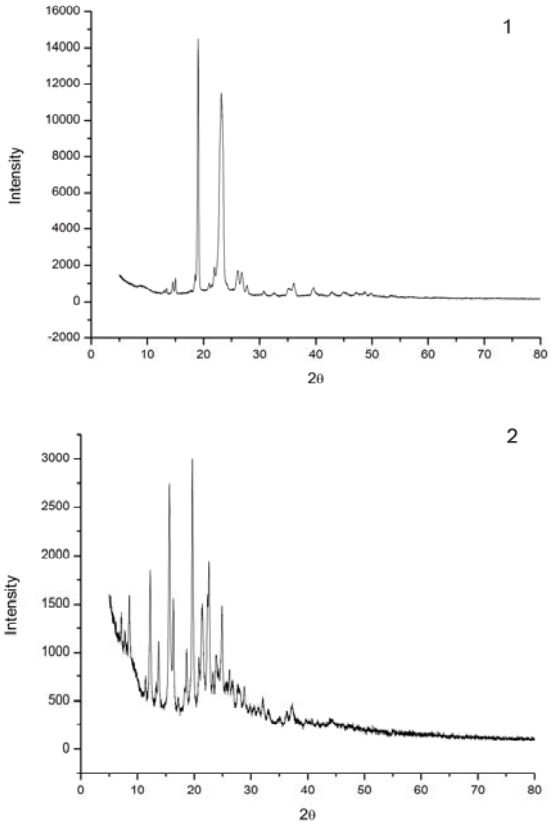


Fig. 2: IR spectra of PEG 6000, hesperidin, complex and mixture.

(1. PEG6000; 2. hesperidin; 3. complex; 4. mixture)

XRD Analysis



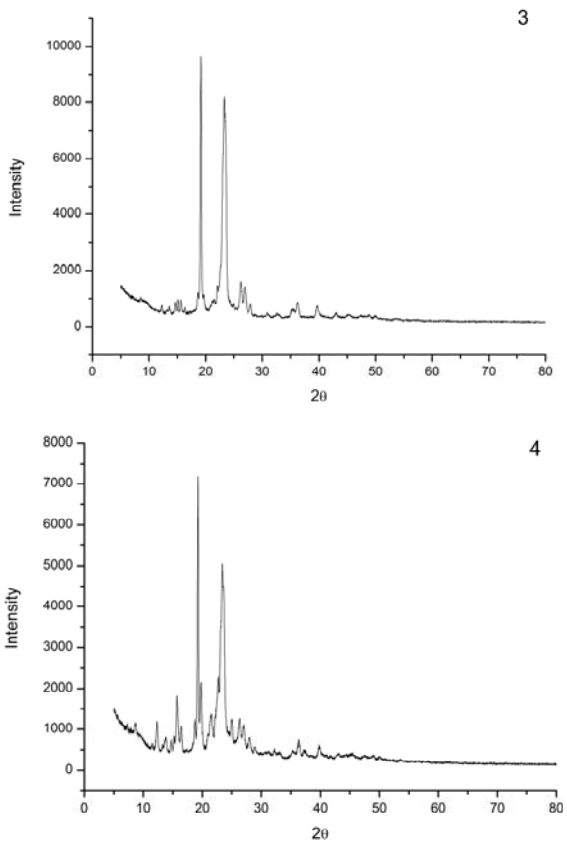


Fig. 3: X-ray diffraction patterns of PEG 6000, hesperidin, complex and mixture.

(1. PEG6000; 2. hesperidin; 3. complex; 4. mixture)

DSC Analysis

Fig. 4 showed the DSC curves of PEG 6000, hesperidin and their complex and mixture samples. Two endothermal peaks were obtained from the the DSC curve of hesperidin. The first endothermal peak with onset at 103 °C could be due to the removal of crystal water from hesperidin molecules. The second endothermal peak with onset temperature at 252 °C was due to the melting of hesperidin. Three endothermal peaks was obtained in the DSC curve of the mixture sample and this might be attributed to the effect of PEG 6000 and hesperidin. But there was only one peak in the DSC curve of the complex and similar to that of PEG 6000. This indicated that the characteristic endothermal peaks of hesperidin disappeared in the complex samples. According to the above results, it implied that hesperidin had been completely dispersed in PEG 6000, and the combination of hydrogen bonds or van der Waals force might happened during their interaction.

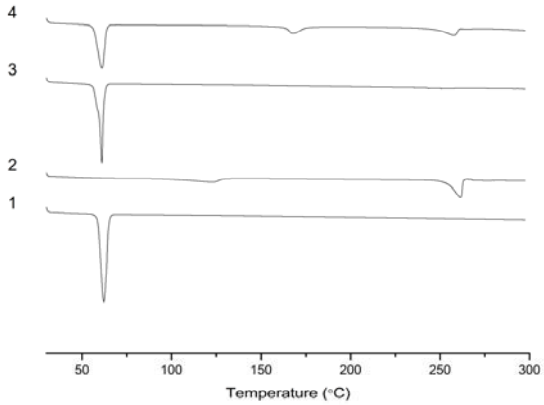


Fig. 4: DSC curves of PEG 6000, hesperidin, complex and mixture.

(1. PEG6000; 2. Hesperidin; 3. Complex; 4. Mixture)

Experimental

Materials and Chemicals

Hesperidin (purity 97%), polyethylene glycol (PEG) 6000 and other chemical were of analytical grade and purchased from Aladdin Reagent Co. Ltd. (Shanghai, China).

Preparation of Hesperidin - PEG 6000 Solid Dispersion

Hesperidin was mixed with melting PEG 6000 at 70 °C and stirred for 1 h. The obtained sample was cooled, ground and collected as hesperidin - PEG 6000 complex. According to our previous research, the proportion of hesperidin and PEG 6000 was 1:2, 1:10 and 1:20.

Preparation of Hesperidin - PEG 6000 Mixture

Hesperidin was mixed with PEG 6000 and ground by a triturator. The obtained sample was collected as hesperidin - PEG 6000 mixture. The proportion of hesperidin and PEG 6000 was 1:2, 1:10 and 1:20.

Solubility

Solubility of hesperidin, its complex and mixture was measured by adding excess of the sample to 5 mL of water at 25 °C. The obtained samples were agitated for 24 h, and then centrifuged

(15 min, 4000 rpm). The supernatant was diluted 20 times and determined the absorbance at 285nm. The content of hesperidin in the supernatant was calculated according to the equation (1).

$y=0.0165x+0.0679$ ($R^2=0.9986$) (1)

Note: y-absorbance; x-content of hesperidin.

UV analysis

0.02 g sample was dissolved by polyethylene glycol and transfered into a 100 mL volumetric flask and diluted with polyethylene glycol to 100 mL. UV analysis was performed on a TU-1810PC UV-visible spectrophotometer (Purkinje, China). The sample was scaned from 200 nm to 400 nm.

IR analysis

IR analysis was performed on a TENSOR 27 infrared spectrophotometer (Bruker, Germany) by the KBr method.

X-ray Diffractometry

Monochromatic Cu Ka radiation (wavelength = 1.54056 A˚) was produced by a D8 Advance X-ray diffractometer (Bruker, Germany). All the samples were packed tightly in a rectangular aluminum cell, and exposed to the X-ray beam. The scanning ranges of the diffraction angle (2θ) were 5-80°. Radiation was detected with a proportional detector.

Differential Scanning calorimetry (DSC)

Differential scanning calorimeter (Q200, TA, and USA) was used to determine the thermal properties of the hesperidin, complex and mixture samples. The samples were sealed in the standard aluminum cell. The heating rate was at 10 °C / min over the temperature range 30 - 300 °C in the atmosphere of nitrogen (flow rate 50 mL/min). The graph was obtained and analysed by TA Universal Analysis 2000 software (TA, USA).

Conclusion

The solubility of the complex formed by hesperidin and PEG 6000 in water was significantly increased. The highest solubility was obtained in the complex sample with the proportion of hesperidin and PEG 6000 1:20 and the solubility increased from 0.019 to 0.399 mg/mL. The UV, IR, DSC, XRD analysis showed that hesperidin could be completely dispersed in the PEG 6000 matrix during forming complex samples and not forming a new compound. In view of its solubility in water, the application of the hesperidin - PEG 6000 complex in food and medicine industries could be expected.

Acknowledgements

This study was funded by Program for Innovative Research Team (in Science and Technology) in University of Henan Province (13IRTSTHN006).

References

1. C. Jungsook. *Archives of Pharmacal Research*, **29**, 699 (2006).
2. F. Ding, Y. Sun, J. X. Diao, X. N. Li, X. L. Yang and Y. Sun. *Photochemistry and Photobiology*, **106**, 53 (2012).
3. A. Garg, S. Garg, L. J. Zaneveld and A. K. Singla. *Phytotherapy Research*, **15**, 655 (2001).
4. M. Hussein and S. Othman. *International Journal of Pharmaceutical Research and Development*, **3**, 19 (2011).
5. N. G. Amado, D. M.Cerqueira and F. S. Menezes. *Anticancer Drugs,* **20**, 543 (2009).
6. A. Crozier, I. B.Jaganath and M. N. Clifford. *Natural Product Reports,* **26**, 1001 (2009).
7. B. Ameer, R. A. Weintraub, J. V. Johnson, R. A. Yost and R. L. Rouseff. *Clinical Pharmacology and Therapeutics*, **60**, 34 (1996).
8. H. Y. Yu, S. W. Park, I. M. Chung and Y. S. Jung. *Food Chemistry Toxicology*, **49**, 3018 (2011).
9. S. T. Ahmad, W. Arjumand, S. Nafees, A. Seth, N. Ali, S. Rashid and S. Sultana. *Toxicology Letters*, **208**, 149 (2012).
10. P. Selvaraj and K. V.Pugalendi. *Experimental and Toxicologic Pathology*, **64**, 449 (2012).
11. Z. Rong, R. Pan, Y. Xu, C. Zhang, Y. Cao and D. Liu, *Neuroscience*, **255**, 292 (2013).
12. L. Boughen, J. Liggat and G. Ellis. *Clinical Biochemistry*, **43**, 750 (2010).
13. S. Janssens, H. Novoa de Armas, W. D'Autry, A. V. Schepdae and G. V. den Mooter. *Eurpean Journal of Pharmaceutics and Biopharmaceutics*, **69**, 1114 (2008).
14. N. Zerrouk, C. Chemtob, P. Arnaud, S. Toscani and J. Dugue. *International Journal of Pharmaceutics*, **225**, 49 (2001).
15. J. O. Eloy and J. M. Marchetti. *Powder Technology,* **253***,* 98 (2014).