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Utilization of Supercritical Carbon Dioxide for the Preparation of 3-Hydroxyflavone and β-Cyclodextrin Complex

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Abstract

The solubility of 3-hydroxyflavone in water can be enhanced by forming complex with β-cyclodextrin (β-CD). In this study we introduced supercritical carbon dioxide as a substitute solvent to prepare 3-hydroxyflavone-β-cyclodextrin complex. The maximum drug loading determined by UV spectroscopy in complex was 14.7 wt.%, which was comparable to that of 1:1 complex (17.34 wt.% of 3-hydroxyflavone). The solubility of the drug loaded in the complex was better than that of pure drug in water. The enhanced solubility was attributed to the formation of the complex and the solubility of the β-cyclodextrin in water.

Introduction

Flavonoids such as flavone and 3-hydroxyflavone have been attracting much attention in pharmacology and related industries, because clinical and biological studies have indicated that flavonoids may play a significant role in the prevention of many forms of disease [1]. But the poor solubility of them in water limited their use in many aspects. In the pharmaceutical industry, many drugs have similar disadvantage, so various methods have been developed to increase the solubility of the drugs in water, which include micronisation, modification of the physicochemical properties of the drug, and formation complex with cyclodextrin and their derivatives.

Cyclodextrins (CDs) are cyclic oligosaccharides produced by an enzymatic degradation of starch by a glucosyltransferase derived from Bacillus macerans [2]. Cyclodextrin are water-soluble due to the hydroxyl groups which located on the outside surface of rings. However, its unique character is that its internal cavity is relatively non-polar, which allow some guest molecules with appropriate size to be included fully or partially in the hydrophobic cavity [3–5]. The formation of inclusion may improve the solubility and bioavailability of the guest molecule [6].

Several techniques have been used to prepare complex, which include grinding, kneading, coprecipitating and freeze drying [7–10]. However, all of them have disadvantages, such as time-consuming, necessitate multistage processing and residual solvent in the product. Recently, supercritical fluids (SCF) were used to prepare complex between β-CD and non-polar drugs [11–13]. SCF have gaslike viscosities, diffusivities, and promoting mass transfer, while its densities are similar to that of liquid solvent [14, 15]. The solvent power can be manipulate by adjusting the temperature and pressure. Supercritical carbon dioxide is the most widely used SCF due to its relatively low critical value and moderate critical pressure. Moreover, it is non-toxic, non-flammable, and inexpensive. Furthermore, it is easily removed from the system after reaction and proceeding [16, 17].

In this paper we studied the feasibility of preparing complex between 3-hydroxyflavone and β-CD using supercritical carbon dioxide ($SCCO_2$) as the solvent. The solubility of the drug loaded in the complex was determined and the result indicated that the drug loaded in the complex has higher solubility in water than the pure drug does. The formation of the complex was confirmed by XRD and DSC and the effect of operating pressure and contacting time on the complex formation was also studied.

Experimental

Materials

Both 3-hydroxyflavone (99.8% purity) and β-cyclodextrin were purchased from Aldrich. Acetonitrile (HPLC grade) was purchased from Beijing Chemical Reagent Factory. Carbon dioxide (>99.9%) was supplied by Beijing Analytical Instrument Factory.

Experimental apparatus and procedure

The experimental apparatus for preparing the complex is illustrated in Figure 1. It consisted mainly of a high-

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pressure view cell with volume of 40 cm^3, a constant temperature water bath, a high-pressure syringe pump (DB-80), a pressure gauge, a magnetic stirrer, and a gas cylinder. The temperature of the water bath was controlled by a Haake-D3 temperature controller, and the temperature fluctuation of the water bath was less than 0.1 °C. The pressure gauge was composed of a pressure transducer (FOXBORO/ICT, Model 93) and an indicator, which was accurate to 0.025 MPa in the range of 0–20 MPa. A tube with ethanol was used as a cold trap.

In a typical experiment, the 3-hydroxyflavone and β-CD were loaded on the bottom and in the middle of the cell, respectively. The two reactants were separated by a board covered with filter paper to prevent the contact before the reaction. After thermal equilibrium had reached, the valve 3 was opened and the liquid CO_2 was charged into cell to a desired pressure, then the valve 3 was closed and the pressure of the system was kept constant for a period of time, which was called as contacting time. When the reaction ended, the valve 6 was opened, the system was depressurized to atmospheric pressure and the mixture was obtained.

Characterization of the complex

Solubility experiments were performed for pure 3-hydroxyflavone and the complex formed by $SCCO_2$. For the complex, the sample was dissloved in water and the solution was passed through a 0.45 μm filter after stirring 8 h, then solution was dried under vacuum (70 °C, 700 mm Hg) and white powder was obtained. Then the powder was dissolved in acetonitrile and the concentration of 3-hydroxyflavone was calculated by detecting the absorbance at 408.4 nm in UV spectroscopy. The Solubility experiment of the pure 3-hydroxyflavone was performed with similar procedure.

X-ray power diffraction (XRD) analysis was carried out by a D/MAX.RB diffraction-meter (made in Japan, Rigaku) with CuKα radiation ($\lambda = 0.154$ nm) at 40 kV and 200 mA. The scanning speed was 2°/min, the step was 0.02°, and the beginning 2θ was 2°.

A matter TC11 TA processor DSC apparatus was used for the thermal analysis of the complex. Sample (6–15 mg) was scanned between 60 and 230 °C under a nitrogen gas stream at the heating rate of 10 °C/min.

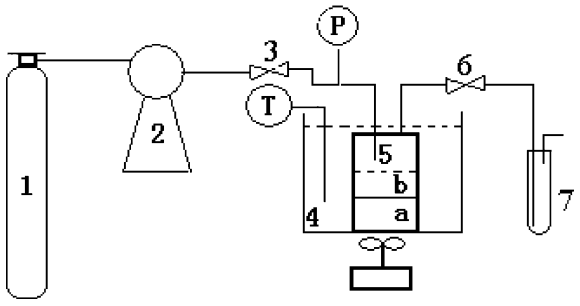

Figure 1. Schematic diagram of the apparatus for the reaction 1. Gas cylinder, 2. high-pressure syringe pump, 3 and 6. valve, 4. water bath, 5. high-pressure view cell, 7. cold trap, a. 3-hydroxyflavone, b. β-CD.

Results and discussion

In the experiment of solubility of the complex in water, The UV curve of complex is shown in Figure 2. The absorption peak at 408.4 nm is the representative absorption peak of the 3-hydroxyflavone. The spectroscopy of the β-CD does not interfere with the spectroscopy of the complex because β-CD does not have absorbance at that wavelength. Through the calibrated curve we can calculate that the drug loading in complex is 14.7 wt.%, which is smaller than that of 1:1 complex (17.34 wt.% of 3-hydroxyflavone). But in the experiment of solubility of the pure 3-hydroxyflavone in water, we did not detect absorbance at 408.4 nm in UV spectroscopy, this evidence also indicated that pure 3-hydroxyflavone has little solubility in water. The formation of the complex is an equilibrium process which loading and unloading occurred simultaneously. We investigated the different drug loading in different time and conclude that the drug loading has reached the maximum in our experimental condition.

In addition to the above results, we also use X-ray diffraction to character the product. X-ray diffraction can give useful information about complex of CDs and other substances [18, 19]. The X-ray diffraction patterns of pure 3-hydroxyflavone and β-CD were shown in Figure 3a and b, respectively. It can be seen that there are some crystalline structures in the two samples. But when they form complex, the XRD patterns of the sample changed obviously, as shown in Figure 3c. The disappearance of the 3-hydroxyflavone diffraction patterns may be credited to the formation of the inclusion complex, in which 3-hydroxyflavone was entrapped in the β-CD cavity.

Typical DSC thermograms of the both physical mixture and the complex are depicted in Figure 4. The sharp peak in the DSC curve of the physical mixture (Figure 4a) represented the melting of 3-hydroxyflavone. For the complex (Figure 4b), the endothermic peak of 3-hydroxyflavone disappeared despite it has proximately 15 wt.% of 3-hydroxyflavone. This evidence may

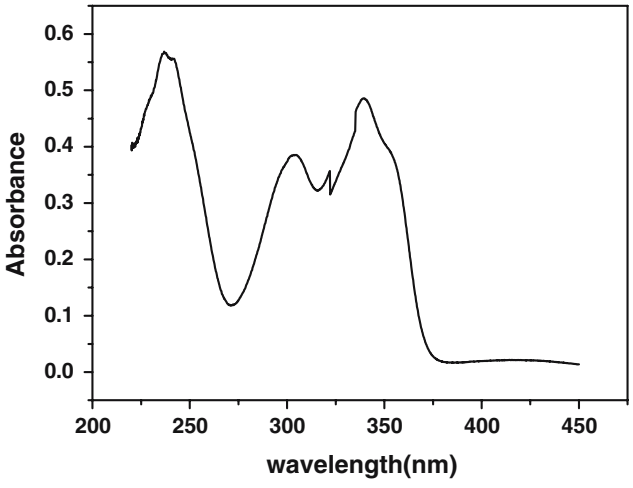

Figure 2. The UV curve of the complex in acetonitrile.

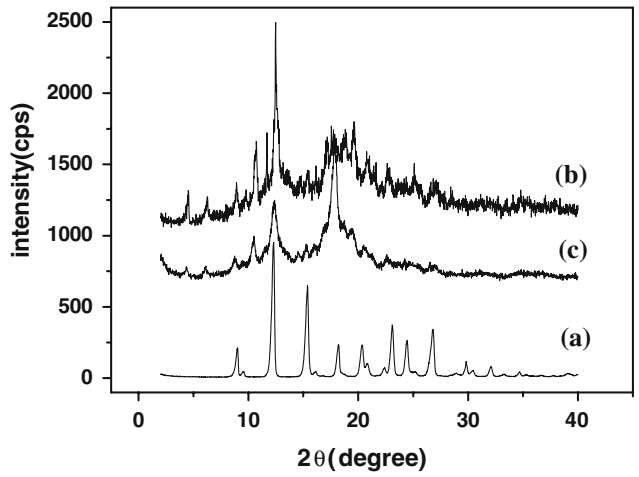

Figure 3. XRD patterns of (a) pure 3-hydroxyflavone (b) pure β-CD (c) the complex.

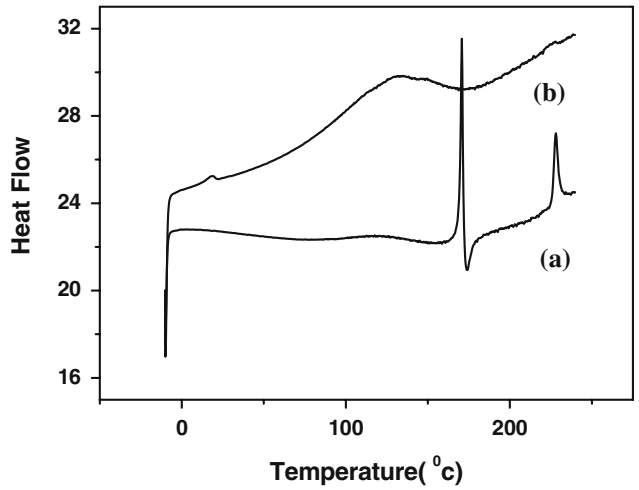

Figure 4. DSC thermograms of (a) the physical mixture (b) complex formed by $SCCO_2$.

be attributed to the transformation of β-CD into an amorphous state or the formation of complex, or a combination of the both phenomena. The disappearance of the endothermic peak in the complex may indicate that there was no crystalline 3-hydroxyflavone in the complex.

The effect of the contacting time and pressure on the drug content in complex was also investigated. The contacting time varied from 2 to 5 h and the pressure was maintained at 16 MPa. The results are listed in Table 1. It can be seen that the contacting time has no obvious effect on drug content in the complex. This

Table 1. Effect of the contacting time on the drug content

Contacting time (h)	Wt.% of 3-hydroxyflavone in the complex
2	14.7
3	13.9
4	15.2
5	14.0

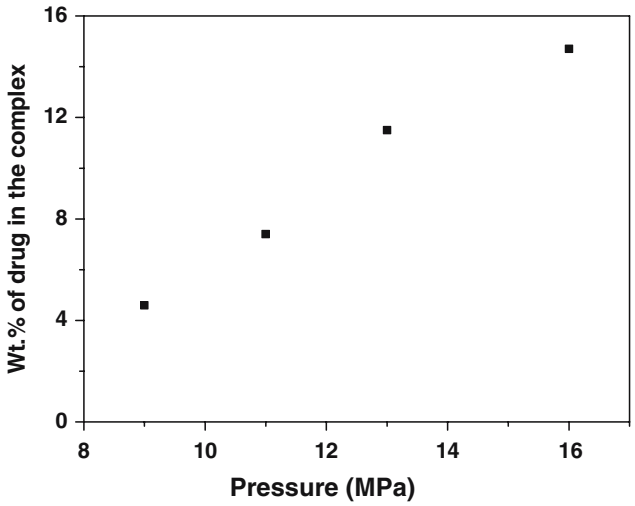

Figure 5. Effect of the pressure on the drug content loaded in β-CD at 40 °C and 2 h contacting time.

result indicated that mass transfer was not a limiting factor for the content of 3-hydroxyflavone loaded in β-CD. Perhaps contact areas affected mostly the content of the drug in the complex.

The effect of pressure on the drug content in complex was investigated between 9 and 16 MPa. The contacting time and temperature was kept constant at 2 h and 40 °C, respectively. As shown in Figure 5, increasing the pressure causes higher drug content in the complex. The concentration of 3-hydroxyflavone in the supercritical solution was increased as the pressure increase [20]. In addition, the β-CD becomes amorphous due to higher amount of CO_2 dissolved into β-CD matrix at higher pressure [21]. We conclude that both the higher amount of 3-hydroxyflavone and more contact areas cause the higher 3-hydroxyflavone content in the complex.

Conclusions

A complex between 3-hydroxyflavone and β-CD was prepared in $SCCO_2$. This process was free of organic solvent and the maximum drug content in this work was approximately 15 wt.%, which close to that of 1:1 complex (17.4 wt.%). Solubility of the drug in complex was found to be significantly higher than that of pure 3-hydroxyflavone in water, which will increase the bioavailability of the drug and minimize the dose required. In the process, the pressure has a positive effect on the drug content in the complex, but contacting time has no significant effect. Perhaps contact areas play the most effect in content of the drug in the complex.

Acknowledgments

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References

1. Y. Kimura, H. Okuda, and S. Arichi: *Chem. Pharm. Bull.* **34**, 2279 (1986).
2. L Lasagna: *Eur. J. Rheumatol. Inflamm.* **12**, 3 (1993).
3. M. Pedersen, S. Bjerregaard, J. Jacobsen, and A.M. Sørensen: *Int. J. Pharm.* **176**, 121 (1998).
4. S. Li and W.C. Purdy: *Chem. Rev.* **92**, 1457 (1992).
5. G.M. Escandar and A. de Muñoz la Peña: *Anal. Chim. Acta* **370**, 199 (1998).
6. K. Uekama, F. Hirayama, and T. Irie: *High Performance Biomaterials,* Technomic Publishing Co. Inc., Lancaster, PA (1991), p. 789.
7. Y. Nozawa, K. Suzuki, A. Miyagishima, and S. Hirota: *Powder Technol.* **79**, 269 (1994).
8. M.J. Arias, J.R. Moyano, and J.M. Ginés: *Int. J. Pharm.* **153**, 181 (1997).
9. I. Orienti, T. Cerchiara, V. Zecchi, M.J. Arias Blanco, J.M. Gines, J.R. Moyano, and A.M. Rabasco Alvarez: *Int. J. Pharm.* **190**, 139 (1999).
10. T. Oguchi, M. Okada, E. Yonemochi, K. Yamamoto, and Y. Nakai: *Int. J. Pharm.* **61**, 27 (1990).
11. J.F. Li, Yu. X. Wei, Li. H. Ding, and C. Dong: *Spectroc. Acta Pt. A* **59**, 2759 (2003).
12. J.J. Berzas Nevado, J.A. Murillo Pulgarín, and M.A. Gómez Laguna: *Talanta* **53**, 951 (2001).
13. G. Becket, L.J. Schep, and M.Y. Tan: *Int. J. Pharm.* **179**, 65 (1999).
14. J. Puiggené, M.A. Larrayoz, and F. Recasens: *Chem. Eng. Sci.* **52**, 195 (1997).
15. E.J. Beckman: *J. Supercrit. Fluids* **28**, 121 (2004).
16. C.A. Eckert, B.L. Knutson, and P.G. Debenedetti: *Nature* **383**, 313 (1996).
17. M.A. McHugh and V.J. Krukonis: *Supercritical Fluids Extraction: Principle and Practice*, 2nd ed., Butterworth-Heinemann, Boston (1994).
18. R.K. Mcmullan, W. Saenger, J. Fayos, and D. Mootz: *Carbonhydr. Res.* **31**, 211 (1973).
19. J.Y. Li and D.Y. Yan: *Macromolecules* **34**, 1542 (2001).
20. K. Mishima, S. Yamauchi, M. Ito, M. Ezawa, and D. Tanabe: *Solvent Extr. Res. Dev.-Jpn.* **6**, 171 (1999).
21. M. Charoenchaitrakool, F. Dehghani, N.R. Foster, and H.K Chan: *Ind. Eng. Chem. Res.* **39**, 4794 (2000).