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ORIGINAL ARTICLE

Complex of shikonin and β-cyclodextrins by using supercritical carbon dioxide

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Abstract In this work, the complex of shikonin-methyl-β-cyclodextrin and shikonin-2-hydroxypropyl-β-cyclodextrin were studied in supercritical carbon dioxide (sc CO_2) at moderate pressure and temperature much lower than the melting point of shikonin. For comparing, the complex was also prepared by sealed heating method. Complex efficiency between shikonin and 2-hydroxypropyl-β-cyclodextrin (HPBCD) was quite low. Partly formation of shikonin—methyl-β-cyclodextrin (MBCD) was obtained by sealed heating method. Complete formation of shikonin—MBCD was obtained in sc CO_2 media in short reaction time. This complexation was accelerated and enhanced by the rise in both the reaction temperature and carbon dioxide pressure up to 100 °C 100 bar. The physical state of cyclodextrins in complex reaction has remarkable influence on the complex. The aqueous solubility of shikonin could be enhanced about 75 times by complexing with MBCD.

Keywords Shikonin · Methyl-β-cyclodextrin · 2-hydroxypropyl-β-cyclodextrin · Complex · Supercritical carbon dioxide

Introduction

Shikonin, is a component in lithospermum erythrorhiza roots and arnebia euchroma, effective for antibacterial, anti-inflammation and antivirus [1, 2]. The most important is its anti-tumor and anti-cancer activity [3, 4]. Shikonin can induce the cytoclasis and stunt the proliferation of the cells, thus make the cells of several kinds of tumor/cancer dieing down. At the same time, its side-effect is comparatively low. In the in vitro study, the depression effect was found to be enhanced with the concentration of shikonin solution. Unfortunately shikonin is almost insoluble in water, this restricts its pharmaceutical use and limits its bioavailability.

Cyclodextrins (CDs) are hydrophilic macrocyclic compounds with hydrophobic internal cavities; show good biocompatibility and can include many hydrophobic drugs to enhance their aqueous solubility and bio-availability. The inclusion complex of shikonin and β-cyclodextrin was prepared by Xu et al. by co-precipitation from saturated aqueous solution with a yield about 60–70% [5]. Ethanol was needed to dissolve the shikonin solid in the process, thus may remain in product. Inclusion complex was also obtained by Papageorgiou et al. [6]. In their work, 8 days was needed to get the solid product. In this way, the aqueous solubility of shikonin was enhanced remarkably and shikonin was protected from unnecessary disturbances such as oxidation and hydrolysis. Thus a green chemistry approach with short processing time should be searched.

In recent year supercritical carbon dioxide technique were introduced in pharmacy due to its special characteristics [7–23]. In most of the works, the drugs was firstly dissolved in sc CO_2, then carried into solid cyclodextrins matrix and interacted with the host. In 2002, the ibuprofen-MBCD complex was prepared by Foster et al. by passing ibuprofen/CO_2 solution through melted MBCD bed [8]. In our previous study, the complex formation of cinnamaldehyde—methyl-β-cyclodextrin and muscone—methyl-β-cyclodextrin were investigated both in supercritical carbon dioxide approach and by sealed heating method. Complete complex was obtained by both methods for cinnamaldehyde-MBCD. Cluster complex between muscone and

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methyl-β-cyclodextrin was partly obtained. The choice for the size of guest molecule still existed for MBCD cavity in both situation.

In present work, the formation of complex between shikonin ($T_m = 148$ °C) and MBCD ($T_g = 180$ °C) were studied both in supercritical carbon dioxide media and in sealed heating method at moderate pressure and temperature much lower than the melting point of shikonin. For comparing, complex between shikonin and HPBCD ($T_g = 278$ °C) was also investigated. The solubility of shikonin in sc CO_2 was measured, the effect of sc CO_2 on CDs physical state was observed, and the influence of the physical format of CDs on the complex was discussed.

Materials and methods

Materials

Shikonin, 98% purity, was provided by Chinese National Institute for the Control of Pharmaceutical and Biological Products. MBCD with substitute rate 1.7–1.9 was obtained from the Sigma-Aldrich Co. HPBCD with a purity of 97% was purchased from Acros Organics. Carbon dioxide with a purity of 99.95% was supplied by Beijing Analytical Instrument Factory. Acetonitrile and cyclohexane, analytical grade, was produced by Beijing Chemical Company.

Estimation of solubility of shikonin and observation of the physical format of CDs in sc CO_2

To estimated the solubility in CO_2, 3 mg shikonin was put in a glass tube ($\phi = 5$ mm, $l = 10$ mm). The tube was accurately weighted (±0.1 mg) and put vertically in the same stainless-steel vessel used for the complex reaction. The vessel was sealed and heated to desired temperature; then carbon dioxide was pumped in to desired pressure. The content was left in static for 6 h, then the vessel was slowly depressed to atmospheric pressure in 4 h. The tube was weighted again after equilibrated in air for 10 min. The solubility of shikonin in sc CO_2 was estimated from the weight loss of the tube containing shikonin and the volume of the reaction vessel. This was done with two repetitions.

For observing the physical format of CDs, 100 mg CD was put in the same stainless-steel vessel, and treated with sc CO_2 in the same way as that for the complex reaction. Then the vessel was depressed to 1 bar in 2 min. The physical format of the product in the vessel was observed. If the product was transparent or semi-transparent granules or blocks, the CD was considered to be melted in sc CO_2 media at the corresponding experimental condition. The observation was done for the two CDs at 70, 80, 90 °C up to 150 bar with a pressure interval of 10 bar.

Preparation of the physical mixture

The shikonin and MBCD (or HPBCD) was weighted with a molar ratio of 1.00:1.00, mixed and ground in a mortar with a pestle.

Preparation of the complex in supercritical carbon dioxide

100 mg physical mixture was put into a dry high pressure stainless-steel vessel (10 mL), purged for 10 min with carbon dioxide at 1 bar. The vessel was sealed and heated to the desired temperature. Then carbon dioxide was pumped into the vessel to the desired pressure. The content was left in static for desired period. At the end, the vessel was depressed to atmospheric pressure within 2 min and the solid products in the vessel were collected.

In sealed heating method, the product was prepared in similar way in 1 bar air.

Quantitative analysis

The total and free content of shikonin in the products were analyzed by UV-VIS spectroscopy method with a TU-1901 UV-VIS spectrograph. The absorbency of shikonin peak was calibrated with shikonin/acetonitrile solutions and shikonin/cyclohexane solutions of known concentration.

To determine the total content of shikonin in a product, about 10 mg shikonin-MBCD product was accurately weighted (±0.1 mg) and dissolved in 40.0 mL acetonitrile, the absorbency was determined at 515 nm. For shikonin-HPBCD product, 2:10 (v:v) water-acetonitrile was used as solvent. Thus the amount of total shikonin in the sample was derived; from the mass of the product sample and the total mass of shikonin in the sample, the amount of CDs in the sample was obtained. Then the molar ratio of total shikonin to total CDs (total ratio) in the product could be derived.

To measure the content of free shikonin in a product, the products were crushed. About 10 mg product was accurately weighted (±0.1 mg) and dispersed in 20 mL cyclohexane. The content was ultra-sounded for 5 min and centrifuged at 3,200 rpm for 10 min. The absorbency of the clear solution was measured at 522 nm. Thus the amount of uncomplexed shikonin in sample was derived. The amount of CDs in the sample was calculated from the total ratio of this product and the mass of the product sample. Then the molar ratio of free shikonin to total CDs (free ratio) in the product was obtained.

The molar ratio of complexed shikonin to total CDs (complex ratio) in the product was the difference between the total ratio and the free ratio of shikonin in the product.

Qualitative characterization

Powder X ray diffraction

The structure of shikonin, physical mixture and products were analyzed by a RIGAKU D/MAX 2500 X ray diffractmeter, the 2θ scan rang was 3–60°, the scan rate was 8°/min, with Cu kα radiation (40 kv, 200 mA).

Differential scanning calorimetry analysis

The thermal analysis of shikonin, MBCD and the products were performed by a PERKIN ELEMER diamond DSC from 25 to 200 °C at 10 °C/min, the flow rate of nitrogen gas was 20 mL/min.

Measurement of aqueous solubility

Solubility of pure shikonin and some complexes in water were determined by UV-VIS spectroscopy method with two repetition.

To measure the aqueous solubility of shikonin, excess amount of pure shikonin was put in deionized water, ultrasound for 5 min and stirred thoroughly for 1 day at room temperature, the mixture was centrifuged at 3,200 rpm and the clear solution was used for analysis.

To measure the apparent aqueous solubility of shikonin in the product, certain amount of the product was accurately weighted (±0.1 mg) and put in 5.00 mL deionized water, stirring thoroughly for 20 min, then the mixture was centrifuged at 3,200 rpm and the clear solution was used for analysis.

To measure the enhancement of the complex for the aqueous solubility of shikonin, complexes with molar ratio about 1.0:1.0 were gradually put in deionized water under stirring until obvious indissoluble was observed. The content was also centrifuged and the clear solution was accurately diluted for analysis.

Results and discussion

Different analytical techniques were used to characterize the solid products of shikonin and cyclodextrins.

Solubility of shikonin and physical format of CDs in sc CO_2

Solubility of shikonin in sc CO_2 was display in Fig. 1. At 80 °C, the dissolution was not detectable at pressure up to 100 bar. At 100 °C 1 bar, the solubility was still quite low ($(0.07 \pm 0.03) * 10^{-3}$ M). When carbon dioxide was introduced up to 150 bar, the solubility is no more than

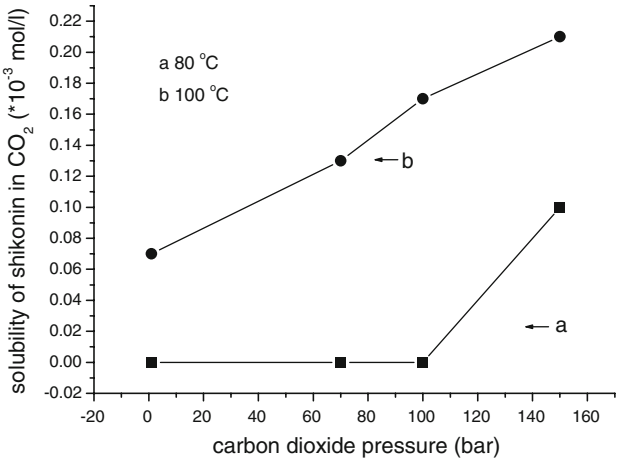

Fig. 1 Solubility of shikonin in supercritical carbon dioxide: (**a**) 80 °C, (**b**) 100 °C

($(0.19 \pm 0.04) * 10^{-3}$ M). Thus at saturate concentration the shikonin in sc CO_2 was lower than 4% of the shikonin used in complex reaction. And shikonin was in powder state similar to the untreated shikonin after all these treatments.

In 50, 70, 80 bar sc CO_2, the liquefying temperature of MBCD was depressed to 90, 80, 70 °C. In 1 bar air, melting of MBCD was not found up to 110 °C and HPBCD could not be liquefied in all experimental condition. Thus MBCD is liquid and HPBCD is solid in our complex reactions in sc CO_2 media.

Contents of shikonin in products

The solubilities of MBCD, HPBCD, shikonin in acetonitrile or cyclohexane were firstly measured. MBCD is easy to be dissolved in acetonitrile ($\geq 1.0 * 10^{-3}$ M) and hard to be dissolved in cyclohexane ($<1 * 10^{-6}$ M). HPBCD is easy to be dissolved in 2:10 water-acetonitrile ($\geq 0.31 * 10^{-3}$ M) and also insoluble in cyclohexane ($<1 * 10^{-6}$ M). The solubility of shikonin is $2.1 * 10^{-2}$ M in acetonitrile and $2.5 * 10^{-3}$ M in cyclodextrin.

The contents of shikonin in products and the physical format of the products were displayed in Tables 1, 2. Distinctive difference between the complex formation of shikonin-MBCD and shikonin-HPBCD was observed.

The complex formation between shikonin and MBCD could be seen from the complex ratio in Table 1. With the sealed heating method, a rise in the complex yield from about 10% to 45% was obtained with the temperature rise from 80 to 100 °C; while the solubility of shikonin in air rise from 0 to $0.07 * 10^{-3}$ M. When carbon dioxide was introduced to 100 bar at 80 °C, the dissolution of shikonin in sc CO_2 was not detectable, but the complex yield increased to 85% and the product was fine granule.

Table 1 Molar ratio of shikonin in shikonin-MBCD products

T (°C)	P (bar)	$T_{contact}$ (h)	Total ratio	Free ratio	Complex ratio	Phy. format
Mix	1		1.00:1.00	0.90:1.00	0.10:1.00	Powder
80	1	6	0.94:1.00	0.87:1.00	0.07:1.00	Powder
80	70	6	0.95:1.00	0.09:1.00	0.86:1.00	Fine grain
80	100	6	0.96:1.00	0.07:1.00	0.88:1.00	Fine grain
100	1	6	1.00:1.00	0.55:1.00	0.45:1.00	Powder
100	1	20	1.02:1.00	0.12:1.00	0.90:1.00	Powder
100	70	20	0.95:1.00	0.008:1.00	0.95:1.00	Fine grain
100	100	20	1.03:1.00	0.003:1.00	1.03:1.00	Fine grain
100	150	20	0.86:1.00	0.004:1.00	0.86:1.00	Fine grain
150	1	20	0.84:1.00	0.002:1.00	0.84:1.00	Dark block

The uncertainty is ±5% of the determined values except for the low free contain ±0.002:1.00

Table 2 Molar ratio of shikonin in shikonin-HPBCD products

T (°C)	P (bar)	Total ratio	Free ratio	Complex ratio	Phy. format
Phy. mix	1	1.00:1.00	0.89:1.00	0.11:1.00	Red powder
100	1	1.00:1.00	0.89:1.00	0.11:1.00	Red powder
100	70	0.96:1.00	0.85:1.00	0.11:1.00	Red powder
100	100	0.95:1.00	0.84:1.00	0.11:1.00	Red powder
100	150	0.88:1.00	0.45:1.00	0.43:1.00	Purple powder

Uncertainty is ±5% of the determined values except for the low free contain ±0.002:1.00

Complete complex for shikonin was obtained in sc CO_2 at 100 °C 70–100 bar. After treated with sealed heating method at 150 °C, the shikonin left in product was all complexed but the total ratio of shikonin was decreased to 0.84:1.00.

The influence of the reaction time on complex yield was displayed in Fig. 2. In sealed heating treatment, the shikonin reacted slowly with MBCD at 100 °C, only half of shikonin was complexed after 9 h reaction. Thus, shikonin must be gradually dissolved in air and gaseous state shikonin molecules penetrated into solid MBCD matrix then complex with MBCD molecules. In supercritical carbon dioxide at 100 °C 100 bar, the complex was already accomplished in 3 h. There are two possible approaches for the complex between shikonin and MBCD in sc CO_2 media due to the melting of MBCD. In the first approach shikonin was dissolved in sc CO_2 then penetrated into MBCD melt and complex with MBCD molecules. As the shikonin dissolved in sc CO_2 media was lower than 4% of the shikonin used in complex reaction, some of the shikonin solid may directly dissolved in MBCD melt and complexed with MBCD. Thus the complex could be completed in such a short reaction time.

For products of shikonin-HPBCD, there was almost no complex formation at pressure up to 100 bar. At 100 °C 150 bar, only small part of shikonin was found to be complexed after 20 h reaction.

Powder X ray diffraction analysis

Diffraction pattern of shikonin, MBCD, physical mixture and the products were displayed in Fig. 3. After treated with sc CO_2 at 100 °C 100 bar, shikonin still have crystalline structure similar with untreated shikonin. In the diffraction pattern of the physical mixture, the characteristic peaks of crystalline shikonin still exited, indicating no obvious interaction between shikonin and MBCD in the physical mixture. These crystalline peaks can still be detected with reduced intensity for product by sealed heating method at 100 °C 20 h. This is in accordance with the quantitative result that only 90% shikonin was complexed in this condition. The crystalline peaks of shikonin could not be detected for product of sealed heating method at 150 °C affirmed that the shikonin left in the product was all complexed. And these crystalline peaks were vanished

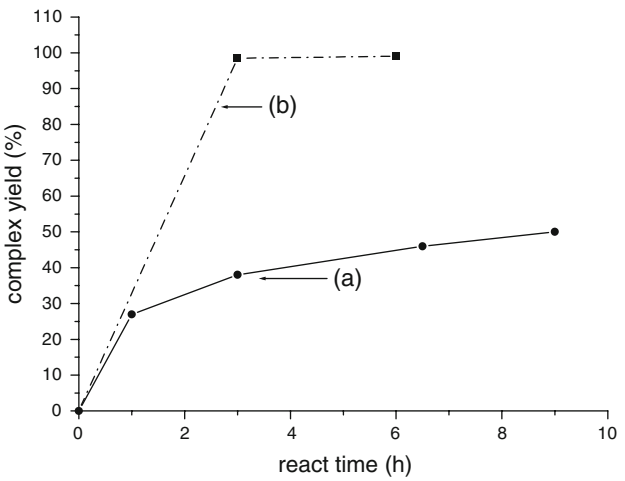

Fig. 2 Effects of reaction time on the complex formation of shikonin-MBCD: (**a**) sealed heating method at 100 °C, (**b**) in 100 °C 100 bar CO_2

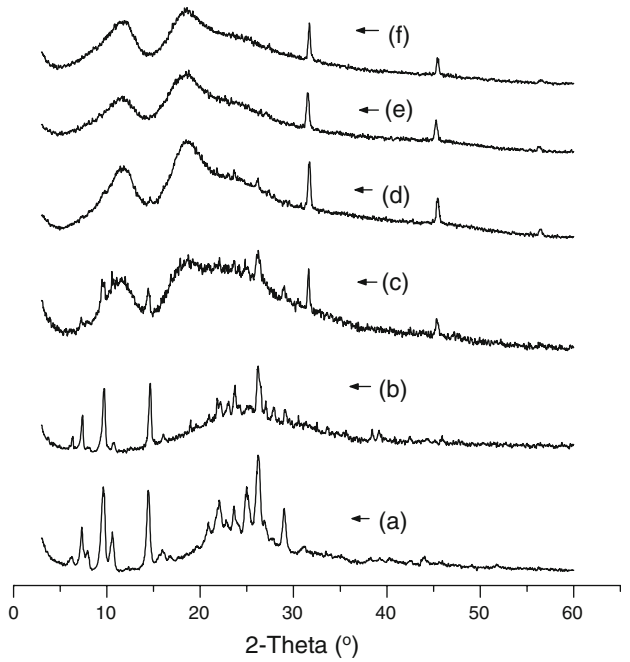

Fig. 3 X ray diffraction pattern of shikonin and shikonin-MBCD: (**a**) untreated shikonin, (**b**) shikonin treated in 100 °C 100 bar CO_2, (**c**) the physical mixture, (**d**) shikonin-MBCD at 100 °C 1 bar (**e**) shikonin-MBCD at 150 °C 1 bar, (**f**) shikonin-MBCD in 100 °C 100 bar CO_2

in the diffraction pattern of the product prepared in supercritical carbon dioxide at 100 °C 100 bar. The absence of the shikonin peaks indicated the complete complexation of shikonin with MBCD at molecular level. And the product has an amorphous structure.

For shikonin-HPBCD products at pressure not higher than 100 bar, the diffraction pattern showed in Fig. 4 is a simple sum of that of the two respective components. For shikonin-HPBCD products at 100 °C 150 bar, the intensity for shikonin crystalline was some reduced. Thus the complex efficiency for shikonin-HPBCD was much lower than that for shikonin-MBCD both by sealed heating method and in supercritical carbon dioxide.

Differential scan calorimeter analysis

The DSC curve for shikonin, MBCD and shikonin-MBCD was showed in Fig. 5. For pure MBCD, the broaden peaks from 30 to 100 °C was resulted from the dehydration and a glass transfer could be observed about 170 °C. Untreated shikonin showed a melting peak at 145 °C. After treated with sc CO_2, the endothermic peak at 145 °C was still there and a small new peak appeared at 135 °C; but in the second DSC scanning of this sample, the small peak was disappeared, the DSC thermo-gram was the same with the untreated shikonin. Thus the granularity of some shikonin was reduced by sc CO_2 processing. For products by sealed heating method, the endothermal peak at 127 °C could be

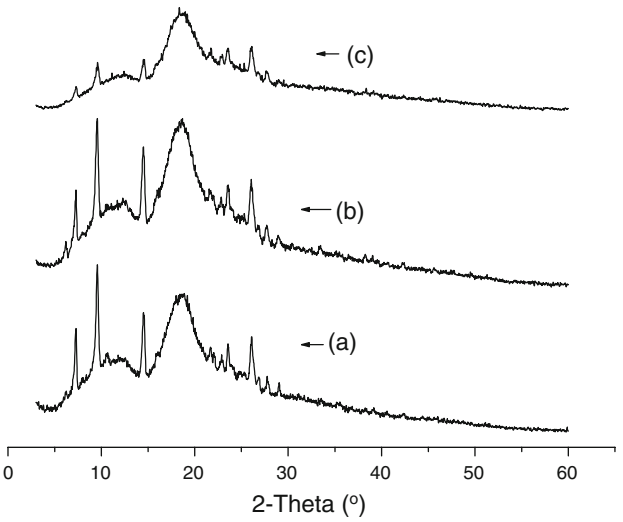

Fig. 4 X ray diffraction pattern of shikonin-HPBCD products (**a**) 100 °C 1 bar, (**b**) 100 °C 100 bar CO_2, (**c**) 100 °C 150 bar CO_2

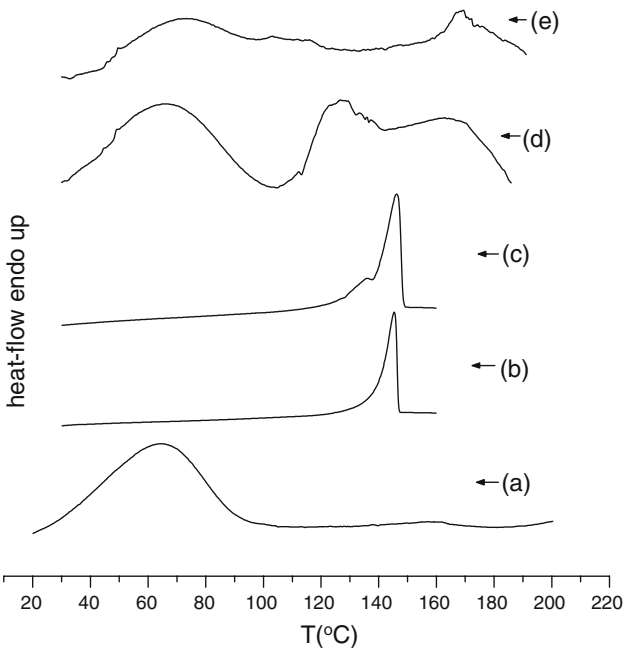

Fig. 5 DSC thermo-gram of MBCD, shikonin, shikonin-MBCD products (**a**) untreated MBCD, (**b**) untreated shikonin, (**c**) shikonin treated in 100 °C 100 bar CO_2, (**d**) shikonin-MBCD treated at 100 °C 1 bar, (**e**) shikonin-MBCD treated in 100 °C 100 bar CO_2

depicted to free shikonin of smaller granularities in MBCD matrix; and the endothermal peak at 160–170 °C may be attributed to the liquefying of the complex. For product by sc CO_2 processing, the peak for crystalline shikonin was no longer observable and the peak at 168 °C was sharpen, this confirmed again the complete complexation between shikonin and MBCD in sc CO_2 media.

Aqueous solubility

Pure shikonin was difficulty to be dissolved in water and the aqueous solubility of untreated shikonin is about $2.0 * 10^{-5}$ M.

The product used to determine the apparent solubility of shikonin in water were the product by sc CO_2 processing at 100 °C 100 bar for 20 h and the product by sealed heating treatment at 100 °C 1 bar for 20 h. The complex was quite easy to be dissolved in water; after 20 min stirring, the concentration of shikonin in water was already constant. When 4.2 mg product was put in 5 mL water, the apparent aqueous solubility of shikonin was $0.41 * 10^{-3}$ M for sc CO_2 product and $0.34 * 10^{-3}$ M for sealed heating product. When 10.0 mg product was put in 5 mL water, the apparent aqueous solubility of shikonin was raised to $0.87 * 10^{-3}$ M for sc CO_2 product and $0.79 * 10^{-3}$ M for sealed heating product. The deviation was ±5% of the determined values. The apparent aqueous solubility of sc CO_2 product was 10–20% higher than that of sealed heating product.

The saturated solubility of shikonin could be raised to $1.7 * 10^{-3}$ M for complex of sc CO_2 processing and $1.8 * 10^{-3}$ M for complex of sealed heating method; almost the same considering the experimental error. The solubility of shikonin could be enhanced about 75 times by complexing with MBCD.

Conclusions

At moderate carbon dioxide pressure and temperature much lower than the melting point of shikonin, the complex between shikonin and MBCD was confirmed. In sealed heating method, shikonin was partly complexed with MBCD and the reaction was slow; thus shikonin was gradually dissolved in air and gaseous state shikonin molecules penetrated into MBCD matrix then complex with MBCD molecules; In sc CO_2 media, the complete complex could be obtained and the reaction was finished in short reaction time; the MBCD is liquefied in complex reaction, some shikonin was dissolved in sc CO_2 then penetrated into MBCD melt and complex with MBCD molecules, some of the shikonin solid may directly dissolved in MBCD melt and react with MBCD molecules; thus the complex reacted quite fast and the complex yield was obviously enhanced. In contrast, for HPBCD with a glass transition temperature 100 °C higher than that of MBCD, which is in solid state in all complex reaction, the complex efficiency of shikonin—HPBCD was very low. Thus the physical format of cyclodextrins in complex reaction has a remarkable influence on the complex between shikonin and cyclodextrins.

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References

1. Chen, X., Oppenheim, J., Zack Howard, O.M.: Shikonin, a component of anti-inflammatory Chinese herbal medicine, selectively blocks chemokine binding to cc chemokine receptor-1. Int. Immunopharmacol **1**, 229–236 (2001). doi:10.1016/S1567-5769(00)00033-3
2. Shen, C.C., Syu, W., Li, S.Y., Lin, C.H., Lee, G.H., Sun, C.M.: Antimicrobial activities of naphthazarins from arnebia euchroma. J. Nat. Prod **65**, 1857–1862 (2002). doi:10.1021/np010599w
3. Ruan, M., Ji, T., Yang, W.J., Duan, W.H., Zhou, X.J., He, J.C., Zhou, J., Chen, W.T., Zhang, C.P.: Growth inhibition and induction of apoptosis in human oral squamous cell carcinoma Tca-8113 cell lines by shikonin was partly through the inactivation of NF-κB pathway. Phytother. Res **22**, 407–415 (2008). doi:10.1002/ptr.2340
4. Singh, F., Gao, D.Y., Lebwohl, M.G., Wei, H.C.: Shikonin modulates cell proliferation by inhibiting epidermal growth factor receptor signaling in human epidermoid carcinoma cells. Cancer Lett **200**, 115–121 (2003). doi:10.1016/S0304-3835(03)00239-8
5. Xu, R.L., Ouyang, H., Deng, H.L.: Optimization of inclusion methods for shikonin and β-cyclodextrin. Acta Acad. Med. Jiangxi **144**, 81 (2004)
6. Assimopoulau, A.N., Papageargiou, V.P.: Encapsulation of isohexenylnaphthazarins in cyclodextrin. Biomed. Chromatogr **18**, 240–247 (2004). doi:10.1002/bmc.310
7. Hees, T.V., Piel, G., Evrared, B., Otte, X., Thunnus, L., Delattre, L.: Application of supercritical carbon dioxide for the preparation of a piroxicam-β-cyclodextrin inclusion compound. Pharm. Res **16**, 1864–1870 (1999). doi:10.1023/A:1018955410414
8. Charoenchaitrakool, M., Dehghani, F., Foster, N.R.: Utilization of supercritical carbon dioxide for complex formation of ibuprofen and methyl-cyclodextrin. Int. J. Pharm **239**, 103–112 (2002). doi:10.1016/S0378-5173(02)00078-9
9. Junco, S., Casimiro, T., Ribeiro, N., Ponte, M.N., Marques, H.C.: A comparative study of naproxen-beta cyclodextrin complexes prepared by conventional methods and using supercritical carbon dioxide. J. Incl. Phenom. Macrocycl. Chem **44**, 117–121 (2002). doi:10.1023/A:1023022008337
10. Lai, S., Locci, E., Piras, A., Porcedda, S., Lai, A., Marongiu, B.: Imazalil-cyclomaltoheptaose (β-cyclodextrin) inclusion complex: preparation by supercritical carbon dioxide and ^{13}C CPMAS and ^{1}H NMR characterization. Carbohydr. Res **338**, 2227–2232 (2003). doi:10.1016/S0008-6215(03)00358-6
11. Locci, E., Lai, S., Piras, A., Marongiu, B., Lai, A.: ^{13}C-CPMAS and ^{1}H-NMR study of the inclusion complexes of β-cyclodextrin with carvacrol, thymol, and eugenol prepared in supercritical carbon dioxide. Chem. Biodivers **1**, 1354 (2004). doi:10.1002/cbdv.200490098
12. Bandia, N., Weib, W., Robertsc, C.B., Kotrac, L.P., Kompellaa, U.B.: Preparation of budesonide and indomethacin-hydroxypropyl-cyclodextrin (HPBCD) complexes using a single-step, organic-solvent-free supercritical fluid process. Eur. J. Pharm. Sci **23**, 159–168 (2004). doi:10.1016/j.ejps.2004.06.007
13. Rodier, E., Lochard, H., Sauceau, M., Letourneau, J., Freiss, B., Fages, J.: A three step supercritical process to improve the dissolution rate of eflucimibe. Eur. J. Pharm. Sci **26**, 184–193 (2005). doi:10.1016/j.ejps.2005.05.011
14. Ibrahim, S., Ali, H., Al, M., Babomcarr, J., Ali, D.: Enhancement of aqueous solubility of itraconazole by complexation with cyclodextrins using supercritical carbon dioxide. Can. J. Chem **83**, 1833–1838 (2005). doi:10.1139/v05-181

15. AL-Marzouqi, A.H., Shehatta, I., Jobe, B., Dowaidar, A.: Phase solubility and inclusion complex of itraconazole with β-cyclodextrin using supercritical carbon dioxide. J. Pharm. Sci **95**, 292–304 (2006). doi:10.1002/jps.20535
16. Wang, B., He, J., Sun, D.H., Zhang, R., Han, B.X.: Utilization of supercritical carbon dioxide for preparation of 3-hydroxyflavone and β-cyclodextrin complex. J. Incl. Phenom. Macrocycl. Chem **55**, 37–40 (2006). doi:10.1007/s10847-005-9015-8
17. Ali, H., Al, M., Baboucarr, J., Ali, D., Francesca, M., Paola, M.: Evaluation of supercritical fluid technology as preparative technique of benzocaine-cyclodextrin complex comparison with conventional methods. J. Pharm. Biomed **43**, 566–574 (2007). doi:10.1016/j.jpba.2006.08.019
18. Arezki, B., Elisabeth, R., Jacques, F.: Maturation of ketoprofen/β-cyclodextrin mixture with supercritical carbon dioxide. J. Supercrit. Fluid **41**, 429–439 (2007). doi:10.1016/j.supflu.2006.11.004
19. Khaled, H., Michael, T., Martin, A.W.: Comparative evaluation of ibuprofen/β-cyclodextrin complexes obtained by supercritical carbon dioxide and other conventional methods. Pharm. Res **24**, 585–592 (2007). doi:10.1007/s11095-006-9177-0
20. Al-Marzouqi, A.H., Jobe, B., Corti, G., Cirri, M., Mura, P.: Physicochemical characterization of drug-cyclodextrin complexes prepared by supercritical carbon dioxide and by conventional techniques. J. Incl. Phenom. Macrocycl. Chem **57**, 223–231 (2007). doi:10.1007/s10847-006-9192-0
21. Moribe, K., Fujito, T., Tozuka, Y., Yamamoto, K.: Solubility-dependent complexation of active pharmaceutical ingredients with trimethyl-β-cyclodextrin under supercritical fluid condition. J. Incl. Phenom. Macrocycl. Chem **57**, 289–295 (2007). doi:10.1007/s10847-006-9175-1
22. Lee, S.Y., Jung, I.L., Kim, J.K., Lim, G.B., Ryu, J.H.: Preparation of itraconazole/HP-B-CD inclusion complexes using supercritical aerosol solvent extraction system and their dissolution characteristics. J. Supercrit. Fluid **44**, 400–408 (2008). doi:10.1016/j.supflu.2007.09.006
23. Al-Marzouqi, A.H., Solieman, A., Shehadi, I., Adem, A.: Influence of the preparation method on the physicochemical properties of econazole-β-cyclodextrin complexes. J. Incl. Phenom. Macrocycl. Chem **60**, 85–93 (2008). doi:10.1007/s10847-007-9356-6