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ORIGINAL ARTICLE

Complex between modified β-cyclodextrins and three components of traditional Chinese medicine in supercritical carbon dioxide medium

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Abstract In this work, the complex between three hydrophobic efficacious components of plants (anisole, asarone, curcumin) and modified cyclodextrin (2-hydroxypropyl-β-cyclodextrin, methyl-β-cyclodextrin) was investigated in supercritical carbon dioxide medium; and compared with the corresponding complex in air circumstance. The effect of the substitute group in the drug molecule on the complex reaction was also discussed.

Keywords Anisole · Asarone · Curcumin · β-Cyclodextrins · Supercritical carbon dioxide · Complex

Introduction

Anisole, asarone and curcumin are three hydrophobic efficacious components of Traditional Chinese Medicine (Fig. 1). Their aqueous solubility is poor; thus their pharmaceutical usage and bioavailability are limited. To improve this property, their cyclodextrin inclusion complexes were prepared. Anisole–β-cyclodextrin (anisole–BCD) complex was prepared with an ultrasound method by Weng et al. [1]. Asarone–BCD complex was made via saturated water solution–ultrasound process by Li et al. [2]. Asarone–BCD and asarone–2-hydroxypropyl-β-cyclodextrin (asarone–HPBCD) complexes were also produced with grinding treatment by Ma et al. [3]. Curcumin–HPBCD complex was derived through freeze-drying way by Gao et al. [4]. Curcumin–methyl-β-cyclodextrin (curcumin–MBCD) complex was gain with saturated solution technique by Liu et al. [5]. Curcumin–HPBCD and curcumin–MBCD complex were also obtained via kneading and common solvent evaporation approach by Yadav et al. [6]. By complex with the cyclodextrins, the aqueous solubility, the dissolution rate and the stability of these hydrophobic compounds were obviously enhanced. But in most of these methods, organic co-solvents were used and might remain in the products, now the organic residue in the drugs was strictly restricted.

Recently, a clean chemical technique-supercritical carbon dioxide ($scCO_2$) approach was developed, and used to prepare the hydrophilic complex between cyclodextrin and some hydrophobic drugs, due to the favorable characteristics of $scCO_2$. There are already more than twenty papers published [7–27]. In most of the reported reactions both in the batch mode and in the continuous flowing way, the hydrophobic compound was dissolved in $scCO_2$, carried into cyclodextrin powders and then formed complex with the cyclodextrin molecule. In 2002, Foster et al. [9] found that the glass transition temperature of methyl-β-cyclodextrin was markedly depressed in $scCO_2$ medium; and got ibuprofen-MBCD inclusion complex by passing ibuprofen/$scCO_2$ gaseous solution through the cyclodextrin melt bed. In recent years, we also investigated the complexing between MBCD and several efficacious components of Traditional Chinese Medicine in $scCO_2$ medium. At temperature much lower than the melting point of shikonin, the MBCD melt can dissolve the shikonin solid and form complete complex in short reaction time [28]. At suitable temperature and carbon dioxide pressure, the MBCD melt can react with the borneol molecules and create crystal inclusion complex, although MBCD itself is an amorphous macrocyclic compound [29]. And the MBCD melt can

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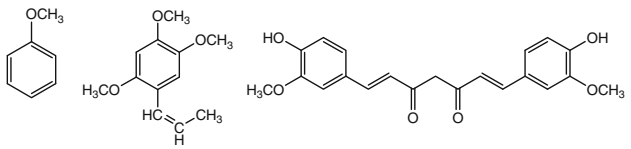

anisole asarone curcumin

Fig. 1 Molecular structure of anisole, asarone and curcumin

form inclusion complex with cinnamaldehyde but only generate cluster associations with muskone, due to the choice of the cavity in cyclodextrin molecule to the size or shape of the guest molecule [30] in $scCO_2$ media.

In this work, we studied the complex of MBCD with anisole, asarone, curcumin (Fig. 1) in $scCO_2$; and compared the results with the corresponding complex of HPBCD in $scCO_2$ medium. The purpose of this research is to observe the influence of the substituent in the guest molecule on their complexing with MBCD or HPBCD matrix in supercritical carbon dioxide media.

Materials and methods

Materials

Anisole, α-asarone and curcumim with 98% purity were provided by Chinese National Institute for the Control of Pharmaceutical and Biological Products. MBCD with 1.7–1.9 mol CH_3 per unit anhydroglucose 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. Ethanol, chlorobutane and cyclohexane (analytical grade) were produced by Beijing Chemical Company.

Estimation of solubility of α-asarone and curcumin in $scCO_2$

The procedure was the same with the process in our previous work [30]. To measure the solubility of α-asarone in $scCO_2$, 5 mg asarone powder was put in the glass tube ($\Phi = 6.0$ mm, $l = 10$ mm, with one end sealed) and the tube was weighted. After equilibrated for 20 h in static state, the CO_2 was slowly discharged from the vessel in 4 h. The tube was weighted again after contacted with air for 10 min. The solubility of asarone in $scCO_2$ was estimated from the weight loss of the tube and the volume of the vessel.

The solubility of curcumin in $scCO_2$ was determined in similar way. And solubility of anisole in $scCO_2$ was referred to the reported values [31, 32].

Preparation of the physical mixture

At a molar ratio of 1.00:1.00, asarone and MBCD were weighted, mixed and ground in a mortar with a pestle; then the asarone–MBCD physical mixture with 1:1 stoichiometry was obtained. The other physical mixtures were made in the same way at the corresponding molar ratio; except that the 1:1 anisole–MBCD mixture was weight at 1.30:1.00 molar ratio and the 1:1 anisole–HPBCD mixture can not be produced with this method.

Preparation of the complex

In preparing most of the complexes in $scCO_2$ media, the process was identical with that described in our previous paper [28], except that the physical mixture added in the reaction vessel was 50 mg. To produce the anisole–MBCD complex, a total of 100 mg physical mixture was put into a 1.0 mL stainless-steel vessel and then treated in similar way [29].

To make the anisole–HPBCD complex, 100 mg HPBCD was added in the 1.0 mL stainless-steel vessel, 12 μL anisole was injected to the HPBCD powders. Then the vessel was sealed at once. After the mixture was left in static in $scCO_2$ at desired temperature and pressure for 20 h, the vessel was depressurized to atmospheric pressure in 2 min and the solid product in the vessel was collected.

In the procedure of the sealed heating treatment, the complex was prepared in similar way in 0.1 MPa air.

Quantitative analysis of the product

At first, the solubility of anisole in ethanol and cyclohexane, the solubility of asarone in ethanol and cyclohexane, and the solubility of curcumin in ethanol and chlorobutane were measured. Then the total content of the drug and the content of free drug in the product were determined by a UV–VIS spectroscopy method with a TU-1901 UV-VIS spectrograph.

For example, the molar absorptivity of the drug was calibrated with corresponding drug/solvent solutions of known concentration. To determine the total content of anisole in a certain anisole–MBCD product, the anisole–MBCD product about 10 mg was accurately weighted (±0.1 mg), dissolved in 10.0 mL ethanol, and the absorbency of this solution at 277 nm was measured. Then the molar ratio of total anisole to total MBCD (total ratio) in this product could be calculated [28]. To measure the content of free anisole in this product, the product about 10 mg was weighted to ±0.1 mg and put in 5.00 mL cyclohexane. The mixture was ultra-sounded for 10 s, stirred for 2 min and centrifuged at 3,200 rpm for 10 min. The absorbency of the clear filtrate solution was determined at 277 nm. Then the

molar ratio of free anisole to total MBCD (free ratio) in this product was obtained [28]. The molar ratio of complexed anisole to total MBCD (complex ratio) in this product was the difference between the total ratio and the free ratio of anisole in this product.

The quantitative analysis for the other complexes was similar with the above one, except that the 1:9 water–ethanol mixture was used as solvent to measure the total content of drug in the drug-HPBCD product and chlorobutane was used as solvent to determine the content of free curcumin in curcumin–MBCD product.

The powder X-ray diffraction

The powder X-ray diffraction of MBCD, HPBCD, the individual drug, the physical mixture and the drug-modified CD product were recorded with a RIGAKU D/MAX 2500 X-ray diffract meter (Rigaku International Corp. Japan), the 2θ range was 3–60° and the scan rate was 8°/min with Cu K_α radiation (40 kV, 200 mA).

The differential scanning calorimetric analysis

The thermal behavior of the sample was observed with a Perkin Elmer diamond DSC (Perkin Elmer, USA) from 25 to 250 °C at scan rate 10 °C/min with a nitrogen flow at 20 mL/min.

The thermo gravimetric analysis

The thermal stability of the sample was analyzed via a Perkin-Elmer-S II Diamond TG-DTA (Perkin Elmer, USA) from 25 to 250 °C at heating rate 10 °C/min with a nitrogen flow of 20 mL/min.

Measurement of aqueous solubility

The aqueous solubility of asarone, curcumin, the physical mixtures and the products were measured by a UV–VIS spectroscopy method, in which the molar absorptivity (ε) of a drug in water was supposed to be equal to the ε of the drug in ethanol. The process was similar with that in reference 28.

For pure asarone and its product of sealed heating treatment, the aqueous mixture was ultrasound for 2 min, thoroughly stirred for 1 h and 5 min respectively; for its product of $scCO_2$ processing, the aqueous mixture was just stirred for 5 min; then the aqueous mixture was centrifuged at 16,000 rpm and the clear solution was diluted for the analysis.

For pure curcumin, the aqueous mixture was ultrasound for 2 min and thoroughly stirred for 6 h; for its physical mixtures and its products, the aqueous mixture was ultrasound for 2 min and stirred for 20 min; during the stirring, the aqueous mixture was protected from light; then the aqueous mixture was centrifuged at 3,200 rpm and the clear solution was used for the analysis.

For anisole oil, as its density was almost equal to the density of water, it is hard to get a completely demixing for its aqueous mixture, thus its solubility in water was not measured.

Results and discussion

Solubility of asarone and curcumin in $scCO_2$

The dissolution of asarone in carbon dioxide at 55–65 °C and 0.1–7 MPa was lower than the detection limit. The solubility of asarone in carbon dioxide at 55–65 °C and 10 MPa was $(2.9 \pm 0.3) \times 10^{-4}$ and $(3.8 \pm 0.4) \times 10^{-4}$ mol/L respectively; the amount dissolved in $scCO_2$ was corresponding to 10% asarone added in the vessel for the complexing.

And the dissolution of curcumin in carbon dioxide at all our experimental condition was not found. Thus the loss of curcumin in the complex reaction should not come from the dissolution.

Solubility of drug in the solvent

In ethanol, the solubility (Cs) of anisole is higher than 5.0×10^{-3} mol/L, the Cs of asarone is higher than 1.8×10^{-3} mol/L, the Cs of curcumim is higher than 0.25×10^{-3} mol/L. While using ethanol or water–ethanol as solvent to determined the total content of the drug in product, the concentration of anisole–CD solution was 0.7×10^{-3} mol/L; the concentration of asarone–CD solution was 0.11×10^{-3} mol/L; the concentration of curcumin in curcumin–MBCD solution was 0.024×10^{-3} mol/L.

In cyclohexane, the solubility of anisole is higher than 6.0×10^{-3} mol/L, the Cs of asarone is higher than 1.0×10^{-3} mol/L; in chlorobutane, the Cs of curcumin is higher than 4.0×10^{-5} mol/L. In the measurement the free content of the drug in the product, the maximum concentration (c_{max}) of anisole in anisole–CD/cyclohexane mixture was 1.4×10^{-3} mol/L; the c_{max} of asarone in asarone–CD/cyclohexane mixture was 0.13×10^{-3} mol/L; the c_{max} of curcumin in curcumin–CD/chlorobutane mixture was 3.8×10^{-5} mol/L.

In all these analysis, the concentration of the drug in the solution was lower than the solubility of this drug in the corresponding solvent.

Anisole–MBCD complex

Content of anisole in the product

The contents of anisole in anisole–MBCD physical mixture and their products, the physical forms of the physical mixture and products were list in Table 1.

In the physical mixture, there was already some interaction between anisole and MBCD. In sealed heating treatment, most of the anisole was complex with the MBCD matrix, only 5% anisole was left free; and the products were aggregated powders. When processed in $scCO_2$ at 50–60 °C and 5.0 MPa, the free anisole was taken away without any loss in the complex yield, and the products were small grains. As the pressure of CO_2 was raised to 7.0 MPa, the total content of anisole in the product began to be cut down.

Powder X-ray diffraction pattern

The X-ray diffraction patterns of pure MBCD, the physical mixture, and the products were shown in Fig. 2.

Compared with that of untreated MBCD, the diffractions at 11.5 and 18.0° relative to the intensity at 23.5° was enhanced for the physical mixture; this change in the MBCD diffraction provided that some interaction already existed between anisole and MBCD. In the diffraction pattern of the products, the comparative intensities at 11.7 and 18.0° were further increased, argued that the interaction in the products were much stronger than that in the physical mixture. These approved the quantitative analysis results.

TG analysis results

The thermo-stability of some products was displayed in Fig. 3. There is some difference between the products made by the two methods.

From 60 °C, the product made at 50 °C and 0.1 MPa began to loss weight; up to 180 °C, the weight decreased about 6%, correspond to 90% anisole in the product. Thus, the interaction between anisole and MBCD was weak in the product by sealed heating treatment.

Up to 80 °C, the gas absorbed in the product prepared in 50 °C and 5 MPa CO_2 was all escaped; from 130 to 180 °C (the glass transition temperature of MBCD), the weight loss was 1.8%, amount to 1/4 anisole in the product. Thus the interaction between anisole and MBCD was much stronger in the product via $scCO_2$ processing; and about 3/4 anisole in this complex was included in the MBCD cavities.

Table 1 Content of anisole in anisole–MBCD product

T (°C)	P (MPa)	Total ratio	Free ratio	Complex ratio	Physical form
T_{room}	0.1	0.95:1.00	0.53:1.00	0.42:1.00	Powder
50	0.1	0.95:1.00	0.05:1.00	0.90:1.00	Powder
50	5.0	0.94:1.00	<0.01:1.00	0.94:1.00	Small block
50	7.0	0.83:1.00	<0.01:1.00	0.83:1.00	Grain
60	0.1	0.94:1.00	0.05:1.00	0.89:1.00	Powder
60	5.0	0.91:1.00	0.01:1.00	0.90:1.00	Small block
60	7.0	0.82:1.00	<0.01:1.00	0.82:1.00	Grain

The uncertainty of the determined value is ±5% except that the uncertainty is ±0.005:1.00 for the low free ratio

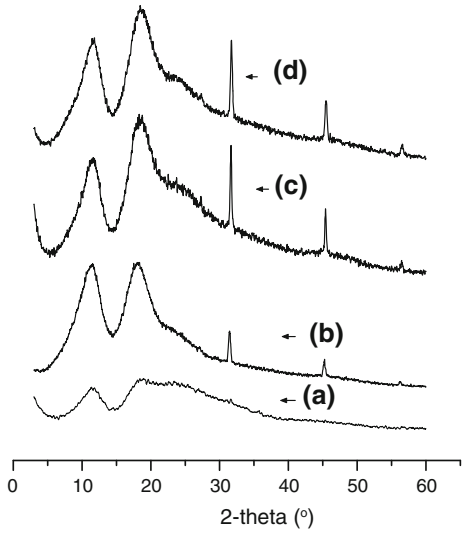

Fig. 2 X-ray diffraction pattern of anisole–MBCD: (*a*) MBCD, (*b*) anisole–MBCD mix, (*c*) anisole–MBCD 60 °C 0.1 MPa, (*d*) anisole–MBCD 60 °C 7.0 MPa

Brief summary

Anisole is a colorless liquid with a boiling point at 155 °C and is easy to react with MBCD matrix. In the physical

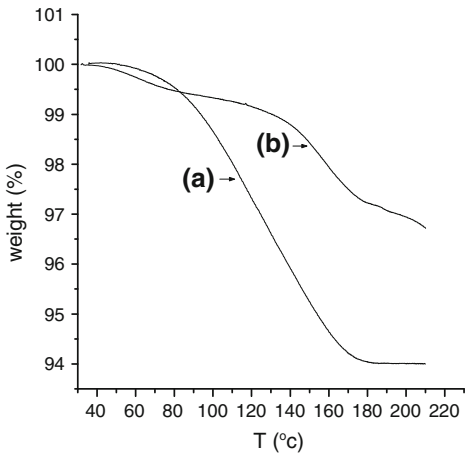

Fig. 3 TG thermogram of anisole–MBCD: (*a*) anisole–MBCD 50 °C 0.1 MPa, (*b*) anisole–MBCD 50 °C 5.0 MPa

mixture, there was already some interaction between anisole and MBCD. In sealed heating treatment, anisole molecules were permeated into MBCD powders and most of them were associated with the MBCD matrix. In the $scCO_2$ processing, the anisole/MBCD mixture was liquefied; the opportunity for the anisole molecule to enter the MBCD cavity was enhanced; the anisole left in the product was all complex with MBCD and majority of them was included in the MBCD cavities.

Anisole–HPBCD complex

Content of anisole in the product

The content of anisole in the products and the physical form of the products were demonstrated in Table 2.

In sealed heating treatment, the complex increased from 56 to 98% as the temperature rise from 50 to 80 °C. At 50 °C, the complex yield was raised to 80% after carbon dioxide was added in. At 7 MPa the total content of anisole in the product was reduced due to its dissolution in $scCO_2$, and the anisole left in the product was all complex. And all the products were in powder state.

X-ray diffraction pattern

The X-ray diffraction of HPBCD and the products were exhibited in Fig. 4.

After treated in dry CO_2, the diffraction of HPBCD was not changed; thus the HPBCD matrix is rigid even in $scCO_2$ medium. In the product's pattern, the intensity of HPBCD at 18.7° contrast to the intensity at 23° was obviously amplified; implied that there are some change in the HPBCD matrix structure. This change affirmed that there are obvious interactions between anisole and HPBCD in the product; and that was consisted with the quantitative analysis results.

Table 2 Content of anisole in anisole–HPBCD product

T (°C)	P (MPa)	Total ratio	Free ratio	Complex ratio	Physical form
50	0.1	0.95:1.00	0.39:1.00	0.56:1.00	Powder
50	5.0	0.94:1.00	0.12:1.00	0.82:1.00	Powder
50	7.0	0.85:1.00	0.04:1.00	0.81:1.00	Powder
80	0.1	0.99:1.00	0.01:1.00	0.98:1.00	Loose block
80	5.0	0.89:1.00	0.03:1.00	0.86:1.00	Loose block
80	7.0	0.84:1.00	0.02:1.00	0.82:1.00	Loose block

The uncertainty of the determined value is ±5% except that the uncertainty is ±0.005:1.00 for the low free ratio

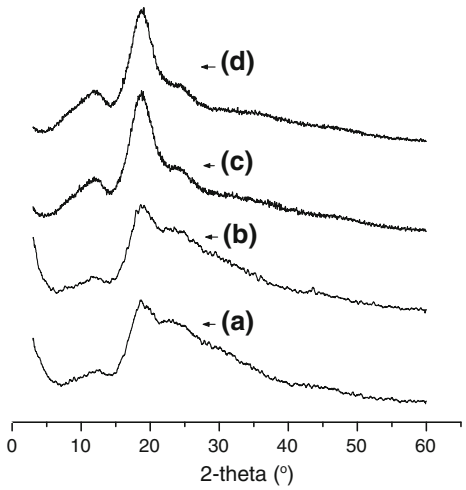

Fig. 4 X-ray diffraction pattern of anisole–HPBCD: (*a*) HPBCD untreated, (*b*) HPBCD 80 °C 7.0 MPa, (*c*) anisole–HPBCD 80 °C 0.1 MPa, (*d*) anisole–HPBCD 80 °C 7.0 MPa

TG analysis results

The stabilities were similar for products both by sealed heating treatment and via $scCO_2$ processing (Fig. 5). Up to 90 °C, the gas all got rid off the product. From 130 °C on, the weight of the product constantly decreased. Up to 250 °C (a little bit lower than the glass transition temperature of HPBCD), the weight loss was about 6%; almost all anisole in the product was got out. Thus most part of the anisole in anisole–HPBCD product was associated with the HPBCD matrix.

Brief comparison

Via both approaches, the anisole can also react with HPBCD at moderate temperature and pressure. In our experiments, the anisole diffused or penetrated into HPBCD

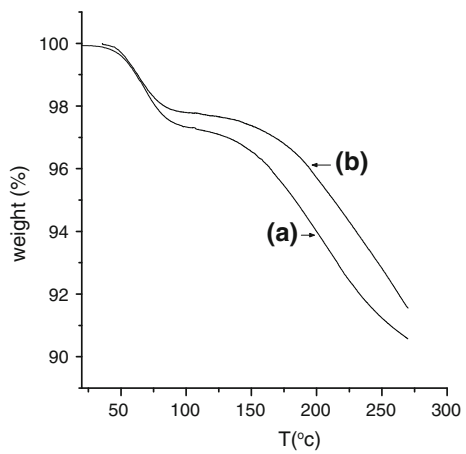

Fig. 5 TG thermogram of anisole–HPBCD: (*a*) anisole–HPBCD 80 °C 0.1 MPa, (*b*) anisole–HPBCD 80 °C 7.0 MPa

powders; as HPBCD matrix was rigid, most of the anisole were located between the HPBCD molecules.

At 55–75 °C 0.1–10 MPa, anisole can easily interact with both MBCD and HPBCD in sealed heating treatment or in $scCO_2$ processing. The complex yield for anisole–HPBCD reaction was a little lower compared with that of anisole–MBCD reaction. In $scCO_2$ medium, the anisole diffused into the HPBCD solid matrix and most of them formed association with HPBCD molecules. But in $scCO_2$ medium, the anisole reacted with the MBCD melt and the main part of the anisole formed inclusion complex with MBCD molecules.

Asarone–MBCD complex

Content of asarone in the product

The contents of asarone in the physical mixture and the products, the physical forms of the physical mixture and the products, were demonstrated in Table 3.

In the physical mixture, no asarone was reacted. After treated in sealed heating method at 55–65 °C for 20 h, about 10% asarone was left free and the product was agglomerated powders. While CO_2 was added in to 5 MPa, the complex was completed without any visible loss and the product was granules. When CO_2 pressure was raised to 10 MPa, the total contain of asarone was evidently reduced and the loss of asarone was proportion to the solubility of asarone in $scCO_2$.

The influence of the reaction time was also investigated for both methods (Fig. 6). After reacted for 1–6 h at 55 °C and 0.1 MPa, the free asarone remained in the products was reduced from 36 to 14%. In 55 °C and 7 MPa $scCO_2$, the complex was already finished in 1 h, and that was similar with the reaction in air at 75 °C. At 55 °C 0.1 MPa,

Table 3 Content of asarone in asarone–MBCD product

T (°C)	P (MPa)	Total ratio	Free ratio	Complex ratio	Physical form
T_{room}	0.1	1.02:1.00	0.96:1.00	0.06:1.00	White powder
55	0.1	1.05:1.00	0.11:1.00	0.94:1.00	White powder
55	5.0	0.95:1.00	0.01:1.00	0.94:1.00	White block
55	7.0	0.95:1.00	0.02:1.00	0.93:1.00	White granule
55	10.0	0.67:1.00	0.02:1.00	0.65:1.00	White granule
65	0.1	1.01:1.00	0.08:1.00	0.93:1.00	White powder
65	5.0	0.97:1.00	0.02:1.00	0.95:1.00	White granule
65	7.0	0.94:1.00	0.02:1.00	0.92:1.00	Light granule
65	10.0	0.51:1.00	0.01:1.00	0.50:1.00	Colored granule

The uncertainty of the determined value is ±5% except that the uncertainty is ±0.005:1.00 for the low free ratio

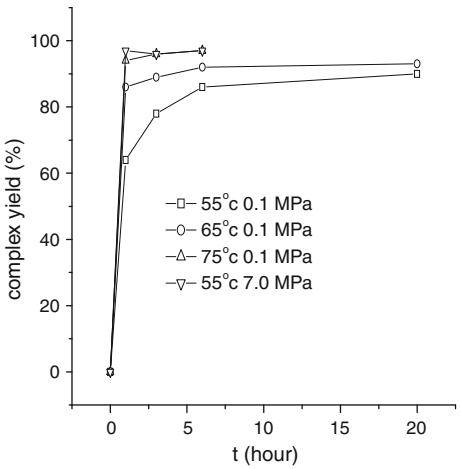

Fig. 6 Influence of reaction time on the complexing between asarone and MBCD

the solid asarone was gasified little by little and diffused into MBCD powders then form complex. In addition to the above approach, at 75 °C 0.1 MPa the asarone could melted and penetrated into MBCD matrix then reacted. In $scCO_2$ media at 55 °C and 7 MPa, the asarone–MBCD mixture was liquefied and complex. Thus at 75 °C 0.1 MPa or in 55 °C 7 MPa $scCO_2$, the complexing was much faster than the reaction at 55 °C 0.1 MPa.

X-ray diffraction pattern

The diffraction pattern of the physical mixture and the products was exhibited in Fig. 7.

The untreated asarone has a series of crystalline diffraction. In the physical mixture, the diffraction intensity of asarone at 14.8° was 4 time for the maximum diffraction of

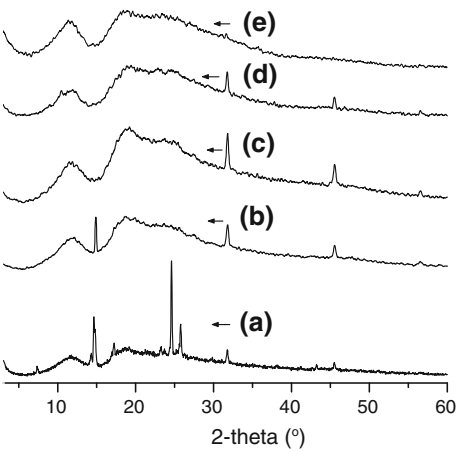

Fig. 7 X-ray diffraction pattern of asarone–MBCD: (*a*) asarone–MBCD mix, (*b*) asarone–MBCD 55 °C 0.1 MPa 1.0 h, (*c*) asarone–MBCD 55 °C 7.0 MPa 1.0 h, (*d*) asarone–MBCD 75 °C 0.1 MPa 1.0 h (*e*) MBCD orig

the MBCD. In the product reacted at 55 °C 0.1 MPa for 1 h, the diffraction of asarone at 14.8° was lowed about 60% and the other peaks was vanished. In the product reacted for 1 h in 55 °C 7 MPa $scCO_2$, all the diffraction of the crystal asarone was disappeared. Thus consisted with the quantitative results, about 60% asarone was complex with MBCD in this sealed heating product, and all the asarone was reacted with MBCD in the $scCO_2$ medium.

TG analysis result

The thermo-stability for the product reacted in air at 55 °C for 3 h or for the product reacted in $scCO_2$ at 55 °C 7 MPa for 3 h was displayed in Fig. 8. Under temperature lower than the glass transition temperature of MBCD, the asarone lost was less than 10% of the total asarone in the product. This result demonstrated that most of the asarone was included in MBCD cavities and a few asarone may be located between MBCD molecules in these products.

Aqueous solubility

The intrinsic and apparent aqueous solubility of asarone was determined and was list in Table 4.

The dissolution of pure asarone in water was slow and the dissolution of the product by $scCO_2$ processing was fast.

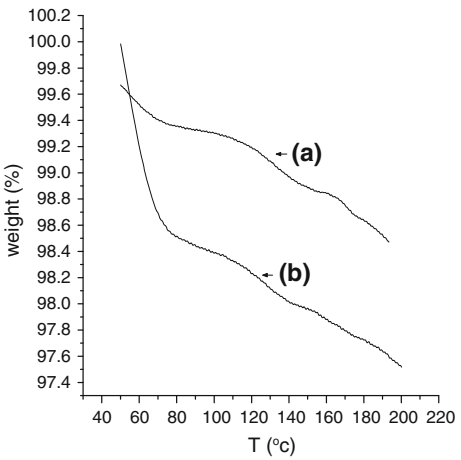

Fig. 8 TG thermogram of asarone–MBCD: (*a*) 50 °C 0.1 MPa 3.0 h, (*b*) 50 °C 7.0 MPa 3.0 h

With 6.6×10^{-2} mol/L MBCD, the aqueous solubility of asarone was enhanced about 3–5 times; in water, the molar ratio of the asarone to the total MBCD was about (0.03–0.05):1.00. Thus only part of the asarone molecule was included in the MBCD cavity and the long substituent on benzene may locate out of the cavity.

Preliminary study for the asarone–HPBCD complex

The complex between asarone and HPBCD was tested through both approaches. At 75 °C, more than 2/3 asarone was not complex with the HPBCD even in the $scCO_2$ media (Table 5).

The UV–vis spectrum of the physical mixture and the products in ethanol were displayed in Fig. 9. The spectrogram of the product processed in $scCO_2$ at 75 °C was the same with that of the mixture. The spectrogram of the product treated in air at 75 °C was quite different, two new absorption bands appeared around 232 and 297 nm. In their work, Yuan et al. [33] found that α-asarone could be dimerized into bisasaricin, the later has two UV absorption at 233 and 294 nm. Thus in our work, some side reaction such as dimerization already happened in air at 75 °C but was prevented in the $scCO_2$ media. When the temperature was raised to 95 °C, the side reaction could not be avoided even in $scCO_2$ media.

But this phenomenon was not found for the asarone–MBCD complex in air or in $scCO_2$ medium.

Table 5 Content of asarone in asarone–HPBCD product

T (°C)	P (MPa)	Total ratio	Free ratio	Complex ratio	Physical form
75	0.1	1.00:1.00	0.98:1.00	0.02:1.00	Aggregate powder
75	5.0	1.01:1.00	0.76:1.00	0.25:1.00	Aggregate powder
75	7.0	1.03:1.00	0.70:1.00	0.33:1.00	Aggregate powder
75	10.0	0.86:1.00	0.59:1.00	0.27:1.00	Aggregate powder

The uncertainty of the determined value is ±5% except that the uncertainty is ±0.005:1.00 for the low free ratio

Table 4 Solubility of asarone with or without MBCD in water

	Pure asarone	1:1 Product in air	1:1 Product in $scCO_2$
C_{MBCD} (10^{-3} mol/L)	0	66	66
$C_{asarone}$ (10^{-3} mol/L)[a]	0.62	2.4	3.4

[a] The uncertainty is ±10%

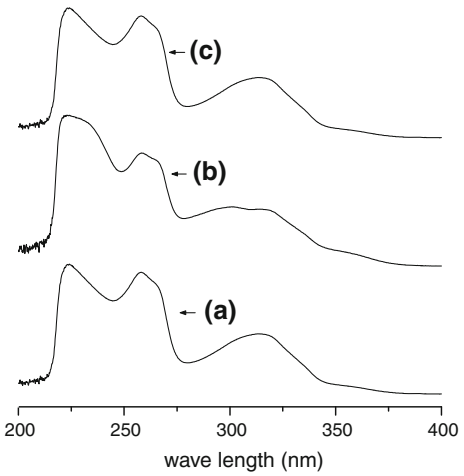

Fig. 9 UV–vis spectrum of asarone–HPBCD: (*a*) asarone–HPBCD mix, (*b*) asarone–HPBCD 75 °C 0.1 MPa, (*c*) asarone–HPBCD 75 °C 5.0 MPa

Brief summary

Asarone was a crystalline compound with a melting point (T_m) at 63 °C. In its molecule, there are three methoxyl groups and one propenyl group substituted on benzene; thus may bring some steric hindrances. Asarone could complete complex with MBCD in short reaction time: in air at temperature higher than its T_m, the asarone liquid can penetrated into flexible MBCD matrix and react; in $scCO_2$ at temperature even lower than its T_m, the asarone/MBCD mixture was melted and complex; for most of the asarone in the product, part of the molecule was included in the MBCD cavity and the long substituent on benzene may locate out of the cavity. When it reacted with HPBCD, which was a rigid solid in our experimental condition, the asarone molecule was difficult to diffuse into the HPBCD matrix and the complex yield was low even in $scCO_2$ medium; thus the steric effect was adequately expressed.

Curcumin–MBCD complex

Content of curcumin in the product

At constant reactant ratio, the effects of temperature and pressure on the complexing were investigated. At constant temperature and a series of pressure, the influence of reactant molar ratio was also studied. The curcumin contents and the physical form of the product were show in Table 6.

At first the molar ratio of curcumin to MBCD was fixed at 1:2. In 0.1 MPa air, the complex yield increased from 13 to 44% as the temperature raise from 100 to 120 °C and the product was in powder state. In $scCO_2$ medium at the same temperature, the complex yield could be improved to 80% without visible curcumin loss and the products were grains in most cases. At 140 °C, the amount of curcumin in the product was obviously decreased due to the unstable of curcumin at high temperature.

In sealed heating treatment, the curcumin solid was gasified bit by bit then enter the MBCD solid matrix and reacted, thus the complex yield was only 65% even at

Table 6 Content of curcumin in curcumin–MBCD product

T (°C)	P (MPa)	React ratio	Total ratio	Free ratio	Complex ratio	Physical form
100	0.1	1.00:2.00	0.95:2.00	0.82:2.00	0.13:2.00	Powder
100	7.0	1.00:2.00	0.99:2.00	0.72:2.00	0.27:2.00	Powder
100	10.0	1.00:2.00	0.98:2.00	0.30:2.00	0.68:2.00	Block
100	12.0	1.00:2.00	0.97:2.00	0.20:2.00	0.77:2.00	Grain
120	0.1	1.00:2.00	0.97:2.00	0.53:2.00	0.44:2.00	Powder
120	7.0	1.00:2.00	0.94:2.00	0.13:2.00	0.81:2.00	Grain
120	10.0	1.00:2.00	0.96:2.00	0.13:2.00	0.83:2.00	Grain
120	12.0	1.00:2.00	0.95:2.00	0.17:2.00	0.78:2.00	Grain
140	0.1	1.00:2.00	0.78:2.00	0.13:2.00	0.65:2.00	Powder
140	7.0	1.00:2.00	0.81:2.00	0.12:2.00	0.69:2.00	Grain
140	10.0	1.00:2.00	0.81:2.00	0.12:2.00	0.69:2.00	Grain
120	0.1	1.00:1.00	0.95:1.00	0.59:1.00	0.36:1.00	Powder
120	7.0	1.00:1.00	0.94:1.00	0.22:1.00	0.72:1.00	Grain
120	10.0	1.00:1.00	0.92:1.00	0.26::1.00	0.66:1.00	Grain
120	0.1	1.00:2.50	1.00:2.50	0.47:2.50	0.53:2.50	Powder
120	7.0	1.00:2.50	1.00:2.50	0.06:2.50	0.94:2.50	Grain
120	10.0	1.00:2.50	0.97:2.50	0.08:2.50	0.89:2.50	Grain

The uncertainty of the determined value is ±5% except that the uncertainty is ±0.005:1.00–0.005:2.50 for the low free ratio

140 °C, as the gasification of curcumin was low. In $scCO_2$ medium, part of the curcumin solid could be dissolved in MBCD melt and then complex, thus the complex yield was much enhanced.

Then the temperature was kept at 120 °C, the molar ratio of curcumin to MBCD was changed from 1:1 to 1:2.5. In 0.1 MPa air, the complex yield was slight increased; in $scCO_2$ at 7 MPa, the complexing was improved from 72 to 94%. A few curcumin was still left free at 1:2.5 reactant ratio, thus the complexing between curcumin and MBCD must be a equilibrate reaction and 1:1 complex was formed in most situation.

In previous work reported [4–6], in water curcumin formed 1:1 inclusion complex with BCD, HPBCD and MBCD; the association constant was only 1×10^2–4×10^2 L/mol. After inclusion, the X-ray diffraction peaks and DSC peak of curcumin were all disappeared for the complex.

X-ray diffraction pattern

The X-ray diffraction of pure curcumin, the physical mixture and 1:2 products was displayed in Fig. 10.

The curcumin has a lot of crystalline diffraction. The diffraction of the physical mixture was the superposition of the two separate, argued that there was no interaction between curcumin and MBCD in the mixture. In the gram of product made at 100 °C 0.1 MPa, the intensity of curcumin diffraction was almost equal to that in the pattern of the physical mixture; thus most of the curcumin in this product was free. In the spectrum of the product prepared at 120 °C 0.1 MPa, some of the curcumin diffraction still existed with reduced intensity and the diffraction of the MBCD was not changed, thus the curcumin must react with MBCD solid matrix and some of them were not complex. In the spectrogram of the product produced in 120 °C and 7 MPa $scCO_2$, the curcumin diffraction was low and the diffraction pattern of MBCD was altered, thus the curcumin should react with the MBCD melt and most of them were complex. These were in accordance with the quantitative analysis results.

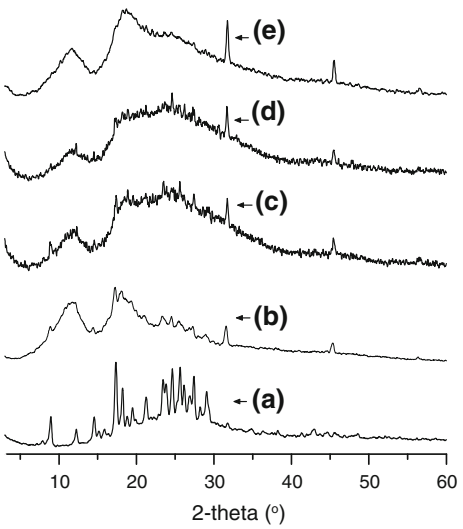

Fig. 10 X-ray diffraction of curcumin–MBCD: (*a*) curcumin untreated, (*b*) curcumin–MBCD mix, (*c*) curcumin–MBCD 1:2 100 °C 0.1 MPa, (*d*) curcumin–MBCD 1:2 120 °C 0.1 MPa, (*e*) curcumin–MBCD 1:2 120 °C 7.0 MPa

DSC analysis results

The thermogram of the product obtained at 120 °C was demonstrated in Fig. 11.

The 1:1 product made in air dehydrated from 40 to 110 °C and the free curcumin in the product melted at 167 °C. As the curcumin's melting peak was broaden and shift from 183 to 167 °C, the particle size of the free curcumin was evidently reduced. After treated with $scCO_2$, a lot of uncomplex curcumin also existed in the 1:1 product. While the molar ratio of curcumin to MBCD was adjusted to 1:2.5, a smaller peak of free curcumin could still be observed. Thus the DSC analysis affirmed that the interaction between curcumin and MBCD was comparatively weak and the complex between curcumin and MBCD was not completed even after reacted at 1:2.5 reactant ratio in $scCO_2$ medium for 20 h.

Aqueous solubility

The aqueous solubility of curcumin with or without MBCD was listed in Table 7.

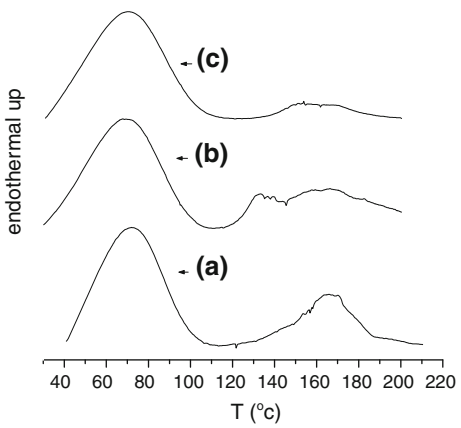

Fig. 11 DSC thermogram of curcumin–MBCD: (*a*) curcumin–MBCD 1:1 120 °C 0.1 MPa, (*b*) curcumin–MBCD 1:1 120 °C 7.0 MPa, (*c*) curcumin–MBCD 1:2.5 120 °C 7.0 MPa

Table 7 Solubility of curcumin with or without MBCD in water

Product	C_{MBCD} (10^{-3} mol/L)	$C_{curcumin}$ (10^{-5} mol/L)[a]	Enhancement
Pure curcumin	0	0.04	
Physical mixture	0.17	0.7	18
	6.8	33	800
1:1 120 °C 7.0 MPa	0.16	1.1	28
1:2.5 120 °C 7.0 MPa	0.17	2.2	55
	6.7	55	1375

[a] The uncertainty is ±10%

The intrinsic solubility of curcumin reported in literature was in 3×10^{-8}–8×10^{-6} mol/L [4–6], the solubility determined in our work was 4×10^{-7} mol/L. In the presence of MBCD at 1.7×10^{-4} mol/L, the solubility of curcumin in water could be enhanced about 17, 27 and 54 times respectively for the physical mixture, the product prepared by sealed heating treatment and the product produced by $scCO_2$ processing. With 6.7×10^{-3} mol/L MBCD, the aqueous solubility of curcumin could be amplified about 800 and 1,400 times separately for the physical mixture and the product made by $scCO_2$ processing. Thus the apparent solubility of curcumin was distinctly improved by complex with the MBCD.

Preliminary study for the curcumin–HPBCD complex

The content of curcumin in the product was showed in Table 8. As HPBCD was a rigid matrix in our experimental condition, the complex yield was lower than 25%, even if curcumin and HPBCD was reacted at 1:2.5 molar ratio in $scCO_2$ medium at 120 °C.

Brief summary

As shown in Fig. 1, two derivatives of benzene have been linked together by a chain of 7 carbon atoms in curcumin molecule. Its melting point is 183 °C and its solubility in $scCO_2$ is low. In both processing at 100–140 °C, the complexing between curcumin and MBCD was a equilibrate reaction and 1:1 complex was formed in most situation. In sealed heating treatment, the curcumin solid was gasified bit by bit and then reacted with MBCD solid matrix. In $scCO_2$ medium, part of the curcumin solid could be dissolved in MBCD melt and complex, thus the complex yield was markedly enhanced. As one of the benzene derivatives in curcumin molecule was outside the MBCD cavity, its apparent solubility in water was still low even after it was enhanced for about 1×10^3 times by complexing with MBCD.

As HPBCD was a rigid matrix, the complex yield of curcumin was quite low in our experimental condition.

Conclusion

Anisole, asarone and curcumin are three derivatives of benzene. Anisole is a sheet benzene substitute; asarone is a benzene molecule substituted with three methoxyl groups and one propenyl group; curcumin is two benzene derivatives linked together by a chain of 7 carbon atoms. In this work, the complexing between these hydrophobic efficacious components of Traditional Chinese Medicine and modified-β-cyclodextrin were investigated both in air and in $scCO_2$ medium.

Via sealed heating treatment and $scCO_2$ processing, anisole can easily complex with both MBCD and HPBCD at 50–80 °C and 0.1–7 MPa. At the same condition, the complex yield for anisole–HPBCD reaction was a little lower compared with that of anisole–MBCD reaction. In $scCO_2$ medium, the main part of the anisole formed inclusion complex with MBCD molecules and most of the anisole formed association with HPBCD matrix. Asarone could complete complex with MBCD in short reaction time in air at temperature higher than its T_m or in $scCO_2$ at temperature even lower than its T_m. For most of the asarone in the product, the aromatic part of the molecule was included in the MBCD cavity and the long substituent on benzene was out of the cavity. When it reacted with HPBCD, which was a rigid solid in our experimental condition, the asarone molecule was difficult to enter the HPBCD matrix even in $scCO_2$ medium at temperature higher than its T_m. For curcumin, the complexing between it and MBCD was a equilibrate reaction and 1:1 complex was formed in most cases. The complex was not completed

Table 8 Content of curcumin in curcumin–HPBCD product

T (°C)	P (MPa)	React ratio	Total ratio	Free ratio	Complex ratio	Physical form
120	0.1	1.00:2.50	0.98:2.50	0.81:2.50	0.17:2.50	Powder
120	7.0	1.00:2.50	0.96:2.50	0.79:2.50	0.17:2.50	Powder
120	10.0	1.00:2.50	0.97:2.50	0.78:2.50	0.19:2.50	Powder
120	12.0	1.00:2.50	1.00:2.50	0.76:2.50	0.24:2.50	Powder

The uncertainty of the determined value is ±5% except that the uncertainty is 0.005:2.50 for the low free ratio

even after reacted at 1:2.5 curcumin–MBCD molar ratio for 20 h in $scCO_2$. At least one of the benzene derivatives in curcumin molecule was outside the MBCD cavity in the product. And the complex yield for curcumin was quite low, even if curcumin and HPBCD was reacted at 1:2.5 molar ratio in $scCO_2$ medium.

In $scCO_2$ media, when the three drugs reacted with MBCD, they all can complex with the MBCD molecule, but the complex yield and the structure of the product was different; the substitute effect was partly exhibited due to the liquefaction of MBCD. In $scCO_2$ when the drugs reacted with HPBCD, the sheet benzene substituent—anisole was easy to be complex; the benzene derivate substituted with three methoxyl groups and one propenyl group—asarone was difficult to enter the rigid matrix; and the complex between curcumin and HPBCD was quite low, as curcumin is two benzene derivatives linked together by a chain of 7 carbon atoms. As HPBCD was a rigid solid in our experimental condition, the substitute effect was adequately expressed due to the space hinders.

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References

1. Weng, H.X., Wang, C.L., Ma, L.: Comparison between two approaches to determine the recovery of volatile oil from their β-cyclodextrin inclusion complex. Her. Med. **25**(1), 63–64 (2006)
2. Li, S.Z., Zhao, H.X., Bai, W.G.: The study for the methodology to prepare the inclusion complex between asarone volatile oil and β-cyclodextrin. Chin. J. Basic Med. Tradit. Chin. Med. (Chin.) **9**(2), 63 (2003)
3. Ma, R., Liu, Q., Xiao, L.: Preparation technology of volatile oils obtained from asarum and lily magnolia complexed with β-cyclodextrin and hydroxypropyl-β-cyclodextrin and its impacts of transdermal absorption. Chin. J. Inf. TCM (Chin.) **15**(6), 52 (2008)
4. Gao, Z.S., Wang, L.: Studies on preparation and physicochemical properties of inclusion complex of curcumin with HP-β-cyclodextrin. China Pharm. (Chin.) **18**, 999–1000 (2007)
5. Liu, Y.S., Wang, X.L.: Formulation and preparation of curcumine methyl-β-cyclodextrin inclusion complex. Med. J. Chin. People's Armed Police Forces (Chin.) **18**(5), 337–339 (2007)
6. Yadav, V.R., Suresh, S., Devi, K., Yadav, S.: Effect of cyclodextrin complexation of curcumin on its solubility and antiangiogenic and anti-inflammatory activity in rat colitis model. AAPS PharmSciTech. **10**(3) (2009). doi:10.1208/s12249-009-9264-8
7. Kamihira, M., Asai, T., Yamagata, Y., Taniguchi, M., Kobayashi, T.: Formation of inclusion complex between cyclodextrins and aromatic compounds under pressurized carbon dioxide. J. Ferment. Bioeng. **69**, 350–353 (1991)
8. 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)
9. Charoenchaitrakool, M., Dehghani, F., Foster, N.R.: Utilization of supercritical carbon dioxide for complex formation of ibuprofen and methyl-β-cyclodextrin. Int. J. Pharm. **239**, 103–112 (2002)
10. 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)
11. 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)
12. 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)
13. Bandia, N., Weib, W., Robertsc, C.B., Kotrac, L.P., Kompellaa, U.B.: Preparation of budesonide and indomethacin-hydroxypropyl-β-cyclodextrin (HPBCD) complexes using a single-step, organic-solvent-free supercritical fluid process. Eur. J. Pharm. Sci. **23**, 159–168 (2004)
14. Wu, M., Yuguchi, Y., Kumagai, T., Endo, T., Hirotsu, T.: Nanocomplex formation of cyclodextrin and azobenzene using supercritical carbon dioxide. Chem. Commun. 1288–1289 (2004). doi:10.1039/b400289j
15. 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)
16. 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)
17. 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)
18. 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)
19. 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)
20. Arezki, B., Elisabeth, R., Jacques, F.: Maturation of ketoprofen/β-cyclodextrin mixture with supercritical carbon dioxide. J. Supercrit. Fluids **41**, 429–439 (2007)
21. 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)
22. 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)
23. 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)
24. 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. Fluids **44**, 400–408 (2008)
25. 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)
26. Sauceau, M., Rodier, E., Fages, J.: Preparation of inclusion complex of piroxicam with cyclodextrin by using supercritical carbon dioxide. J. Supercrit. Fluids **47**, 326–332 (2008)
27. Al-Marzouqia, A.H., Elwya, H.M., Shehadib, I., Ademc, A.: Physicochemical properties of antifungal drug–cyclodextrin complexes prepared by supercritical carbon dioxide and by conventional techniques. J. Pharm. Biomed. Anal. **49**, 227–233 (2009)
28. He, J.: Complex of shikonin and β-cyclodextrins by using supercritical carbon dioxide. J. Incl. Phenom. Macrocycl. Chem. **63**, 249–255 (2009)
29. He, J., Li, W.J.: Complex formation of cinnamaldehyde-methyl-β-cyclodextrin and muscone-methyl-β-cyclodextrin by supercritical carbon dioxide processing and sealed heating method. J. Incl. Phenom. Macrocycl. Chem. **63**, 61–68 (2009)
30. He, J., Li, W.J.: Preparation of borneol–methyl-β-cyclodextrin inclusion complex by supercritical carbon dioxide processing. J. Incl. Phenom. Macrocycl. Chem. **65**, 249 (2009)
31. Reilly, J.T., Kim, C.H., Clark, A.B., Donohue, M.D.: High pressure vapor-liquid equilibria of aromatic hydrocarbons with carbon dioxide and ethane. Fluid Phase Equilib. **73**, 81–107 (1992)
32. Cortesi, A., Kikic, I., Alessi, P., Turtoi, G., Garnier, S.: Effect of chemical structure on the solubility of antioxidants in supercritical carbon dioxide: experimental data and correlation. J. Supercrit. Fluids **14**, 139–144 (1999)
33. Yuan, Y.S., Wang, C.W., Zhou, X.Y.: The study for the active component in rhizome acori graminei. Chin. Tradit. Herb. Drugs **13**(9), 3–4 (1982)