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ORIGINAL ARTICLE

Complex formation of cinnamaldehyde-methyl-β-cyclodextrin and muscone-methyl-β-cyclodextrin by supercritical carbon dioxide processing and sealed heating method

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Abstract Firstly, the interaction between cinnamaldehyde (CIN) and methyl-β- cyclodextrin (MBCD) was studied in aqueous solution. 1:1 inclusion complex was formed and the association constant was $187 \pm 9\ M^{-1}$. Then the complex of CIN–MBCD and muscone–MBCD was prepared both by sealed heating method and by supercritical carbon dioxide (sc CO_2) approach. Complete complex was obtained by both methods for CIN–MBCD. Some CIN molecules was weakly associated with MBCD molecules in products by sealed heating method, all CIN molecules was strongly associated with MBCD molecules in products by sc CO_2 processing. Complete complex between muscone and MBCD was not obtained. The choice for the size of guest molecule still existed for MBCD cavity in sealed heating method and sc CO_2 processing.

Keywords Cinnamaldehyde · Muscone · Methyl-β-cyclodextrin · Complex · Supercritical carbon dioxide

Introduction

Cinnamaldehyde (CIN), a major effective component of cinnamon oil, is a viscous liquid effective for antibacterial, anti-inflammation, antivirus, antitumour and anticancer [1–6]. Its aqueous solubility is low and it is easy to be oxidized when exposed to air for long time. This restricts its pharmaceutical use and its bioavailability. To improve the properties and to enhance the aqueous solubility, CIN has been included in some cyclodextrins. Inclusion complex of CIN and β-cyclodextrin (β-CD) was prepared by Zhang via co-precipitation method [7]. Inclusion complex between CIN and modified γ-CD (ethylcarbonate-γ-CD and octylcarbonate-γ-CD) was obtained using freeze-drying method by Carlotti et al. [8]. These complex all need multi-step processing. In recent year, some one step and clear chemistry methods were proposed, such as the sealed heating method and the supercritical carbon dioxide (sc CO_2) technique [9–27].

In most sc CO_2 processing, β-CD with a melting point about 300 °C was used as the host and most drugs were solid; thus, the drugs interacted with cyclodextrins through gas–solid approach. In some cases, high temperature or pressure was needed to get enough dissolution for the drugs in carbon dioxide. In other cases, some water was needed to maturate the drug/cyclodextrin mixture. The imazalil (T_m = 55 °C)–β-CD complex was prepared at 65 °C and 150–200 bar by Lai et al. and characterized by ^{1}H-NMR in aqueous solution and ^{13}C-NMR in solid state [13]. The complex of geraniol (or mustard oil) and cyclodextrins (α-CD, β-CD, γ-CD) was obtained at 20 °C and pressure not higher than 50 bar by Kobayashi et al. [9]. They found that 16–28% CD moisture content was necessary for the complex formation and almost no complex formation between the oils and modified CDs (hydropropyl-β-CD and methyl-β-CD) was found in this condition. The ibuprofen–MBCD complex was produced by Foster et al. by passing ibuprofen/CO_2 solution through MBCD bed, the melting temperature of MBCD was found to be depressed a lot in carbon dioxide media [11]. The complex between some active pharmaceutical ingredients and crystalline trimethyl-β-CD was investigated by Moribe et al. [24]. Drug's complex depended on both the sc CO_2 treatment time and

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drug solubility in CO_2, suggesting the complex processed after dissolving of both components in sc CO_2 media.

In our previous work, inclusion complex of borneol and MBCD was successfully obtained in sc CO_2 media. In this work, the interaction between CIN and MBCD in aqueous solution was first studied; due to the higher solubility of MBCD in aqueous solution, the complex of CIN and MBCD was then investigated both in sc CO_2 approach and in sealed heating method to search possible approaches to get solid complex between viscous liquid drugs and MBCD. For comparing, muscone, a viscous liquid drug with large flexible alicyclic structure (Fig. 1), was also used as guest; the choice of the host cavity for the size of the guest molecule was observed.

Materials and methods

Materials

Cinnamaldehyde 98.8%, was produced by Beijing Chemical Company. Muscone, 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. Carbon dioxide with a purity of 99.95% was supplied by Beijing Analytical Instrument Factory. Ethanol and cyclohexane, analytical grade, were produced by Beijing Chemical Company.

Phase solubility study

The stoichiometry and association constant between CIN and MBCD in aqueous solution were determined by phase solubility method described by Higuchi and Connors [28] at room temperature. The CIN–MBCD mixture was stirred and protected from light for 20 h. The solubility of CIN in MBCD solution (0–50 $\times$ 10^{-3} M) was determined by UV absorbency at 289 nm with two replicates. Assuming that the stoichiometry of CIN–MBCD complex was 1:1, the association constant (K_{assoc}) was calculated by using the following equation:

$$K_{assoc} = \frac{slope}{Co(1 - slope)}$$

where the slope is obtained from the linear regression of the molar concentrations of CIN in solution versus the molar concentration of cyclodextrin in the solvent, Co is the intrinsic CIN solubility (13 $\times$ 10^{-3} M) in water.

^{1}H-NMR study

^{1}H-NMR spectra of deuterated aqueous solutions were recorded at 400 MHz, on a Bruker AV-400 spectrometer, at 20 °C. The chemical shift of DOH ($\delta = 4.79$ ppm) was used as internal reference. The samples were CIN (12 $\times$ 10^{-3} M), MBCD (20 $\times$ 10^{-3} M) and CIN (26 $\times$ 10^{-3} M)–MBCD (20 $\times$ 10^{-3} M), and were bubbled with N_2 before being determined.

Preparation of the physical mixtures

The CIN and MBCD with a molar ratio of 1.00:1.00 was mixed and ground in a mortar with a pestle. The physical mixtures of muscone and MBCD with a molar ratio of 1.00:1.00 and 1.00:2.00 were prepared in the same way.

Preparation of the complex in sc CO_2

100 mg physical mixture was put into a high pressure stainless-steel vessel (10 mL), the vessel was sealed and heated to the desired temperature. Then carbon dioxide was

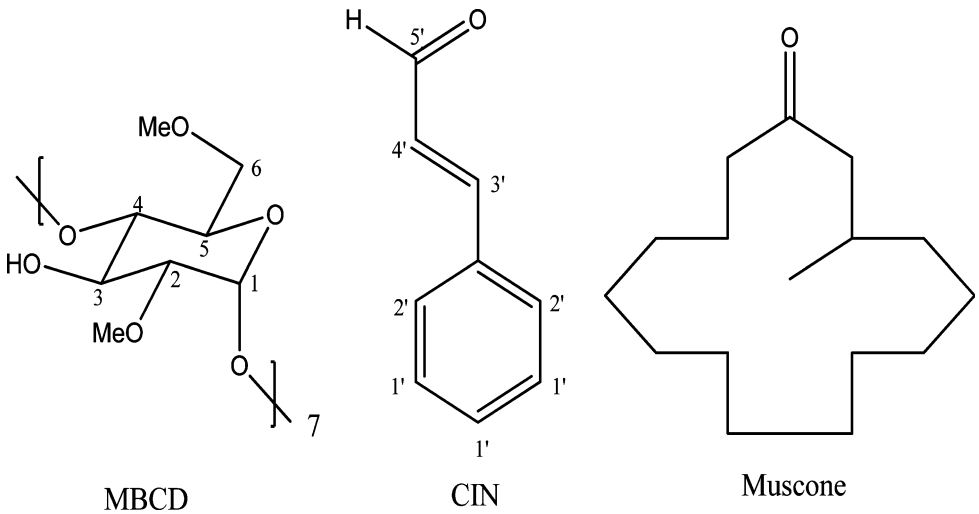

Fig. 1 Molecular structure of MBCD, CIN and muscone

pumped into the vessel to the desired pressure. The content was left in static for 20 h except for the study of the reaction time effect. At the end, the vessel was depressed to atmospheric pressure within 2 min and the solid products in the vessel were obtained.

In sealed heating method, the complex was prepared in similar way but without carbon dioxide.

Quantitative analysis

The total and uncomplexed CIN content in the products were analysed by UV–VIS spectroscopy method with a TU-1901 UV–VIS spectrograph. The absorbency of CIN peak was calibrated with CIN/ethanol solutions and CIN/cyclohexane solutions of known concentration.

To determine the total content of CIN in product, accurately weighted product (about 10 mg) was dissolved in 100 mL ethanol and stirred thoroughly, the absorbency was determined at 285 nm.

To measure the content of free CIN in product, suitable amount of products were crushed. Accurately weighted product (about 10 mg) was dispersed in 50 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 281 nm.

The total and uncomplexed content of muscone in the products were analysed in similar way. To determine the total content of muscone in product, accurately weighted product (about 50 mg) was dissolved in 4 mL ethanol and determined at 206 nm. To measure the free muscone content, about 50 mg crushed product was dispersed in 4 mL cyclohexane and determined at 216 nm.

Power X-ray diffraction (PXRD)

The structure of MBCD and the products were analysed by a RIGAKU D/MAX 2500 X-ray diffract-meter, the 2θ scan range was 3–60°, the scan rate was 8°/min, with Cu kα radiation.

Differential scanning calorimetric analysis

The thermal analysis of some products was 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.

Thermal stability of the products

Some crushed product was put in glass bottle covered with alumina film and kept in a thermostat oven at 60 °C for desired period, and then the total CIN content was analysed.

Aqueous solubility determination

Solubility of pure CIN and some complexes in water were determined by UV–VIS spectroscopy method.

Excess amount of pure CIN was put in deionized water in a glass conical bottle with a glass stopple, the content was protected from light, stirred thoroughly for 8 h, left in static at room temperature for 4 days, and then the aqueous phase was centrifuged at 3,200 rpm for 20 min and was used for analysis.

Products of CIN–MBCD with molar ratio about 1.0:1.0 were put in deionized water under stirring until obvious indissoluble oil was observed. The content was also centrifuged and the aqueous phase was accurately diluted for analysis.

The solubility of muscone and its products was analysed in similar way.

Results

CIN–MBCD

Phase solubility studies

First, the UV–VIS absorption spectra of CIN in aqueous solutions containing increasing concentration of MBCD ($0–5 \times 10^{-2}$ M), keeping the concentration of CIN fixed (2×10^{-5} M), were registered. No change was found for the maximum peak (at 289 nm) intensity of CIN. Then the phase solubility studies were carried out and the result was displayed in Fig. 2. R of the linear regression was 0.9987, thus, the stoichiometry of CIN–MBCD complex was 1:1. And, the calculated association constant was $187 \pm 9\ M^{-1}$.

^{1}H-NMR results

The ^{1}H-NMR spectrum for MBCD with or without CIN in deuterated aqueous solution was shown in Fig. 3 and Table 1. The ^{1}H-NMR spectrum for CIN with or without MBCD in deuterated aqueous solution was shown in Fig. 4 and Table 2. The ^{1}H-NMR spectrum for pure MBCD was the same with ^{1}H-NMR spectrum for 2,6-dimethyl-β-cyclodextrin reported in the literatures [29–31]. Thus, the ^{1}H-NMR peaks were assigned to different H atoms in MBCD as the same as that in the literatures. With CIN, the ^{1}H resonance for H-3 and H-5 located at the inner portion of MBCD cavity were shifted upfield, while the ^{1}H chemical shift for H-6 outside the MBCD cavity was unmoved. At the same time, with MBCD the ^{1}H resonance for H-1′, H-2′, H-3′, H-4′ of CIN were obviously shifted upfield while the ^{1}H chemical shift for H-5′ of CIN was constant. Thus, in aqueous solution, the aryl group of CIN was included in the

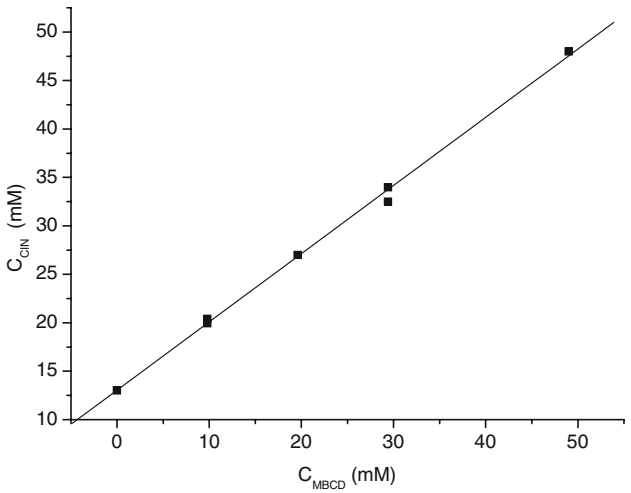

Fig. 2 Solubility of CIN in MBCD aqueous solution

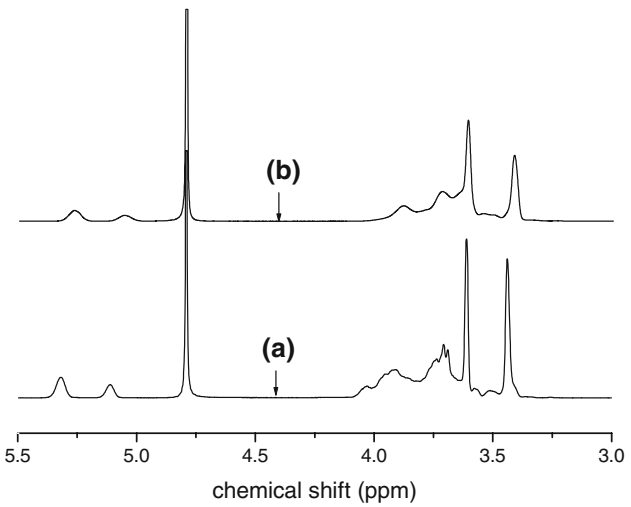

Fig. 3 ^{1}H-NMR spectrum for MBCD: (**a**) MBCD (20 mM) and (**b**) CIN (26 mM)–MBCD (20 mM)

Table 1 ^{1}H-NMR chemical shift for MBCD with or without CIN in aqueous solution

	1-H	3-H	5-H	6-H	2-OCH_3	6-OCH_3
MBCD	5.319	4.023	3.929	3.711	3.610	3.439
CIN–MBCD	5.262	3.876		3.712	3.603	3.409
$\Delta\delta$ (ppm)	−0.057			0.001	−0.007	−0.030

$\Delta\delta = \delta_{CIN-MBCD} - \delta_{MBCD}$

MBCD cavity and the carbonyl group of CIN was outside the MBCD cavity. The 1:1 inclusion complex between CIN and MBCD was formed in aqueous solution.

Content of CIN in solid products

The MBCD is high soluble in ethanol ($\geq$0.2 M) and almost insoluble in cyclohexane ($<1.5 \times 10^{-6}$ M). CIN can mixed

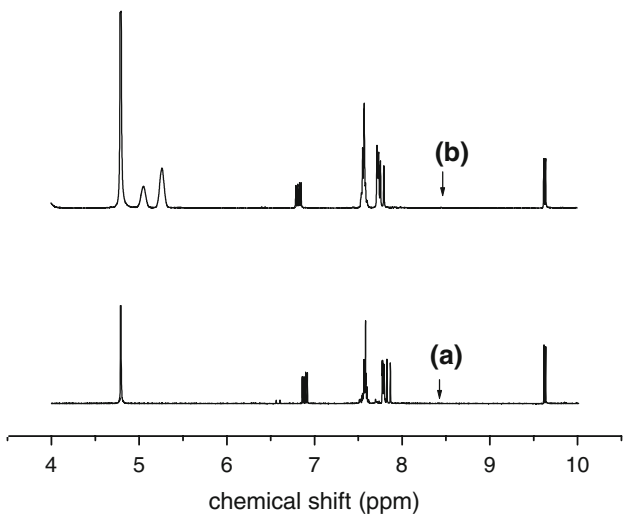

Fig. 4 ^{1}H-NMR spectrum for CIN: (**a**) CIN (12 mM) and (**b**) CIN (26 mM)–MBCD (20 mM)

Table 2 The ^{1}H-NMR chemical shift for CIN with or without MBCD in aqueous solution

	1′-H	2′-H	3′-H	4′-H	5′-H
CIN	7.577	7.784	7.845	6.889	9.629
CIN–MBCD	7.559	7.718	7.764	6.819	9.633
$\Delta\delta$ (ppm)	−0.018	−0.066	−0.081	−0.070	0.004

$\Delta\delta = \delta_{CIN-MBCD} - \delta_{CIN}$

with ethanol at any ratio and is high soluble in cyclohexane ($\sim$0.8 M). Muscone can mix with ethanol and cyclohexane at any ratio. The UV spectrums were given in Fig. 5.

The content of CIN in CIN–MBCD products was shown in Table 3. In the physical mixture, there was already some interaction between CIN and MBCD. The complex efficiency increased with temperature rise from 50 to 70 °C, almost complete complexation was obtained at 70 °C 1 bar. When CO_2 was added in up to 70 bar, the free CIN was dissolved in sc CO_2 and the complex yield was almost kept constant, then the complex efficiency decreased at higher pressure. At 50–70 °C, the product was still in powder state by sealed heating method and the products was transparent granule or blocks by sc CO_2 processing.

The influence of the reaction time on the complex yield and the physical format of the product was listed in Table 4. At 100 °C 1 bar, the complex was almost completed in 1 h. The CIN molecules penetrated into the MBCD solid first and then gradually swelled and melted the MBCD matrix. In sc CO_2 media, the MBCD was melt, mixed with the CIN liquid/gas and form CIN–MBCD complex. At the end of reaction, the CO_2 was discharged from the system rapidly and the structure of the products was fixed.

PXRD analysis

The effect of sc CO_2 on the X-ray diffraction pattern of pure MBCD and the CIN–MBCD products was displayed in Fig. 6. After treated with sc CO_2, the comparative intensity of peaks at 11.5 and 18.0° were obviously increased for pure MBCD, resulting from the rearrangement of molecules in MBCD melt. The X-ray diffraction pattern of CIN–MBCD product by sealed heating method was similar with that of untreated MBCD except for a little bit sharpen at 18.0°. The X-ray diffraction pattern of product by sc CO_2 processing was obviously enhanced at 18.0°, similar to that of sc CO_2 treated MBCD, indicating the melting of the mixture in sc CO_2 media.

DSC analysis results

DSC thermo-grams of untreated MBCD and CIN–MBCD products were shown in Fig. 7. The endothermic peak up to 110 °C was resulted from the dehydration of MBCD and the changes around 170 °C could be attributed to the glass-transition of MBCD. The new peaks from 120 to 160 °C

Table 4 Influence of reaction time on CIN–MBCD products

T (°C)	P (bar)	t (h)	Free content	Physical format
100	1	1	0.02:1.00	Aggregated powder
100	1	3	0.007:1.00	Block
100	1	6	0.008:1.00	Wax block

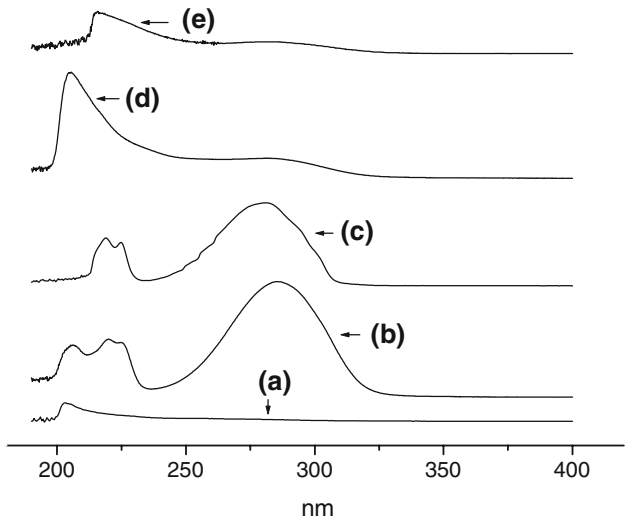

Fig. 5 UV spectra of (**a**) MBCD in ethanol (8.4×10^{-3} M); (**b**) CIN in ethanol (5.3×10^{-5} M); (**c**) CIN in cyclohexane (2.8×10^{-5} M); (**d**) muscone in ethanol (6.4×10^{-3} M); (**e**) muscone in cyclohexane (3.2×10^{-3} M)

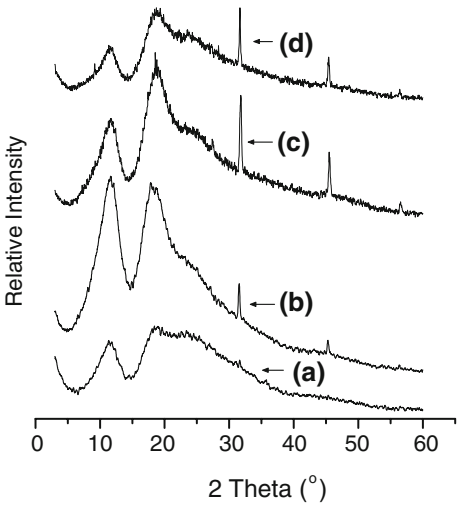

Fig. 6 X-ray diffraction pattern of MBCD and CIN–MBCD products (**a**) untreated MBCD, (**b**) MBCD treated in 80 °C 160 bar sc CO_2, (**c**) CIN–MBCD treated in 50 °C 50 bar sc CO_2, (**d**) CIN–MBCD treated at 60 °C 1 bar

Table 3 Molar ratio of CIN in CIN–MBCD products

T (°C)	P (bar)	Total content	Free content	Complex content	Physical format
Physical mixture	1	1.00:1.00	0.45:1.00	0.55:1.00	Powder
50	1	1.01:1.00	0.13:1.00	0.88:1.00	Powder
50	50	0.93:1.00	0.001:1.00	0.93:1.00	Grain
50	70	0.90:1.00	0.007:1.00	0.90:1.00	Block
60	1	1.04:1.00	0.07:1.00	0.96:1.00	Powder
60	50	0.95:1.00	0.006:1.00	0.95:1.00	Grain
60	70	1.01:1.00	0.009:1.00	1.00:1.00	Grain
60	100	0.80:1.00	0.015:1.00	0.78:1.00	Grain
70	1	1.05:1.00	0.06:1.00	0.97:1.00	Powder
70	50	0.98:1.00	0.003:1.00	0.98:1.00	Grain
70	70	0.85:1.00	0.001:1.00	0.85:1.00	Grain
100	1	0.95:1.00	0.01:1.00	0.94:1.00	Brown block

The uncertainty is ±5% of the determined values, except for the uncertainty for lower free content is ±0.002:1.00

might suggest the formation of complex between CIN and MBCD both in sealed heating processing and in sc CO_2 media.

Thermal stability

As shown in Fig. 8, about half of the CIN was lost for the physical mixture after heated at 60 °C for 6 h, more than 10% loss of CIN was found for sealed heating product and no loss was observed for products by sc CO_2 processing for 6 days. The thermal stability of product by sc CO_2 technique was the same with that of the CIN–β-CD inclusion complex produced by freeze-drying method [27], although the melting temperature of MBCD is about 120 °C lower than that of β-CD. Thus, CIN molecules should also be included in MBCD cavities in sc CO_2 media; and more than 10% of CIN molecules were weakly associated with MBCD molecules in products by sealed heating method.

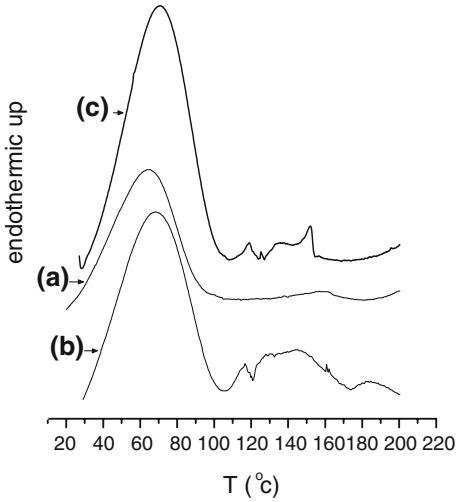

Fig. 7 DSC thermo-gram of MBCD and CIN–MBCD products (**a**) untreated MBCD, (**b**) product of sealed heating method at 60 °C 1 bar, (**c**) product of sc CO_2 processing at 50 °C 50 bar

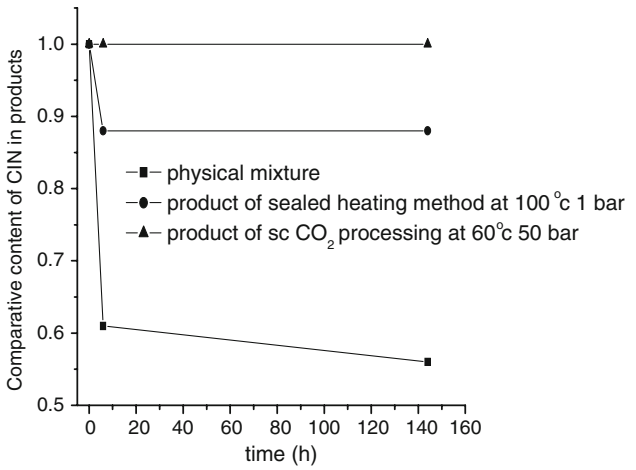

Fig. 8 Thermostability of CIN–MBCD (■) physical mixture,(●) product of sealed heating method at 100 °C 1 bar,(▲) product of sc CO_2 processing at 60 °C 50 bar

Aqueous solubility

The aqueous solubility of CIN is 1.3×10^{-2} M; the aqueous solubility of CIN could be raised to 2.4×10^{-1} M for product with molar ratio of 1.0:1.0; the solubility of CIN in water was enhanced about 20 times by complexing with MBCD.

Muscone–MBCD

As shown in Table 5, no obvious interaction between muscone and MBCD was found in the physical mixtures. The molar ratio of muscone to MBCD is about 2.0:3.0 for 1.0:1.0 reactants, is 0.9:2.0 for 1.0:2.0 reactants both by sealed heating method and by sc CO_2 processing. Complete complexation was not obtained and the products were in powder state similar to that of the physical mixtures.

Complex between muscone and β-CD was obtained by Song et al. via grinding [32]. The molar ratio of muscone to BCD in products is about 1:40 for 1:9 reactants. Complex between muscone and β-CD was also prepared by adding 20×10^{-3} g muscone to aqueous solution containing 10 g BCD but still not completed [33]. Thus, the association between muscone and β-CD was in cluster way.

In sealed heating method and sc CO_2 processing, the complex yield between muscone and MBCD was remarkably enhanced. The aqueous solubility of pure muscone is 2.7×10^{-3} M; the aqueous solubility of muscone could be raised to 1.3×10^{-2} M for our 2:3 products and 2.1×10^{-2} M for our 1:2 products. The solubility was enhanced only 5–8 time, thus, the muscone in our products should also associated with MBCD molecules in cluster way.

Conclusion

Cinnamaldehyde, also called as β-phenylacrolein (M = 132.16, d = 1.045–1.053, Tb = 250–252 °C), has a planar structure showed in Fig. 1 due to the large π bond. The width of the aryl group (the distance between the two H-2′) is small than the diameter of β-CD cavity. Thus, CIN penetrated into MBCD solid or dissolved in MBCD melts, could enter the cavity to form inclusion complex with MBCD. Muscone, 3-methyl-cyclopentadecanone (M = 238.42, d = 0.922, Tb = 328 °C), has a large alicyclic structure larger than the size of β-CD cavity. At the same time, the 3D conformation of muscone may change with time due to the free rotating of –C–C– bond in gaseous and

Table 5 Molar ratio of muscone in muscone–MBCD products

Physical mixture	*T* (°C)	P (bar)	Total content	Free content	Complex content
1.0:1.0	Mix	1	0.85:1.00	0.68:1.00	0.17:1.0
1.0:1.0	70	1	0.87:1.00	0.21:1.00	2.0:3.0
1.0:1.0	70	50	0.87:1.00	0.24:1.00	1.9:3.0
1.0:1.0	70	70	0.87:1.00	0.24:1.00	1.9:3.0
1.0:1.0	100	1	0.87:1.00	0.13:1.00	2.2:3.0
1.0:2.0	Mix	1	1.05:2.00	0.95:2.00	0.10:2.00
1.0:2.0	70	1	0.99:2.00	0.10:2.00	0.89:2.00
1.0:2.0	70	50	1.05:2.00	0.17:2.00	0.88:2.00
1.0:2.0	70	70	1.05:2.00	0.08:2.00	0.97:2.00
1.0:2.0	100	1	0.99:2.00	0.10:2.00	0.89:2.00

The uncertainty is ±5% of the determined values, except for the uncertainty for lower free content is ±0.02:1.00

liquid state. Thus, it is also difficult to form inclusion complex for one muscone molecule with two MBCD molecules in sealed heating method and sc CO_2 processing.

These analyses were consistent with our experimental results. 1:1 inclusion complex of CIN–MBCD was formed in aqueous solution. Complete complex was obtained for CIN–MBCD both by sealed heating method and in sc CO_2 media. Some CIN molecules was weakly associated with MBCD molecules in products by sealed heating method, all CIN molecules was strongly associated with MBCD molecules should be included in MBCD cavities in products by sc CO_2 processing. Complete complexation was not obtained, the muscone molecules still associated with MBCD molecules in cluster way. Thus, the choice for the size of guest molecule still existed for MBCD cavity in both sealed heating method and sc CO_2 processing.

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