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Influence of supercritical CO_2 pressurization on the phase behavior of mixed cholesteryl esters

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ABSTRACT

Evidences indicating the presence of phase transformations in the mixed cholesteryl benzoate (CBE) and cholesteryl butyrate (CBU) under the supercritical CO_2 pressurization, by means of differential scanning calorimetry (DSC) and X-ray diffraction (XRD), are presented in this work. These include (1) the DSC heating curve of pure CBU; (2) the DSC heating curves of CBU/CBE mixtures; (3) the XRD spectra of pure CBU; (4) the XRD spectra of CBU/CBE mixtures; (5) CBU and CBE are miscible in either solid phase or liquid phase over the whole composition range. As a result of the presence of these phase transformations induced by pressurization, it could be deduced that a solid solution of the CBU/CBE mixture might have formed at the interfaces under supercritical conditions, subsequently influencing their dissolving behaviors in supercritical CO_2.

1. Introduction

Cholesterol presents one of the essential biochemical compounds in the living body, and is involved in many biofunctional tasks, including its most notable roles as the precursor for in vivo synthesis of bile acids and steroid hormones, and a regular component of cellular membranes that maintains their flexibility and proper transport properties (Maxfield and Tabas, 2005). However, a large body of evidence (Ludwig et al., 1997; Wilson et al., 1998; Breslow, 2000) has indicated a strong link between elevated blood cholesterol levels and coronary heart disease as reflected by the large amount of low-cholesterol foods available in the market. Hence, it is important to find reliable methods to reduce the cholesterol content in the foods for human consumption. These methods include conventional Soxhlet extraction, chromatographic separation, supercritical fluid extraction and other solvent purification methods. At this regard, benign supercritical CO_2 extraction seems promising as it possesses many desirable features for separating and purifying desired components from natural products and foods. Therefore, a number of recent studies have been contributed to the cholesterol reduction by using supercritical fluid extraction methodology from cattle brain (Vedaraman et al., 2004, 2008), cow brain (Vedaraman et al., 2005), shrimp (Higuera-Ciapara et al., 2005), pork (Lin et al., 1999), chicken meat (Froning et al., 1994), turtle fish egg (Shen et al., 2008), egg yolk (Bringe, 1997; Froning et al., 1998), butter oil (Mohamed et al., 1998, 2000) and squid oil (Kang et al., 2005). These researches have indicated that supercritical fluid extraction technology holds promise for substantially reducing cholesterol contents and thus offers an alternative to current methods of developing low-cholesterol foods. In fact, supercritical fluid technology is currently no longer a laboratory curiosity as it is successfully commissioned for caffeine, tea, spices, hops and flavor extraction process on large commercial scale in USA and Europe.

In order to facilitate the development of efficient supercritical fluid extraction technology, fundamental solubility data for various cholesterols in supercritical fluids are a necessary starting point for any process design. In previous studies (Huang et al., 2008, 2009), we have investigated the dissolving behavior of cholesteryl butyrate and cholesteryl benzoate solid mixture in supercritical CO_2 and found that both of the two solutes have become greatly less soluble in the presence of another solute and the solubility compression is heavily dependent on the feed composition used in the SFE process. These unusual dissolving behaviors might be related to the phase transition induced by high-pressurized supercritical carbon dioxide. However, the formation of a liquid phase is not observed for the solute mixture investigated and the solid particles still remain intact as evidenced from visual examination after each experimental run. Thus, it is necessary to understand the thermal behavior and structural properties of the chosen cholesteryl ester binary systems before and after processed by high-pressure CO_2.

In this work, we have examined the influence of supercritical CO_2 pressurization on the phase behaviors of the CBU/CBE solid mixture through the techniques of differential scanning calorime-

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try and X-ray diffraction analyses. The results thus obtained may be useful to better understand the observed mutual solubility diminution of both CBU and CBE in supercritical CO_2.

2. Experimental

2.1. Materials

The compounds used in this study were purchased from commercial suppliers: cholesteryl butyrate (Fluka Chemicals, USA, TLC grade, 99+ %), cholesteryl benzoate (Aldrich Chemicals, USA, 98+ %), and carbon dioxide (Beijing Ya-Nan Gas Pte Ltd. China, China, 99.95%). These materials were directly used as received without further purification.

2.2. Sample preparations

For fully investigating the supercritical CO_2 on the thermal behavior of two cholesteryl esters, two series of the samples were prepared as follows.

The samples in series A, physically mixed CBU and CBE at different mass ratios under ambient condition, were prepared by weighing out the required amount of each component and putting it into a container, which was then stirred vigorously for at least one hour to make sure that the powders were well mixed before measurements.

The samples in series B, pressurized by supercritical CO_2, were prepared by physically mixing CBU and CBE in a vigorous way, then loading the mixture into a high-pressure vessel, followed by feeding supercritical CO_2 into the vessel at around 20 MPa and 318.1 K and holding it for about 1 h.

For the sake of convenience, we define X_u as the mass fraction of cholesteryl butyrate in the solid mixture. Thus the chosen compositions used in the study were 25, 33, 40, 50, 60, 67 and 75, respectively.

2.3. DSC measurements

The phase behavior of binary cholesteryl ester solid mixtures was fully studied with differential scanning calorimeter apparatus Netzsch DSC 200. The standard sample of indium with a melting point of 429.7 K was used for routine instrument calibrations. Note that the calibration was carried out under identical conditions required for the CBU/CBE mixture. A sample of ca. 1.0–2.0 mg was measured, transferred to an aluminum pan, then hermetically sealed with a presser before placed in the furnace chamber. The analysis was carried out at a scanning rate of 5 K/min from room temperature, uniformly heated up, then directly cooled down to room temperature by using liquid nitrogen. High purity N_2 at a rate of 50 ml/min was employed as a purge gas as well as a protective gas.

Apart from phase behavior investigation by DSC measurements, a study of the melting-point depression of CBU and CBE was also carried out using a static technique. A glass tube loaded with about 1 g of the solute was placed inside a 10 cm^3 high-pressure view cell with sapphire windows for phase transition visualization. Carbon dioxide after liquefied was fed into the view cell in a temperature-controlled oven and the system was pressurized to 10, 15, 20 and 25 MPa, respectively. The temperature was initially increased very fast to approach the melting point and then continuously increased at 2 K increments until the last solid particle melted. The system was equilibrated for at least 10 min after each temperature increment. The melting point was determined by visual observation and the temperature at which the whole solute melted was then recorded.

2.4. XRD characterization

In concert with DSC analysis, powder XRD analysis, recorded on a Shimadzu XRD-6000 spectrometer, was performed to study the effect of moderately high pressure used in the SFE process on the phase crystalline structure of CBU/CBE solid mixtures. The XRD spectra measurements were carried out at room temperature by using Cu Kα radiation of 1.5406 å, 40 kV, and 40 mA with a scanning rate of 0.02°/s. The diffraction angle, 2θ, used ranges from 5° to 35°.

3. Results and discussion

3.1. DSC results

Starting with transitions occurring on cooling process, it was observed that there exist two distinct transitions for all the samples in Series A and B as well as the pure esters. It is well known (Gray, 1962; Davis et al., 1970; Merritt et al., 1971; Galanti and Porter, 1972; Griffen and Porter, 1973; Saeva, 1979) that cholesteryl esters represent one type of important liquid crystals – cholesteric liquid crystals, thus making their thermal responses more complex than simple eutectic mixtures. Upon cooling the ester mixture was observed to have experienced in the sequence of isotropic liquid → cholesteric mesophase → solid phase changes. As seen from

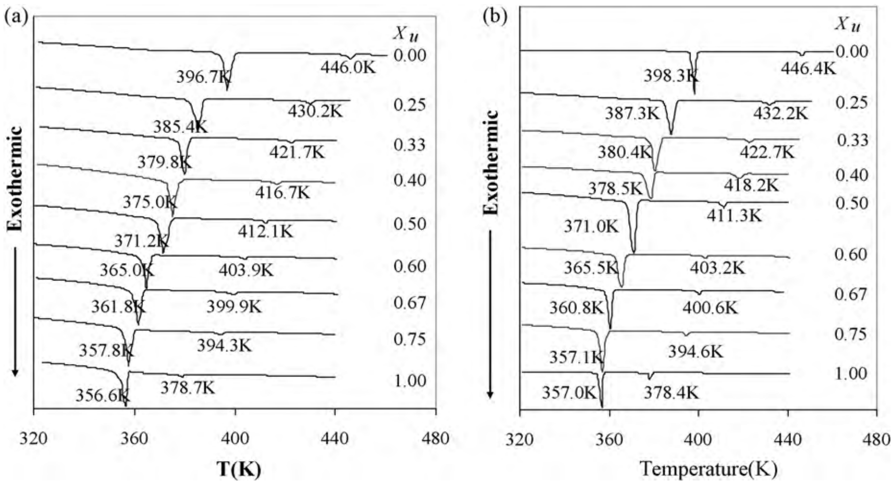

Fig. 1. DSC cooling thermo-diagrams of the CBU/CBE binary system before (a) and after (b) pressurized by supercritical CO_2.

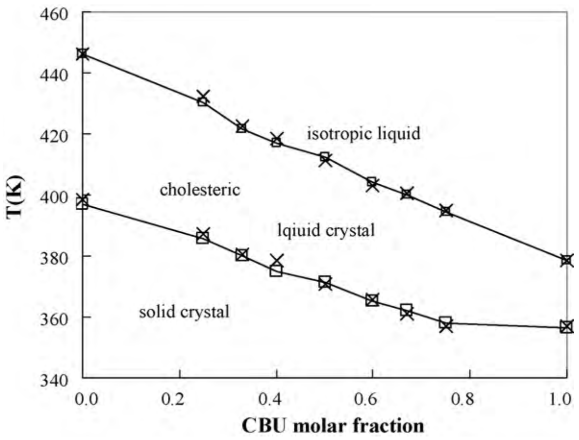

Fig. 2. Phase diagram of the CBU/CBE binary system established from cooling profiles: before (□) and after (×) pressurized by supercritical CO_2.

Fig. 1a, the two transition temperatures are 356.6 and 378.7 K for pure CBU, 396.7 and 446.0 K for CBE, respectively. For the CBU/CBE mixtures, it can also be seen from Fig. 1a, their transition temperatures lie between those of pure esters and they approach those of pure CBE as the benzoate content increases. The cooling thermograms for the sample after processed by high-pressure supercritical CO_2 are shown in Fig. 1b. A careful comparison of Fig. 1a and b shows that the transition temperature for the samples considered is not varied before and after supercritical CO_2 treatment. The phase diagram thus established is shown in Fig. 2. It can be clearly observed that there are three phase domains, i.e., solid phase, cholesteric liquid crystal phase and isotropic liquid phase, over the entire range of composition. Therefore, it can be deduced from the phase diagram that CBE and CBU are miscible to each other either in the liquid or solid phase. Besides, the two transition temperatures vary approximately linearly with the content of benzoate. The results shown in Fig. 2 also suggest that the transition temperatures determined for the samples in Series A and B are identical to each other within experimental errors, indicating that the two compounds are miscible since they exhibit the same phase transitions as they are cooled from the isotropic liquid state.

The multiple transitions obtained by heating are more complicated than the case for cooling. We may be aware of that a significant temperature lag is observed in the cooling process and thus the transition temperatures obtained are obviously lower than those obtained from the heating process. Fig. 3 shows the DSC curves for the mixed solids without supercritical CO_2 pressurization, i.e., the samples in Series A. It is evident that pure CBE has two mesomorphic transition temperatures at 422 and 451 K, which correspond to solid–cholesteric liquid crystal and cholesteric liquid crystal–isotropic liquid transitions, respectively. The values agree well with those previously reported (Merritt et al., 1971; Saeva, 1979). Similarly, based on the heating process, pure CBU exhibits a solid–cholesteric transition temperature at 372 K and a cholesteric–isotropic transition temperature at 383 K, which are close to those earlier reported (Davis et al., 1970; Merritt et al., 1971; Saeva, 1979).

It can be found from Fig. 3a that pure CBU experiences a delicate dual solid–cholesteric mesophase transition over a few degrees of at 370–375 K, which may have not been reported previously and probably be due to the small nature of the textural transition. It is also found that these dual cholesteric textural transitions were broadened in the presence of CBE. As the benzoate content increases, its solid–cholesteric transition could be observed. This transition peak is very broad but seems to narrow down as more CBE is mixed. Following it, a transition of cholesteric–isotropic liquid for the whole sample is observed at a higher temperature. These two solid–cholesteric and cholesteric–isotropic liquid transition temperatures are observed to move to higher values, approaching those of pure CBE as more benzoate is added. It may be noted that the broadness of transition peaks for the binary mixture is due to the presence of the other component.

For the samples in series B that have been subjected to high pressures through supercritical CO_2, their thermal responses are seen to be quite different from those not exposed to high pressures, as shown Fig. 3b. For pure CBU, its dual cholesteric mesophase changes at 370–375 K cannot be observed and the transformations observed are only solid–cholesteric mesophase and cholesteric mesophase–isotropic liquid transitions, indicating that pressurized supercritical CO_2 has significantly affected the crystal structure of pure CBU. Similarly, the pressure effect on the polymorphic transitions for cholesteryl esters has also been reported in early work (Gray, 1962), where, e.g., the solid–smectic transition of cholesteryl myristate is found to be 341 K at 8 MPa but 345 K under 22 MPa. Recently, several studies (Lin et al., 2000; Bettini et al., 2001; Tozuka et al., 2003; Shinozaki et al., 2006) have shown that the polymorphs of pharmaceuticals could be varied in high-pressure supercritical CO_2 and thus supercritical CO_2 treatment may be useful to realize the polymorph conversion of desired compounds.

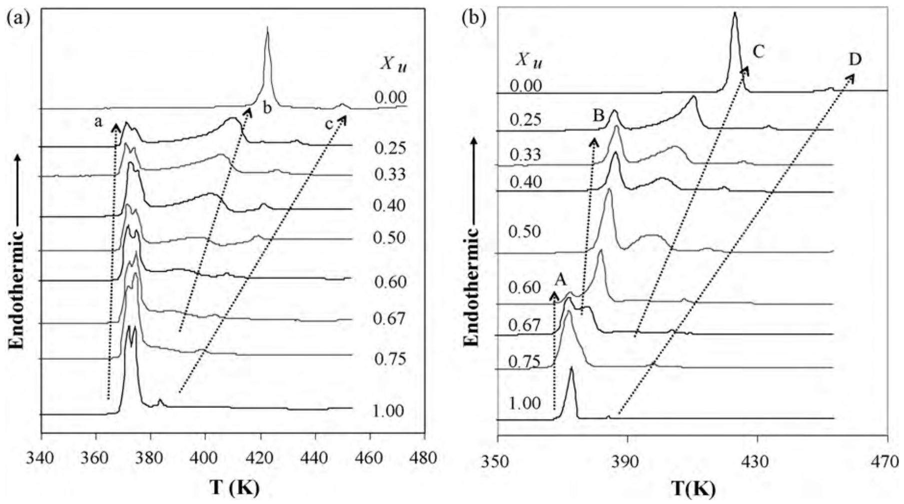

Fig. 3. DSC heating thermo-diagrams of the CBU/CBE binary system before (a) and after (b) pressurized by supercritical CO_2.

For solid mixtures, when the benzoate content is below 25 mass%, two transitions are observed but the solid–cholesteric transition peak is broadened with CBE serving as the impurity, as compared with that for pure CBU. When the CBU content decreases to 67 mass%, a new third transition, as marked by "B" arrow in Fig. 3b, is observed between the other two transition temperatures. With more benzoate present in the mixture, its solid–cholesteric transition could be clearly observed whereas the solid–cholesteric transition peak of CBU becomes weak and is no longer observed at higher CBE content. When the benzoate content exceeds 50 mass%, three separate transitions are clearly seen. The two endothermic transitions observed at the higher temperatures unambiguously correspond to the solid–cholesteric and cholesteric–isotropic liquid transitions of CBE. The other transition peak obtained at about 382–387 K, as marked by arrow "B", is not seen for the sample without supercritical CO_2 pressurization. This new transition is possibly due to the presence of a CBU/CBE solid solution, which may have been partially formed after subjecting the CBU/CBE mixed solids to the high-pressure CO_2. However, the mechanism for crystal polymorph transformation is not so far clearly understood. The possible explanation to it may be that the molecules of two compounds have similar molecular sizes and chemical structures so that they may have interacted strongly with each other at the interfaces of their respective crystal phases under high pressure of around 20 MPa. Consequently, the appreciated interactions may render the butyrate and benzoate molecules reorder on the crystallographic sites of the interfacial region, then leading to the formation of the CBU/CBE solid solution at the interface region during the highly pressurizing process.

Presented in Fig. 4 are the phase diagrams constructed from heating profiles for the CBU/CBE mixtures with or without high pressurization. A careful examination of Fig. 4 indicates that the phase diagrams built from high transition temperatures are basically similar for the two cases and the solid–cholesteric liquid crystal and cholesteric liquid crystal–isotropic liquid transitions vary linearly between those of pure cholesteryl butyrate and benzoate. For the samples in Series A, the dual cholesteric transitions characteristic of pure CBU are retained, indicating the existence of cholesteryl butyrate in the CBU/CBE solid mixture.

In contrast, for the samples in Series B, the phase diagram appears to be quite different. As described above, a solid solution of CBU/CBE may be formed after subjected to high-pressure supercritical CO_2. When the butyrate content varies between 60 and 67 mass%, four transformations can be observed in the order of increasing temperature: solid–cholesteric liquid crystal transition of CBU, solid–cholesteric liquid crystal transition of possibly formed CBU/CBE solid solution, solid–cholesteric liquid crystal transition of CBE, and cholesteric liquid crystal–isotropic liquid transition of CBU/CBE mixture. When butyrate content is below 50 mass%, its solid–cholesteric liquid crystal transition is not observed any longer but the other three transitions still retain.

The dissimilarity in phase diagrams observed for the CBU/CBE mixture in two series implies that subjected to high-pressure supercritical CO_2 treatment, the strong interactions between butyrate and benzoate molecules may have resulted in the partial formation of a solid solution at interfacial regions of CBU/CBE solid mixture, which in turn affect their thermal transitional behaviors.

Besides the above DSC measurements, the phase transition of CBU or CBE was also studied in the presence of highly compressed CO_2. Subsequently, the melting-point depression was visually obtained for two compounds. The melting point (i.e., solid–cholesteric liquid crystal transition temperature) of CBU was depressed to 361, 354, and 352 K while its cholesteric liquid crystal–isotropic liquid transition was observed to be 371, 363 and 360 K at 10, 15, and 20 MPa, respectively. In the case of CBE, the melting temperature was decreased to 410, 407 and 401 K when the solute was present in pressurized CO_2 at 15, 20, and 25 MPa, respectively. As for its cholesteric liquid crystal–isotropic liquid transition, the temperature obtained was 438, 429, and 423 K, respectively. The melting-point depression in supercritical CO_2 has been reported to readily lead to the occurrence of a phase transition and sometimes produce a liquid phase in the system investigated (Chung and Shing, 1992). Thus the melting-point depression observed for CBU/CBE may indicate it is possible for the partial formation of a binary solution in the presence of highly compressed supercritical CO_2.

3.2. X-ray diffraction result

X-ray diffraction was used primarily to identify structure differences in the pure and mixed crystals for samples in two series of A and B. Fig. 5 shows the XRD patterns for pure cholesteryl butyrate, benzoate and the mixed solids without going through high CO_2 pressurization. The benzoate has three strong characteristic peaks around $2\theta = 16.9^\circ$, 17.6° and 18.9° while the butyrate has three strong peaks at $2\theta = 6.1^\circ$, 8.6° and 17.6°, respectively. Thus the two esters have different crystal parameters and crystal structures. The XRD results for pure esters are consistent with earlier study (Merritt et al., 1971). For the mixed samples, their XRD patterns are regularly varying between those of pure esters and can be simply obtained by a weighted average of the contribution from two esters. It is worthwhile to note that the strong peak observed

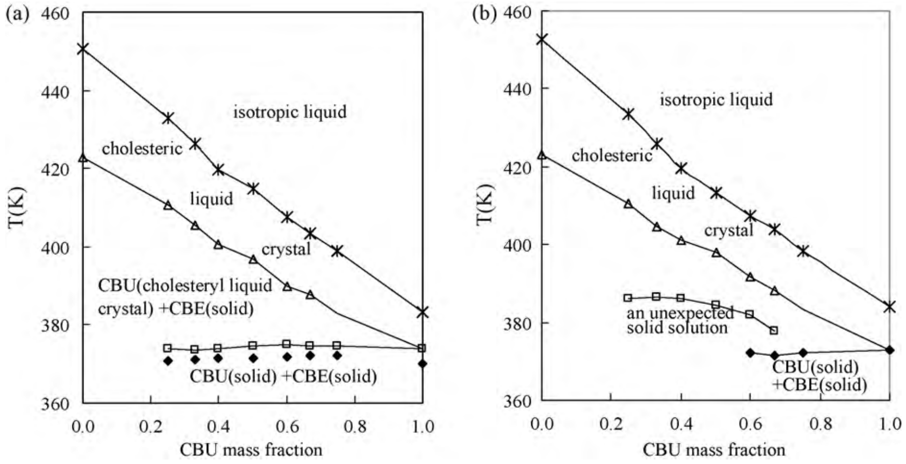

Fig. 4. Phase diagram of the CBU/CBE binary system established from heating profiles: before (a) and after (b) pressurized by supercritical CO_2.

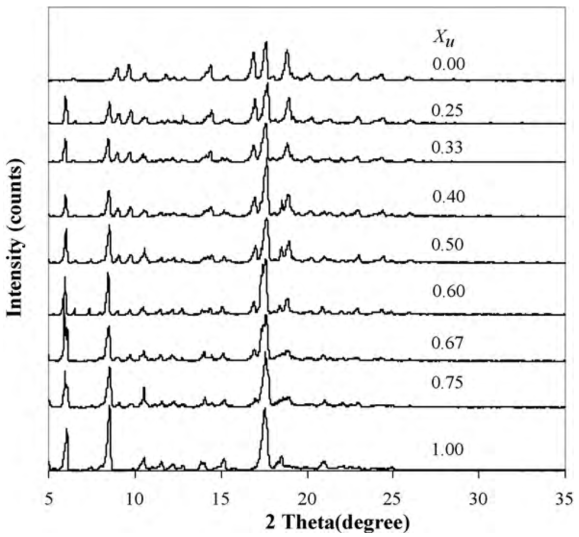

Fig. 5. XRD patterns of the physically mixed CBU/CBE samples.

at about $2\theta = 6.1^\circ$ applies to all the samples in Series A, except the pure benzoate.

Fig. 6 shows the XRD patterns of the samples in series B that are subjected to high-pressure supercritical CO_2. As compared with Fig. 5, it is readily observed that XRD patterns of pure CBU are considerably different before and after high-pressure treatment. This in turn suggests that, in the process of supercritical CO_2 pressurization, pure CBU solid crystal sites have reoriented to form a new crystal structure, and then experienced a phase transition. However, the XRD patterns of pure CBE show little change, indicating that its crystal structure has not been affected by CO_2 pressurization. An examination of Fig. 6 shows the XRD patterns for the mixtures vary systematically with composition between those of two pure solids. When the butyrate content increases the patterns shift to those of pure CBU whereas the patterns become more similar to those of pure CBE when the benzoate content increases. A comparison of Figs. 5 and 6 reflects that the strong peak noticeably observed at $2\theta = 6.1^\circ$ disappears but a new strong peak as marked by “A” appears at around $2\theta = 18^\circ$ for all the solid mixtures treated by the supercritical CO_2. These XRD pattern differences seemingly imply that the crystal structure of the mixture has changed and a phase transition thus may have occurred after the CO_2 pressurization treatment.

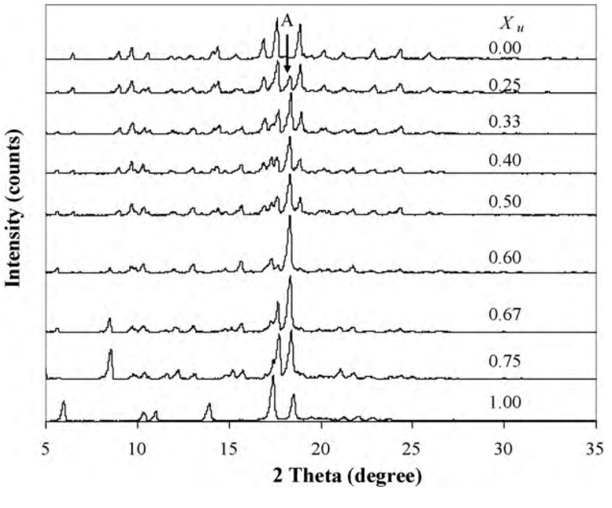

Fig. 6. XRD patterns of the physically mixed CBU/CBE solid samples processed by high-pressure supercritical CO_2.

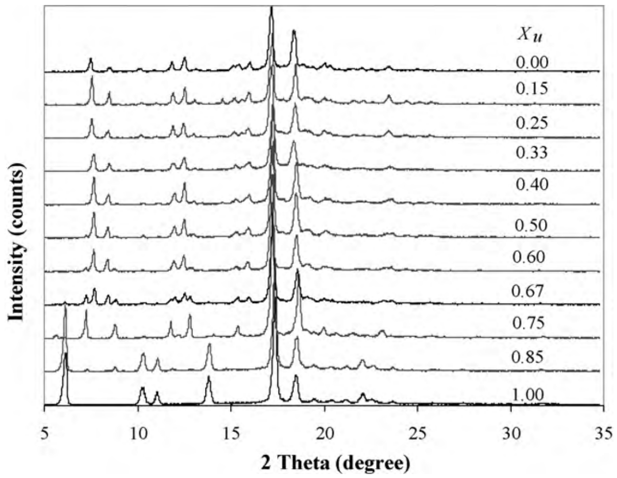

Fig. 7. XRD patterns of the melted CBU/CBE samples.

For better understanding the above XRD results, we have also measured the samples obtained from the melted solid mixtures, and the resultant XRD patterns are shown in Fig. 7. It can be seen from Figs. 5–7 that XRD spectra of the melted butyrate are almost identical with those treated by the CO_2 pressurization but distinct from those without CO_2 processing. For the melted CBE, its spectra are greatly different from those obtained for commercial ones with or without CO_2 treatment. These analysis results confirm that these two esters are polymorph materials (Merritt et al., 1971), and high-pressure CO_2 can render butyrate converse its crystal polymorph. The dissimilar XRD patterns observed for the two compounds further indicate that their crystal structures are not exactly the same. It can also be seen from Fig. 7 that all the samples show two strong peaks around $2\theta = 17.2^\circ$ and 18.4°, and these two peak values shift from those of pure CBU to CBE basically in a linear way. This characteristic pattern seems to be consistent with our previous DSC results that cholesteryl butyrate and benzoate are miscible in all range of composition investigated. A further analysis of the XRD patterns shows that, when the butyrate content increases from around 50 mass%, some changes in the diffractional patterns are seen in the range $6^\circ < 2\theta < 9^\circ$. For example, the shoulder peak ($2\theta = 7.4^\circ$) of the peak at $2\theta = 7.7^\circ$ is clearly seen at equal mass of CBU/CBE. When the butyrate content increases to 67 mass%, the two peaks observed for the butyrate content <50 mass% are seen to split into four peaks. When the butyrate content increases to 85 mass%, the XRD patterns are almost identical as those for pure cholesteryl butyrate.

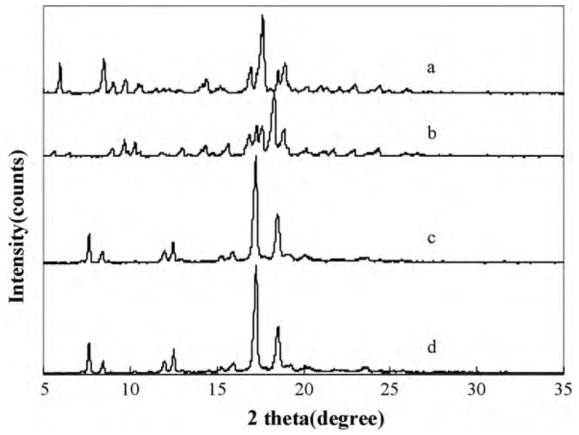

Fig. 8. XRD patterns of the mixture with 40 mass% CBU: (a) physically mixed; (b) physically mixed and followed by CO_2 treatment; (c) melted; (d) melted and followed by CO_2 treatment.

Fig. 8 compares the XRD results for the mixture with 40 mass% butyrate prepared by different conditions. It can be seen that the melted sample retains its XRD spectra after pressurization treatment. However, for the physical mixed sample, its XRD spectra are seen to have experienced a very obvious change after supercritical CO_2 treatment. The treated sample is observed to exhibit a peak at $2\theta = 18.4^\circ$, which is also seen in the melted samples. The presence of this unique XRD peak seems to suggest a polymorphic conversion after treated by highly pressurization. These results agree with our previous DSC result, evidencing that the physically mixed CBU/CBE solids may have formed a solid solution under supercritical CO_2 treatment.

4. Conclusions

In this study, we have made a detailed analysis of the CBU/CBE binary mixtures by using DSC and XRD to systematically examine the effect of high-pressure supercritical CO_2 on their thermal behaviors. The main conclusions of this work can be summarized as follows:

(1) DSC analysis results evidence that cholesteryl butyrate and benzoate are miscible to each other in either solid phase or liquid phase in the entire composition range.
(2) The thermal behavior of physically mixed CBU/CBE is found to have changed rather drastically after supercritical CO_2 pressurization as compared with those samples without treatment. The pressurization effect has resulted in the occurrence of the new exothermic peak, suggesting that the phase transformation of the mixtures has significantly varied and that a solid solution might have been formed at the interfaces under high supercritical pressure.
(3) The XRD results reflect that the XRD characteristic spectra are regularly varying between those of pure esters based on their respective mass ratios for the physically mixed CBU/CBE samples. Similar observations also apply to the melted samples.
(4) But for the physically mixed samples after supercritical CO_2 treatment, their XRD spectra are quite different from those of the other two groups, in very good consistence with DSC results. This further evidences that the crystal structure of the solid mixtures could have changed through the high pressurization, and formed a solid solution at mixed solid interfaces.

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