Complex Formation between Deoxycholic Acid and Menadione by Grinding and Sealed Heating Methods

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Complex formation between deoxycholic acid (DCA) and menadione (MND) by grinding and sealed heating was investigated. The complex between DCA and MND was prepared by coprecipitation and showed a stoichiometry of 2:1 (DCA:MND). The IR spectrum of the complex suggested that MND molecules were located in the channel formed by DCA molecules. The 10 min ground mixture of DCA–MND gave the same X-ray diffraction pattern as the DCA–MND coprecipitate. Differential scanning calorimetry (DSC) and IR spectroscopy also demonstrated the identical results between the ground mixture and the complex, confirming the complex formation between DCA and MND by grinding. The DSC data suggested that the complex formation was expected during heating process of the DCA–MND physical mixture. The sealed heated samples were prepared and compared with the complex prepared by coprecipitation. After heating in a sealed ampoule at 110 °C for 10 min or at 80 °C for 24 h, samples demonstrated the same X-ray diffraction patterns, DSC curves and IR spectra as the complex. These results indicated that the complex formation took place between DCA and MND by sealed heating as well as by grinding.

Key words deoxycholic acid; menadione; complex; grinding; sealed heating; sublimation

Deoxycholic acid (DCA), 3α,12α-dihydroxy-5-β-cholan-24-oic acid, is one of bile acids which have an ability to form complexes with a variety of chemical compounds such as fatty acid,[1] aromatic compound,[2] ketone,[3] and organometallic compound.[4] The crystal structures of DCA–guest inclusion compounds can be classified into three crystal systems, orthorhombic, tetragonal and hexagonal.[5] The orthorhombic system is characterized by a pleated antiparallel bilayer of DCA molecules stabilized by hydrogen bond network between carboxyl groups and hydroxy groups. The outer surface of the bilayer, covered with the methyl group of DCA, is hydrophobic channel in which guest molecules (preferably non polar) can be accommodated. This channel size can be adjusted depending on the size of guest molecules resulting in the effective complexation with many compounds. The chemical stability of some compounds can be changed by the complexation. Lach and Pauli reported the stabilization of benzocaine by complexation with DCA against hydrolysis.[6] The stability of the autooxidizable compounds, vitamin K[7] and vitamin A[8] was also improved by complexation with DCA.

Menadione (MND), vitamin K_3, is a vitamin K compound used in the treatment and prevention of hemorrhage associated with vitamin K deficiency.[9] MND was reported to form a complex with DCA with a stoichiometric ratio of 2:1 (DCA:MND) by Guillory *et al.*[10] and Pathipvanich.[11] Few other details have been investigated in depth.

Up to now, DCA–guest complexes have usually been prepared by coprecipitation from solution. This method, however, has some disadvantages because some solvents may be included in the channel in place of the guest compounds and some guest compounds may be decomposed during the preparation process.

Grinding was proposed as an alternative means of preparing cyclodextrin complexes.[12-14] A sealed heating method was recently introduced as a new method for preparing technique and the effect of cyclodextrin types, heating temperature, moisture content and guest–host molar ratio were studied.[15-17]

The aim of the present study was to investigate the possible complex formation between DCA and MND by grinding and sealed heating and to compare with the coprecipitate. Details of the DCA–MND complexes were also extensively evaluated by X-ray diffractometry, thermal analysis and IR spectroscopy.

Experimental

Materials DCA and MND were purchased from Nacalai Tesque, Kyoto (Fig. 1). All other chemicals were of analytical reagent grade and used without further purification.

Preparation of Complex by Coprecipitation Method DCA and MND (2:1 molar ratio) were dissolved in methanol at 55 °C. The solution was cooled to 4 °C to allow coprecipitation. The coprecipitate was filtered out with a filter paper and dried over phosphorous pentoxide at room temperature. The content of MND in the complex was assayed by UV measurement (λ_{max} 331 nm) after dissolving the complex in ethanol.

Preparation of Physical Mixture and Ground Mixture A physical mixture of DCA–MND (molar ratio 2:1) was prepared by mixing DCA and MND in a bottle using a vortex mixer for 5 min. A ground mixture was prepared by grinding the physical mixture (3.0 g) using a vibrating mill (Heiko Seisakusho, TI 200, Tokyo).

Preparation of Sealed Heated Sample The physical mixture of DCA–MND (300 mg) was placed in a 2.0 ml glass ampoule. After sealing, the ampoule was heated to a desired temperature and kept isothermally for a predetermined period.

Powder X-Ray Diffractometry A Rigaku Denki diffractometer (model 2027, Tokyo) was used. The measurement conditions were as follows: target, Cu; filter, Ni; voltage, 30 kV; current, 5 mA; time constant, 0.5 s; scanning speed, 4 degree/min.

Differential Scanning Calorimetry (DSC) A Du Pont thermal analysis system (model TA 9900, U.S.A.) was used. Samples (about 3 mg) were

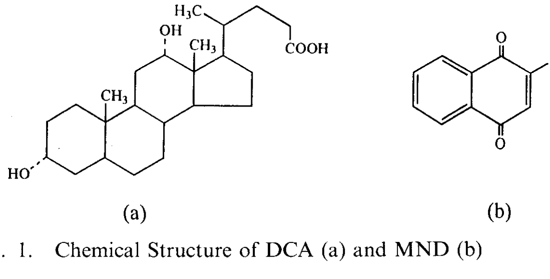

Fig. 1. Chemical Structure of DCA (a) and MND (b)

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sealed in an aluminum hermetic pan and measured at a scanning speed of 5 °C/min from 30 to 250 °C under nitrogen gas flow.

IR Spectroscopy The measurement was carried out by the KBr method using a Nicolet FT-IR spectrophotometer (model 5ZDX, U.S.A.).

Sublimation Experiment The sublimation behaviour of MND from the coprecipitate, physical mixture, ground mixture and sealed heated sample were compared. Samples (about 10 mg) were heated isothermally at 50 °C in a vacuum. The weight loss was recorded up to 6 h using a thermal gravimetric module of Du Pont TA 9900.

Results and Discussion

Complex Formation by Grinding As a reference to investigate the possibility to form complex between DCA and MND by grinding and sealed heating, the complex was first prepared by coprecipitation. The coprecipitate was evaluated comparatively with the ground mixture and sealed heated sample by X-ray diffraction, IR spectroscopy, thermal analysis and sublimation method. Figure 2 shows the powder X-ray diffraction patterns of DCA, MND and DCA–MND complex prepared by coprecipitation. Characteristic diffraction peaks were observed at $2\theta = 10.7°$ and $19.0°$ for DCA crystals and at $2\theta = 11.8°$ for MND crystals. The complex showed diffraction peaks at $2\theta = 13.7°$, $18.3°$ and $21.4°$, while neither the original peaks of DCA crystals nor MND crystals were observed. It was found that different molecular arrangement was accomplished in the coprecipitate due to complex formation. The MND content in the complex was assayed as 17.6% by UV spectroscopic method which was in good agreement with the calculated value (18.0%) of 2:1 (DCA : MND) stoichiometry.

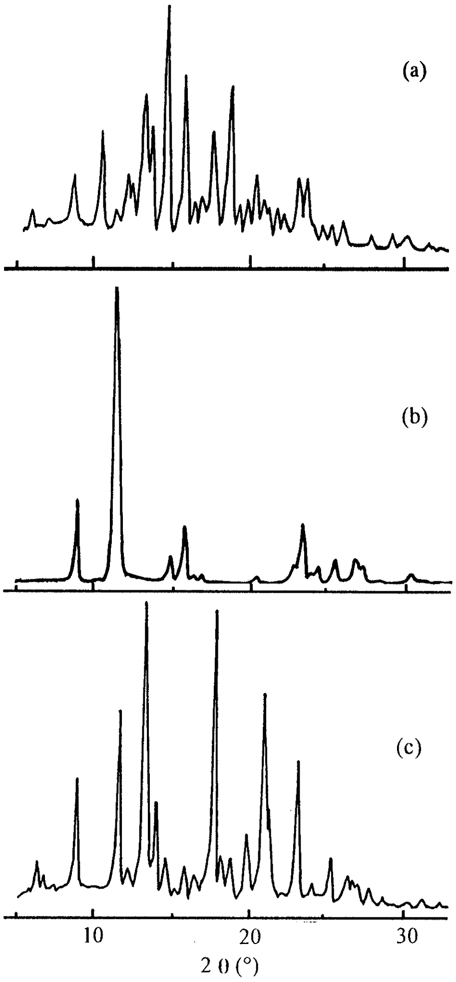

Fig. 2. Powder X-Ray Diffraction Patterns of DCA–MND Systems
(a) DCA, (b) MND, (c) coprecipitate.

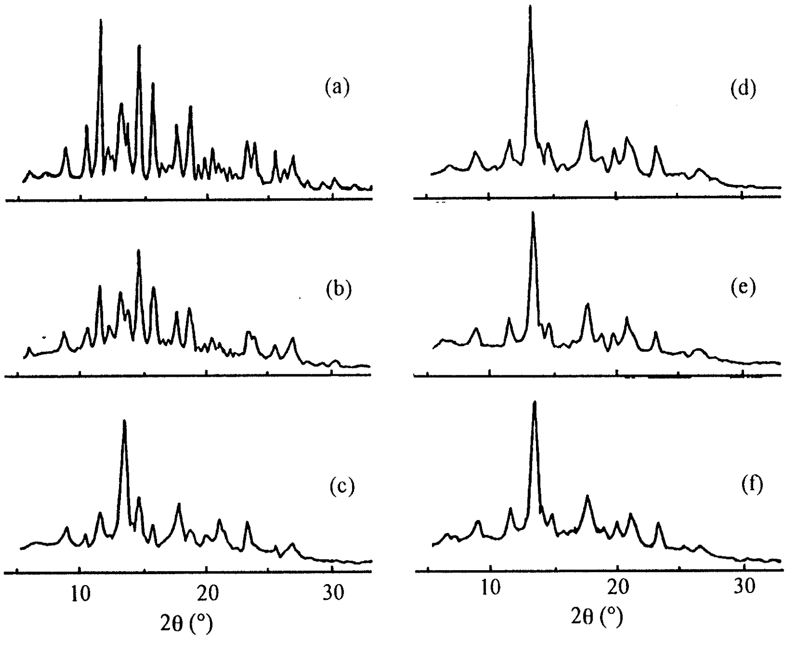

Fig. 3. Effect of Grinding on the Powder X-Ray Diffraction Patterns of DCA–MND Physical Mixture (Molar Ratio 2: 1)
(a) Physical mixture, (b) ground for 1 min, (c) ground for 3 min, (d) ground for 5 min, (e) ground for 10 min, (f) DCA–MND coprecipitate after grinding for 10 min.

The powder X-ray diffraction patterns of DCA–MND ground mixtures are shown in Fig. 3. The physical mixture showed the diffraction peaks of both components at $2\theta = 10.7°$ and $11.8°$ due to DCA and MND crystals, respectively. After grinding for 3 min, diffraction peaks characteristic of the DCA–MND complex were observed at $2\theta = 13.7°$, $18.3°$ and $21.4°$. The intensities of these peaks increased with prolonged grinding, whereas diffraction peaks of DCA crystals and MND crystals gradually decreased in the intensity. After grinding for 10 min, the X-ray diffraction peaks of DCA crystals and MND crystals disappeared and the powder X-ray diffraction pattern changed to the same pattern as the complex. It should be noted that orientation effects were observed thus leading to differences in relative intensities between ground mixture and complex intact.

We also performed thermal analysis and IR spectroscopy to confirm the complex formation by grinding. Figure 4 shows DSC curves of DCA–MND systems. DCA crystals, MND crystals and DCA–MND complex showed endothermic peaks due to the fusion at 175, 107 and 173 °C, respectively. The physical mixture (curve c) showed two endothermic peaks at 104 and 173 °C which were considered to be due to the fusion of MND crystals and the complex, respectively. Since the area of MND fusion peak was smaller than that expected from the MND content, the complex formation would occur during heating process. The effect of heating on the complex formation is discussed further in the next section. The ground mixture (curve e) showed only one endothermic peak at 173 °C presumably due to fusion of the complex, indicating the complex formation of MND with DCA by grinding.

Since IR spectral patterns are affected by the molecular environment, many applications of IR spectral method for studying complexation have been reported.12,14,17–19) Figure 5 shows IR spectra of DCA–MND systems. DCA crystals (curve a) showed free O–H stretching vibration bands at 3567 and 3553 cm^{-1}, and carbonyl stretching vibration bands at 1716 and 1699 cm^{-1}, respectively. MND crystals (curve b) showed the two bands at 1623 and 1594 cm^{-1} which were due to double bond stretching in the naphthoquinone ring. A peak at 780 cm^{-1} resulting from out of plane aromatic C–H bending was also observed. The pattern of the physical mixture (curve c) was the superimposition of spectral patterns of DCA and MND crystals. In DCA–MND complex, the peaks of free O–H stretching band of DCA crystals (3567, and 3553 cm^{-1}) were not observed whereas the broad band due to hydrogen bond O–H stretching was observed at 3500—3200 cm^{-1}. The carbonyl stretching vibration band of DCA was also shifted to 1698 cm^{-1}. Giglio5) reported that when the complex was formed between DCA and guests, the carbonyl groups of DCA molecules formed hydrogen bond with the OH groups of adjacent DCA molecules to build up the typical bilayer structure of DCA–guest complex. The shift in carbonyl peak of DCA would thus be due to hydrogen bonding among DCA molecules. The IR peak shift of MND by complexation was also observed. The double bond stretching peaks in naphthoquinone ring were shifted from 1623 and 1594 cm^{-1} to 1627 and 1598 cm^{-1}, respectively. The band of out of plane aromatic C–H bending was shifted from 780 to 774 cm^{-1}. The shift in MND peaks might be caused by van der Waals interaction or interaction between π charge cloud between DCA and MND molecules as reported by Candeloro De Sanctis *et al.*2) and Jones *et al.*20) in other DCA complexes. DCA–MND ground mixture (curve e) showed the same IR pattern as the DCA–MND complex.

Consequently, the powder X-ray diffraction pattern and IR spectra demonstrated that the complex prepared by grinding had the same crystal structure and molecular states as the complex prepared by coprecipitation.

Complex Formation by Sealed Heating The sealed

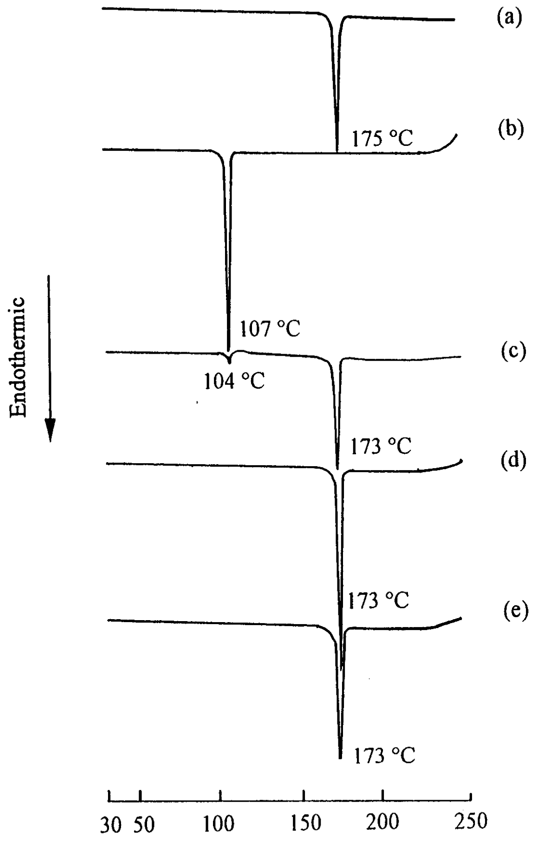

Fig. 4. DSC Curves of DCA–MND Systems (Molar Ratio 2:1)

(a) DCA, (b) MND, (c) physical mixture, (d) coprecipitate, (e) ground mixture (ground for 10 min).

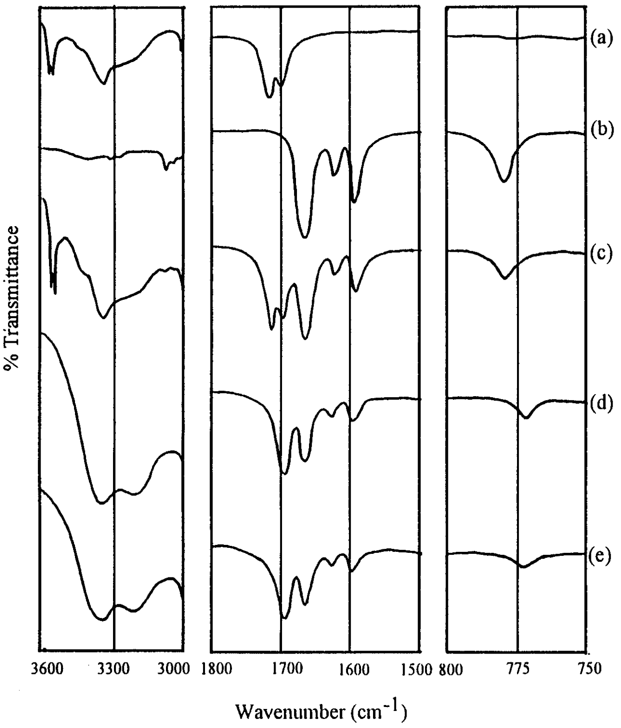

Fig. 5. IR Spectra of DCA–MND Systems (Molar Ratio 2:1)

(a) DCA, (b) MND, (c) physical mixture, (d) coprecipitate, (e) ground mixture (ground for 10 min).

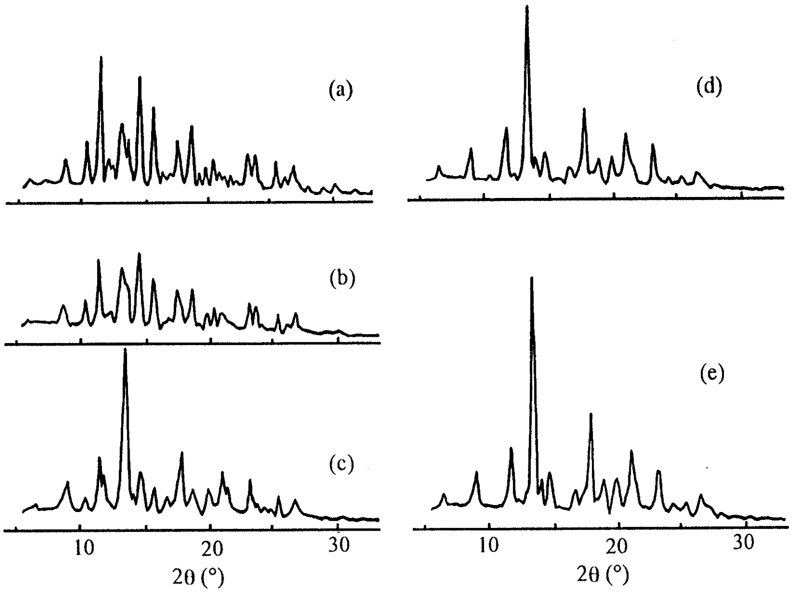

Fig. 6. Powder X-Ray Diffraction Pattterns of DCA–MND Sealed Heated Samples Prepared at Various Temperatures for 10 min (Molar Ratio 2 : 1)

(a) Physical mixture, (b) sealed heated at 80 °C, (c) sealed heated at 90 °C (d) sealed heated at 100 °C, (e) sealed heated at 110 °C.

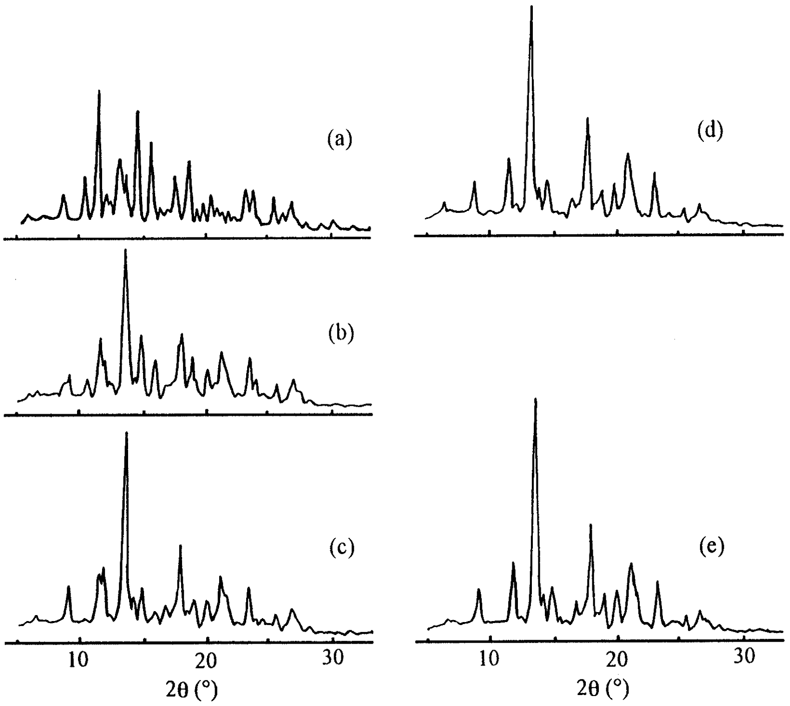

Fig. 7. Changes in the Powder X-Ray Diffraction Pattern of DCA–MND Physical Mixture during the Process of Sealed Heating at 80 °C (Molar Ratio 2 : 1)

(a) Physical mixture, (b) sealed heated for 1 h, (c) sealed heated for 5 h (d) sealed heated for 12 h, (e) sealed heated for 24 h.

heating method has been developed to prepare the complex of cyclodextrins and guest compounds.[15–17] Since DSC data in the previous section suggested that the complex was formed during heating process, we attempted to use this method to prepare DCA–MND complexes. The effects of heating temperature and heating time on the complex formation were assessed using X-ray diffractometry, IR spectroscopy and thermal analysis.

As shown in Fig. 6, the intensities of X-ray diffraction peaks of both DCA and MND crystals were decreased and the diffraction peaks due to DCA–MND complex were observed after sealed heating of the physical mixture at 90 °C for 10 min. These changes were accelerated by increasing the heating temperature, and the X-ray diffraction pattern was completely changed to the pattern of the inclusion compound after heating at 110 °C.

Nakai *et al.* reported that the sublimation of guest molecules should be the first step in complex formation in the sealed heating method.[15] Figure 7 shows the changes of powder X-ray diffraction patterns of DCA–MND during the sealed heating process at 80 °C. The X-ray diffraction peak intensities of DCA and MND crystals were gradually decreased with prolongation of the heating time and had completely disappeared after heating for 24 h. The diffraction peaks due to the DCA–MND complex were observed at $2\theta = 13.7°$, $18.3°$ and $21.4°$ after heating over 1 h (Fig. 7b—e). From these results, it can be concluded that the complex formation also occurred at 80 °C which was below the melting point of guest compound.

The DSC measurements of sealed heated samples were also carried out. After sealed heating at 110 °C for 10 min and at 80 °C for 24 h, no peak of MND fusion and only one endothermic peak due to the melting of the complex was observed in the DSC curve in a similar manner to that shown in Fig. 4, curves d and e. The sealed heated samples of DCA–MND showed the same IR patterns as the DCA–MND complex. These results confirmed that the complexation was accomplished by the sealed heating method. Below the melting point of MND, the complex formation would occur through the sublimation of MND.

A sublimation experiment was also conducted to study the difference of molecular state of MND in the physical mixture and in the complex. When the guest forms complex with DCA, the guest molecules are included into the channel of DCA sheet and interact with DCA molecules. Because of this stabilization, the sublimation of MND from the complex should occur at a higher temperature compared with MND intact crystals. In the conventional TG curve of MND crystals measured at a heating rate of 5 °C/min, the weight loss of MND started at about 90 °C whereas the complex demonstrated weight loss from 110 °C. No clear difference was observed between the complex and the physical mixture, because the complex formation would proceed during TG experiment. Therefore, we developed an alternative technique to investigate the difference between the complex and the physical mixture.

Figure 8 shows sublimation curves of MND from various DCA–MND systems at 50 °C in a vacuum. The temperature was fixed at 50 °C because we found that MND intact could be sublimed in a vacuum at this temperature. The physical mixture (curve f) showed weight loss of 17.9% which equaled the MND content. This result indicated that all of the free MND which had no interaction with DCA could completely sublimed under this condition. The weight loss from the complex (curve a) was as small as 0.1%, indicating that the sublimation of MND was suppressed by forming complex with DCA. The weight loss from the coprecipitate was increased to 1.5% by grinding. Grinding reduced the crystallinity of the complex, resulting in the acceleration of MND release. In the cases of the sealed heated samples and the ground mixture, the weight loss were almost 2%.

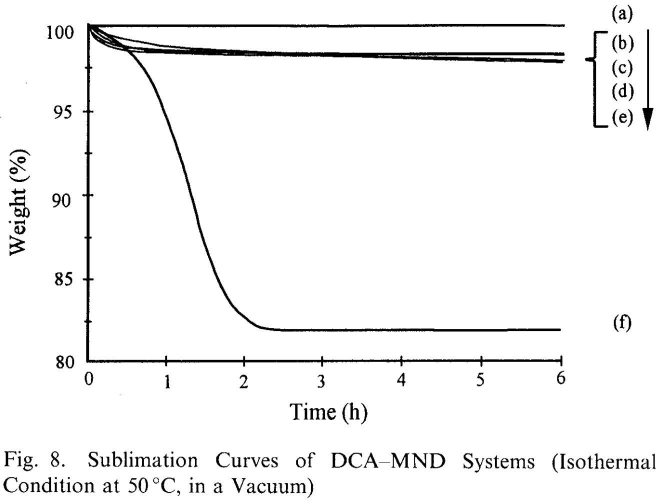

Fig. 8. Sublimation Curves of DCA–MND Systems (Isothermal Condition at 50 °C, in a Vacuum)

(a) Coprecipitate (0.1%), (b) ground coprecipitate (1.5%), (c) sealed heated at 110 °C for 10 min (1.6%), (d) sealed heated at 80 °C for 24 h (2.0%), (e) ground mixture (2.2%), (f) physical mixture (17.9%). Data in parentheses indicate weight loss after sublimation for 6 h.

From all the results described above, it was concluded that DCA can form complex with MND by both grinding and sealed heating methods.

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References

1) Coiro V. M., D' Andrea A., Giglio E., *Acta. Cryst.*, **B36**, 848—852 (1980).
2) Candeloro De Sanctis S., Giglio E., Pavel V., Quagliata C., *Acta Cryst.*, **B28**, 3656—3661 (1972).
3) Popovitz-Biro R., Tang C. P., Chang H. C., Lahav M., Leiserowitz L., *J. Am. Chem. Soc.*, **107**, 4043—4058 (1985).
4) Miki K., Kasai N., Tsutsumi H., Miyata M., Takemoto K., *J. Chem. Soc., Chem. Commun.*, **1987**, 545—546.
5) Giglio E., "Inclusion Compounds," Vol. 2, ed. by Atwood J. L., Davies J. E. D., MacNicol D. D., Academic Press, London, 1984, pp. 207—229.
6) Lach J. L., Pauli W. A., *Drug Std.*, **27**, 104—108 (1959).
7) Cilento G. J., *J. Med. Pharm. Chem.*, **2**, 241 (1960).
8) Wakely R. D., U.S patent 2,758,923 (1956).
9) Reynolds J. E. F. (ed.), "Martindale, the Extra Pharmacopoeia," Pharmaceutical Press, London, 1989.
10) Guillory J. K., Hwang S. C., Lach J. L., *J. Pharm. Sci.*, **58**, 301—307 (1969).
11) Pathipvanich M., Ph. D. Thesis, University of Iowa, Iowa City, Iowa, (1971).
12) Ahmed M. O., Nakai Y., Aboutaleb A. E. S., Yamamoto K., Rahman A. A. Z. A., Saleh S. I., *Chem Pharm. Bull.*, **38**, 3423—3427 (1990).
13) Yamamoto K., Nakai Y., *Siberain J. Chem.*, **5**, 51—55 (1991).
14) Nakai Y., Yamamoto K., Oguchi T., Yonemochi E., Hanawa T., *Chem. Pharm. Bull.*, **39**, 1532—1535 (1991).
15) Nakai Y., Yamamoto K., Terada K., Watanabe D., *Chem. Pharm. Bull.*, **35**, 4609—4615 (1987).
16) Nakai Y., Yamamoto K., Terada K., Oguchi T., Watanabe D., Saito H., *Chem. Pharm. Bull.*, **37**, 1055—1058 (1989).
17) Nakai Y., Yamamoto K., Oguchi T., Yonemochi E., Hanawa T., *Chem. Pharm. Bull.*, **38**, 1345—1348 (1990).
18) Watanabe D., Ohta M., Yang Z. J., Yonemochi E., Oguchi T., Yamamoto K., *Chem Pharm Bull.*, **44**, 833—836 (1996).
19) Nakai Y., Aboutaleb A. E., Yamamoto K., Ahmed M. O., *Chem. Pharm. Bull.*, **38**, 728—732 (1990).
20) Jones J. G., Schwarzbaum S., Lessinger L., *Acta Cryst.*, **B38**, 1207—1215 (1982).