Effect of Pore Size on the Gaseous Adsorption of Ethenzamide on Porous Crystalline Cellulose and the Physicochemical Stability of Ethenzamide after Storage

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The effect of pore size on the gaseous adsorption of ethenzamide (EZ) on porous crystalline cellulose (PCC) in a mixture of PCC–EZ, and the physicochemical stability of EZ in a mixture of PCC–EZ after storage at various relative humidities (RHs) were investigated by powder X-ray diffractometry, differential scanning calorimetry, specific surface area and pore size distribution measurements. After heating a mixture of PCC–EZ, which contained less than 7% EZ, at 70 °C at reduced pressure, the EZ became amorphous. The specific surface areas and pore size distribution curves of PCC and the heated mixtures of PCC–EZ were calculated from the nitrogen gas adsorption isotherms. The specific surface area of PCC was 82.3 m^2/g, while the specific surface areas of the heated mixtures of PCC–3% EZ, PCC–5% EZ and PCC–7% EZ were 74.1, 72.7 and 63.8 m^2/g, respectively. The specific surface area of the heated mixture of PCC–EZ decreased with an increase in the mixing ratio of EZ. In the pore size distribution curves of the heated mixtures of PCC–EZ, the pore volumes in the range of pore diameters from 2 to 40 nm decreased compared with the pore volume of PCC. The volumes of pores with diameters ranging from 3 to 10 nm decreased markedly with an increase in the mixing ratio of EZ. These results indicate that the adsorption of EZ on the pore surfaces of PCC occurred principally in the range of diameters from 3 to 10 nm. After storage of the heated mixtures of PCC–7% EZ at various RHs for 7 d at 30 °C, recrystallization of EZ was observed in the X-ray diffraction spectrum when the mixtures were stored above RH 72.8% due to condensation of water vapor in pores.

Key words porous crystalline cellulose; ethenzamide; adsorption; amorphism; specific surface area; pore size distribution

Many studies have been carried out on the adsorption of drugs on adsorbents such as controlled pore glass (CPG),[1)] activated charcoal,[2)] magnesium aluminum silicate,[3)] porous calcium silicate[4)] and porous clay.[5)] In a mixture of drug crystals and adsorbents, the drugs become amorphous due to their adsorption on the pore surfaces of the adsorbents and exhibit unusual properties of suppressed sublimation,[1b)] increased dissolution[6)] and instability.[1e,5,7)]

We reported on the interactions between porous crystalline cellulose (PCC) and drugs.[6,7)] After heating a mixture of PCC and ethenzamide (EZ), EZ became amorphous and exhibited a high dissolution rate in polar and nonpolar solvents.[6)] Aspirin also became amorphous after mixing with PCC and the decomposition of aspirin when mixed with PCC fitted a first-order equation. The hydrolysis rate constant of the aspirin in the mixture of PCC–aspirin was comparable with the hydrolysis rate constant of the reaction of nonionic aspirin and H_2O among six element reactions in aqueous solution.[7)]

Only a few studies have been conducted so far on the effect of adsorbent pore diameter on the adsorption of drugs on adsorbents.[1c,e)] In this study, the effect of pore size on the gaseous adsorption of ethenzamide on PCC was investigated by powder X-ray diffractometry, differential scanning calorimetry (DSC), specific surface area and pore size distribution measurements. Powder X-ray diffraction patterns of the mixtures of PCC–EZ after storage at various relative humidities (RHs) were investigated to determine the physicochemical stability of EZ in an amorphous state in the mixture of PCC–EZ.

Experimental

Materials PCC and microcrystalline cellulose (MCC, PH-101) were obtained from Asahi Chemical Industrial Co., Ltd. (Tokyo, Japan) and dried at 100 °C for 3 h at reduced pressure before use. EZ (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was used as received from the supplier.

Preparation of PCC–EZ and MCC–EZ Mixtures Physical mixtures of PCC–EZ and MCC–EZ were prepared by simply blending the components. Heated mixtures of PCC–EZ were obtained by heating the physical mixtures at 70 °C at reduced pressure. For the mixtures of PCC–3% EZ and PCC–5% EZ the heating time was 5 h and for the mixture of PCC–7% EZ the heating time was 24 h. The EZ content of the mixture was constant during heating.

Powder X-Ray Diffractometry A Rigaku Denki RINT 1100 diffractometer (Tokyo) was used under the following conditions: Cu target; Ni filter; voltage, 40 kV; current, 40 mA; receiving slit, 0.15 mm; time constant, 0.5 s and scanning speed, 4°/min.

DSC The mixture samples were sealed in liquid sample pans and DSC measurements were made on a Seiko DSC-220C instrument (Chiba, Japan). The heating rates were 2 or 5 °C/min in a nitrogen atmosphere. In the measurements at a heating rate of 5 °C/min, the temperature was increased from 20 to 170 °C at 5 °C/min then decreased to 20 °C at 5 °C/min, and the measurements were repeated. The first temperature increase was termed the 1st run and the repeated increase was termed the 2nd run.

Measurement of Nitrogen Gas Adsorption Isotherm Nitrogen gas adsorption isotherms of PCC, heated mixtures of PCC–EZ and MCC were determined at −196 °C using a Micromeritics ASAP 2000 instrument (Shimadzu, Kyoto, Japan). Dead space was determined using helium gas. The specific surface area and pore size distribution of samples were calculated by the BET method[8)] and BJH method,[9)] respectively. In the heated mixtures of PCC–EZ, these calculations were made using the values of the sample weights of PCC alone.

Storage at Various RHs PCC, heated mixtures of PCC–7% EZ and MCC were stored in desiccators at various RHs. A temperature of 30 °C was maintained using an air bath. Constant RHs were generated using saturated aqueous solutions of LiCl (RH 11.8%), $MgCl_2$ (RH 32.8%), $Mg(NO_3)_2$ (RH 52.0%), $NaNO_3$ (RH 72.8%), KCl (RH 84.6%) and

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KNO_3 (RH 90.7%).

Determination of Amount of Sorbed Water Amount of sorbed water of PCC, heated mixtures of PCC–7% EZ and MCC after storage at various RHs were determined by the Karl–Fischer method on a Hiranuma AQ-6 aquacounter (Ibaraki, Japan).

Results and Discussion

Figure 1 shows the powder X-ray diffraction patterns of EZ, PCC and the mixtures of PCC–EZ containing 3%, 5% and 7% EZ before and after heating at 70 °C at reduced pressure. For the physical mixtures of PCC–EZ, X-ray diffraction peaks were observed at $2\theta = 9.6°$, 19.3° and 25.3° and were due to EZ crystals. For the heated mixtures of PCC–EZ, none of the X-ray diffraction peaks were observed. As no variation in EZ content in the mixtures occurred during heating, EZ must be physically adsorbed on the pore surfaces of PCC in the molecular state.[6)]

Figure 2 illustrates DSC curves obtained for the physical mixtures of PCC–EZ and MCC–EZ containing various ratios of EZ performed at a heating rate of 2 °C/min. The effect of pores of PCC on the physical properties of EZ in the mixture with PCC was investigated using MCC as non porous crystalline cellulose. For the physical mixtures of MCC–EZ, the endothermic peak due to the melting of EZ crystals was observed at about 123 °C. The area of this peak increased with an increase in the mixing ratio of EZ. The physical mixture of PCC–EZ showed a different thermal behavior from that of MCC–EZ. No peak was

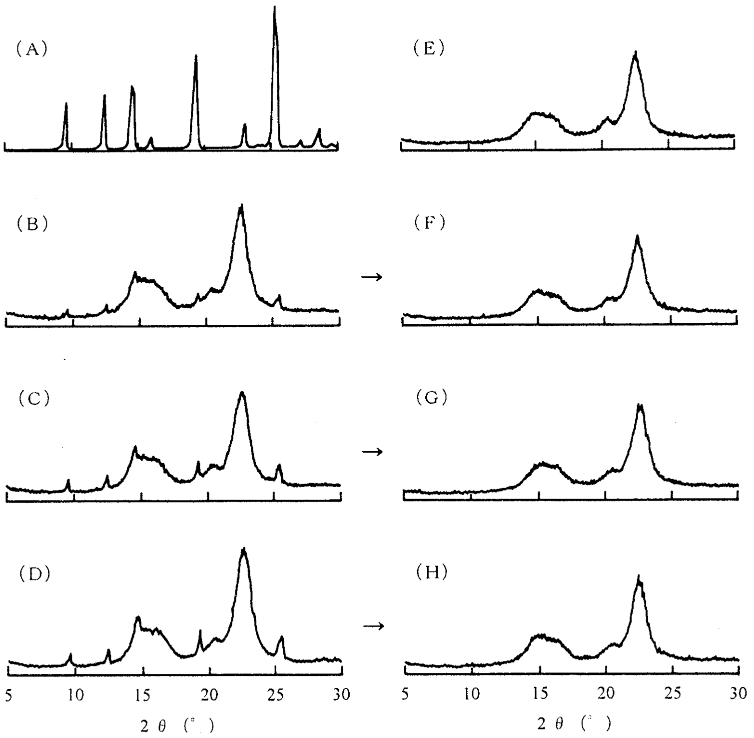

Fig. 1. Powder X-Ray Diffraction Patterns of EZ, PCC, Physical Mixtures and Heated Mixtures of PCC with EZ

(A) EZ, (B) PCC–3% EZ physical mixture, (C) PCC–5% EZ physical mixture, (D) PCC–7% EZ physical mixture, (E) PCC, (F) PCC–3% EZ heated mixture, (G) PCC–5% EZ heated mixture, (H) PCC–7% EZ heated mixture.

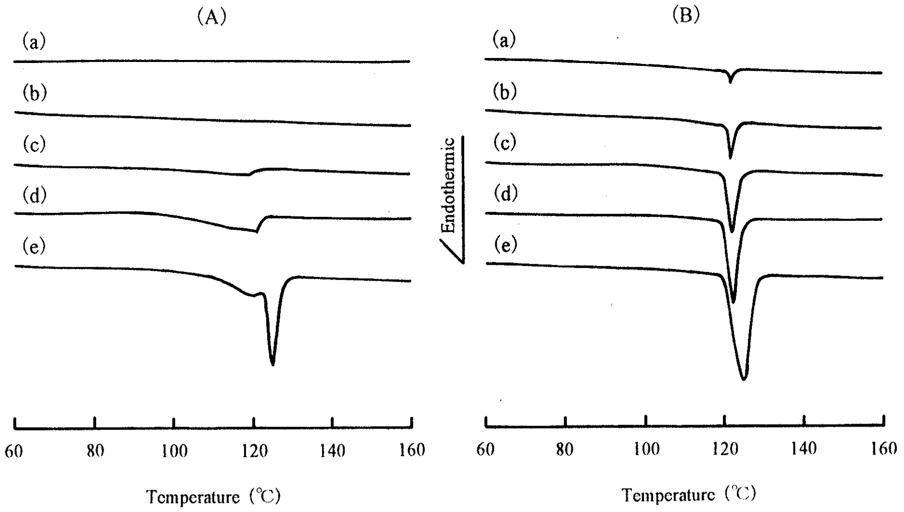

Fig. 2. DSC Curves of Physical Mixtures of PCC or MCC with EZ

(A) PCC–EZ mixture, (B) MCC–EZ mixture; (a) 5% EZ, (b) 10% EZ, (c) 20% EZ, (d) 30% EZ, (e) 50% EZ; heating rate, 2 °C/min.

observed for the physical mixtures of PCC–EZ containing 5% and 10% EZ, while a broad endothermic peak at 100—125 °C was observed for those of PCC–EZ containing 20% and 30% EZ. For the physical mixture of PCC–50% EZ, two overlapping endothermic peaks were observed: the first was broad and observed at a lower temperature than the melting point of EZ crystals and the second was due to the melting of EZ crystals. The total area of these peaks increased with an increase in the mixing ratio of EZ. Nakai *et al.* reported that three different states of benzoic acid may exist in a mixture of benzoic acid and CPG having a pore diameter of 7 nm.[1a] These are the crystalline state (phase 1), the disordered crystalline state for which a broad endothermic peak is observed at a lower temperature than the melting point (phase 2), and the amorphous state for which no peak on the DSC curve was observed (phase 3). Since the physical mixture of PCC–5% EZ showed the X-ray diffraction peaks due to EZ crystals before DSC measurement (Fig. 1), the results of DSC measurements indicated the EZ phase changed from phase 1 to phase 3 during the heating process in the physical mixtures of PCC–EZ containing 5% and 10% EZ. The EZ transformed from phase 1 to a mixture of phase 2 and phase 3 during the heating process in the physical mixtures of PCC–EZ containing 20% and 30% EZ. In the physical mixture of PCC–50% EZ, the EZ transformed from phase 1 to a mixture of phase 1, phase 2 and phase 3 during the heating process.

DSC curves for the physical mixtures of PCC–EZ and MCC–EZ obtained at a heating rate of 5 °C/min are illustrated in Fig. 3. In the 1st run, the physical mixture of PCC–5% EZ exhibited no peak. The physical mixtures of PCC–EZ containing 10% and 20% EZ exhibited a broad endothermic peak at a lower temperature than the melting point of EZ crystals, while the physical mixtures of PCC–EZ containing 30% and 50% EZ exhibited two overlapping endothermic peaks. The total area of these peaks increased with an increase in the mixing ratio of EZ. In the 2nd run, no peak was observed for the physical mixtures of PCC–EZ containing 5% and 10% EZ. Those of PCC–EZ containing 20% and 30% EZ exhibited only a broad peak at a lower temperature than the melting point of EZ crystals, while the physical mixture of PCC–50% EZ exhibited two overlapping endothermic peaks. These results suggest that after heating the physical mixture of PCC–EZ the EZ existed in phase 3 at a low concentration, and that phases 2 and 1 appeared in turn as mixing ratio of EZ increased. That is, in the mixtures with low EZ content, all the EZ molecules adsorbed on the pore surfaces of PCC (phase 3). As the mixing ratio of EZ increased, the EZ molecules which could not adsorb on the pore surface condensed in pore spaces (phase 2); in the mixture of PCC–50% EZ, the EZ existed in crystalline state (phase 1) in addition to phases 2 and 3.

The specific surface areas of PCC and the heated mixtures of PCC–EZ containing 3%, 5% and 7% EZ were calculated by the BET equation using nitrogen gas adsorption isotherms. The specific surface area of PCC was 82.3 m^2/g (100), while that of the heated mixtures of PCC–3% EZ, PCC–5% EZ and PCC–7% EZ were 74.1 m^2/g (90), 72.7 m^2/g (88) and 63.8 m^2/g (78), respectively. The number in parenthesis represents the relative ratio of the specific surface area of the heated mixture of PCC–EZ to that of intact PCC. The specific surface area of the heated mixture of PCC–EZ decreased with an increase in the mixing ratio of EZ, and the specific surface area of the heated mixture of PCC–7% EZ decreased by about 22%. The pore size distribution curves of PCC and the heated mixtures of PCC–EZ containing 3%, 5% and 7% EZ determined by the BJH method using nitrogen gas adsorption isotherms are shown in Fig. 4. PCC has many pores with diameters ranging from 2 to 150 nm, and the maximum pore size distribution curve was located at a pore diameter of 5 nm. In the heated mixtures of PCC–EZ, the volume of pores with diameters from 2 to 40 nm decreased with an increase in the mixing ratio of EZ. Since the size of an EZ molecule is about 1 nm and it is possible to diffuse in pore spaces of PCC, the reduction in pore volumes in the heated mixtures of PCC–EZ is caused by adsorption of EZ on the pore surfaces of

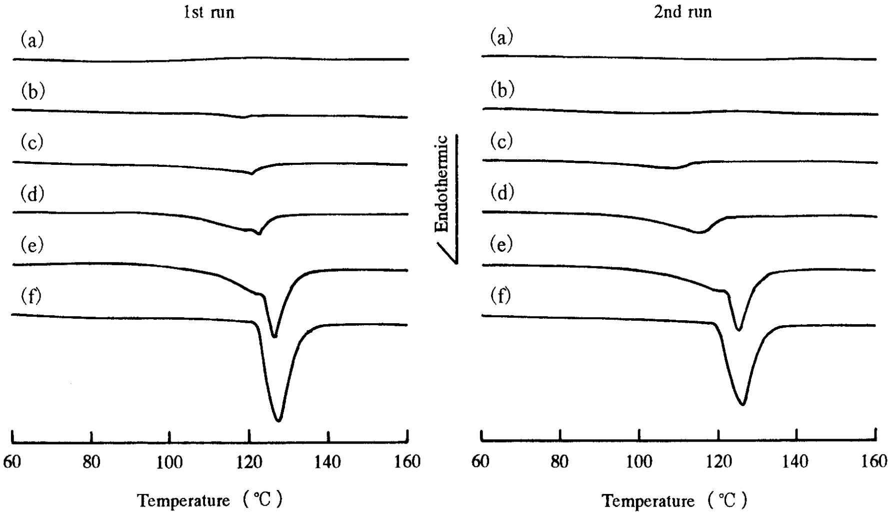

Fig. 3. DSC Curves of Physical Mixtures of PCC or MCC with EZ

(a) PCC–5% EZ, (b) PCC–10% EZ, (c) PCC–20% EZ, (d) PCC–30% EZ, (e) PCC–50% EZ, (f) MCC–50% EZ; heating rate, 5 °C/min.

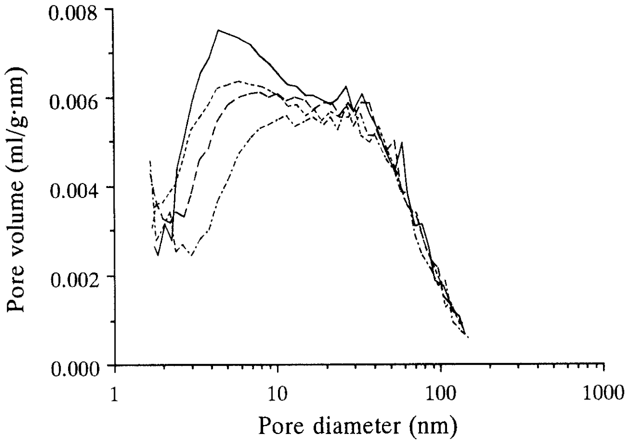

Fig. 4. Pore Size Distribution Curves of PCC and Heated Mixtures of PCC with EZ

——, PCC; ------, PCC–3% EZ; ---, PCC–5% EZ; -----, PCC–7% EZ.

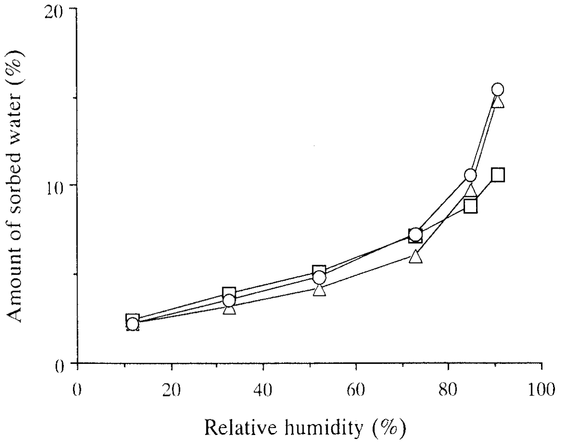

Fig. 6. Water Vapor Adsorption Isotherms of PCC, Heated Mixture of PCC with 7% EZ and MCC

○, PCC; △, PCC–7% EZ; □, MCC.

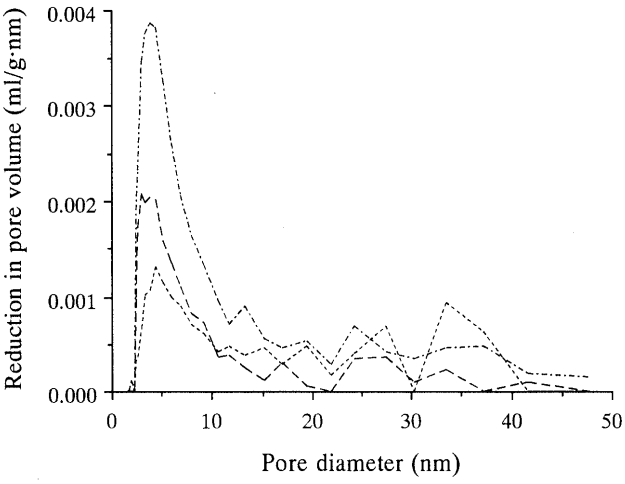

Fig. 5. Reduction Patterns of Pore Volume of Heated Mixtures of PCC with EZ

------, PCC–3% EZ; ---, PCC–5% EZ; -----, PCC–7% EZ.

PCC.[1a)] No reduction in volume was observed in pores with diameters above 40 nm, so that little EZ is believed to be adsorbed on the surfaces of pores of these diameters. The adsorbates adsorbed on adsorbents to reduce the free energy of the adsorbent surfaces, and EZ molecules were not thought to adsorb on surfaces of pores above 40 nm due to a low free energy of those surfaces. Figure 5 shows plots of the reduction in pore volume of the heated mixtures of PCC–EZ compared with that of intact PCC *vs.* the pore diameter. The reduction in pore volume over the diameter range from 3 to 10 nm increased with an increase in the mixing ratio of EZ. The maximum reduction in volume was located at pore diameters of 3—5 nm which was constant regardless of the mixing ratio of EZ. These results seem to indicate that the adsorption of EZ on the pore surfaces of PCC occurred over the diameter range from 2 to 40 nm. When the mixing ratio of EZ in the heated mixtures of PCC–EZ increased, EZ was adsorbed mainly on the surfaces of pores with diameters ranging from 3 to 10 nm.

Yonemochi *et al.* reported on the physicochemical properties of aspirin when it was mixed with various CPGs having a narrow and regulated pore size distribution.[1e)] The hydrolysis rate constants of aspirin mixed with CPG with pores less than 30 nm in diameter were constant in value, while those of aspirin mixed with CPG with pores larger than this decreased with an increase in that diameter. In this report it is proposed that the aspirin exists in amorphous state in the mixtures with CPGs with less than 30 nm diameter pores, that is, the aspirin adsorbs on the surfaces of these pores. This result is in good agreement with that obtained for the adsorption of EZ on the surfaces of PCC pores with diameters ranging from 2 to 40 nm in this study, for the size of an EZ molecule is about 1 nm and is equal to that of an aspirin molecule, and both types of molecules adsorbed physically on adsorbents.

Figure 6 shows the water vapor adsorption isotherms of PCC, heated mixture of PCC–7% EZ and MCC at 30 °C. The amount of sorbed water in the heated mixture of PCC–7% EZ decreased compared with that in PCC over the whole range of relative humidities, RH 11.8%—90.7%. The amount of sorbed water in MCC was smaller than that in PCC at RH 84.6% and RH 90.7%. The maximum diameters of pores causing water vapor condensation at RH 32.8%, RH 52.0%, RH 72.8%, RH 84.6% and RH 90.7% at 30 °C estimated using Kelvin's equation[6)] were 1.8, 3.1, 6.4, 12.2 and 21.0 nm, respectively. These calculations were made using the following values: molar volume of water, $18.1\,\mathrm{cm^3 \cdot mol^{-1}}$; surface tension of water, $71.3\,\mathrm{dyn \cdot cm^{-1}}$; angle of contact, 0°. Since PCC has pores with diameters ranging from 2 to 150 nm, water vapor condenses little in the pores of PCC stored below RH 52.0%, and the number of condensed pores of PCC increased with an increase in the storage RH above 52.0%. MCC, the specific surface area of which was calculated as $1.1\,\mathrm{m^2/g}$ from the nitrogen gas adsorption isotherms, has very few pores, so that the increase in the amount of sorbed water in PCC at high RHs was caused by water condensed in these pores.

The powder X-ray diffraction patterns of heated mixtures of PCC–7% EZ after storage at various RHs for 7 d are shown in Fig.7. The patterns of the heated mixtures stored below RH 52.0% were the same as those before storage, while after storage above RH 72.8%, diffraction

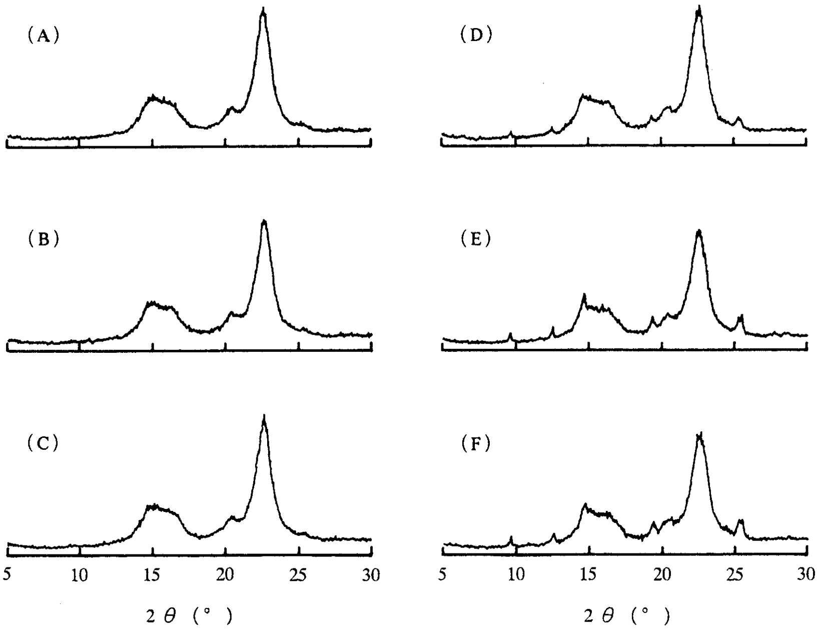

Fig. 7. Powder X-Ray Diffraction Patterns of Heated Mixtures of PCC–7% EZ after Storage for 7 d at Various Relative Humidities at 30 °C
(A) RH 11.8%, (B) RH 32.8%, (C) RH 52.0%, (D) RH 72.8%, (E) RH 84.6%, (F) RH 90.7%.

peaks were observed at $2\theta=9.6°$ and 25.3° due to EZ crystals. It was assumed that EZ recrystallized in the heated mixtures after storage above RH 72.8%. The intensity of diffraction peaks of the crystals in the heated mixtures after storage at RH 84.6% and RH 90.7% was higher than that of crystal diffraction peaks in the heated mixture after storage at RH 72.8%. Furthermore, the intensity of diffraction peaks of the EZ crystals in the heated mixture after storage at RH 90.7% was similar to that of diffraction peaks of these crystals in the heated mixture after storage at RH 84.6%. The maximum pore diameters causing water vapor condensation at RH 52.0% and RH 84.6% at 30 °C were 3.1 and 12.2 nm, respectively. Water vapor hardly condenses in pores with diameters from 3 to 10 nm in the heated mixture stored below RH 52.0%, while in the heated mixture stored above RH 84.6%, water vapor condensed in the pores of which almost all of the EZ had adsorbed on the surfaces. From these results it was assumed that the recrystallization of EZ in the heated mixtures after storage above RH 72.8% was caused by water condensing in the PCC pores. As the intensity of the X-ray diffraction peaks due to the EZ crystals in the heated mixture stored at RH 84.6% was similar to that of the peaks after storage at RH 90.7%, the adsorbed EZ on the surfaces of pores with diameters from 3 to 10 nm recrystallized as a result of water vapor condensation in the pores, and the adsorbed EZ on the surfaces of pores above 10 nm in diameter hardly recrystallized.

It was concluded that EZ adsorbed on the surfaces of PCC pores from 2 to 40 nm in diameter and the amount adsorbed in pores 3 to 10 nm in diameter increased with an increase in the mixing ratio of EZ. It was confirmed that the adsorbed EZ on the surfaces of PCC pores would not recrystallize after storage below RH 52.0%, while it would recrystallize as a result of water vapor condensation after storage above RH 72.8%.

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