

Freeze-Drying of Drug-Additive Binary Systems. II. Relationship between Decarboxylation Behavior and Molecular States of *p*-Aminosalicylic Acid

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The effects of additives on the crystallinity and the stability of freeze-dried *p*-aminosalicylic acid (PAS) have been investigated. Aqueous PAS solutions, to which various amounts of pullulan were added, were freeze-dried. The degradation rates of PAS at 80 °C increased with increasing amount of pullulan added and were also affected by the freezing conditions. It was suggested from the powder X-ray diffractograms that the stability of PAS was closely related to the content of amorphous fraction of PAS in the freeze-dried samples. In the freeze-dried samples containing α -cyclodextrin (α -CD) as the additive, the fastest degradation rate of PAS was observed at the mixing molar ratio (α -CD/PAS) of 0.5 (rapid freezing condition) or 1.0 (slow freezing condition). These results could be explained in terms of the three possible states of PAS in the freeze-dried sample with α -CD: (A) the crystalline state, almost stable; (B) the included state in the α -CD cavity, moderately unstable; (C) the dispersed state in the intermolecular hydrogen-bonding network of α -CDs, unstable.

Keywords *p*-aminosalicylic acid; freeze-dry; pullulan; α -cyclodextrin; solid-state reaction; amorphous; molecular interaction; solid dispersion; degradation

p-Aminosalicylic acid (PAS), an anti-tuberculosis drug, undergoes decarboxylation to *m*-aminophenol. Many studies have been conducted related to the kinetics and the mechanism of PAS decarboxylation in the crystalline state¹⁻⁴⁾ as well as in solution.^{5,6)} Nakai *et al.* reported⁷⁾ that the thermal degradation of PAS was accelerated in the solid dispersion prepared by co-grinding with microcrystalline cellulose.

In the preceding paper,⁸⁾ we reported that freeze-drying with a saccharide transformed a crystalline medicinal to an amorphous state to some extent, and that the amount of amorphous fraction was dependent on the freezing conditions. In present study, the decarboxylation rate of PAS was determined in two-component freeze-dried samples, consisting of PAS and an additive such as pullulan or α -cyclodextrin, to investigate the molecular interaction between the drug and additive in freeze-dried samples. This paper also describes the effects of freezing conditions on the stability of PAS.

Experimental

Materials PAS (Kanto Chemical Co., Inc.) was recrystallized twice from ethanol, dried *in vacuo*, ground in a mortar, and redried. Pullulan and α -cyclodextrin (α -CD) of guaranteed reagent grade were purchased from Nakarai Chem. Ltd. and were used as received. *m*-Aminophenol of special reagent grade was purchased from Wako Pure Chem. Ind., Ltd.

Freeze-Drying Procedure The freeze-drying procedures were the same as those reported in the previous paper.⁸⁾ The aqueous PAS solutions (19.14 mg in 25 ml; 5×10^{-3} M) containing various amounts of additives (0–360 mg) were frozen under the following conditions: (1) liquid nitrogen (required about 3 min for freezing), (2) kept in a bath thermostated at -13 °C for 24 h (required 1–3 h for freezing), followed by immersion in liquid nitrogen for complete freezing. The frozen samples were lyophilized in a vacuum (below 10^{-2} Torr) using a Neo Cool DC 55-B freeze-dryer (Yamato) for 16–20 h at the shelf temperature of *ca.* 20 °C. After the primary drying, the samples were stored in a desiccator with P_2O_5 in a vacuum for 24 h. The freeze-dried samples contained 4–7% water for the α -CD system, and 3–5% water for the pullulan system. During the freeze-drying procedures, the samples were shielded from light by covering the sample holder with aluminum foil.

Powder X-Ray Diffraction Measurement The apparatus and procedures were the same as those reported in the previous paper.⁸⁾

Kinetic Studies Each freeze-dried sample (100 mg) was placed in a vial tube. The vial tubes were kept in an air incubator (Ishii Shouten Ltd.) at 80 °C (± 0.5 °C) in the presence of P_2O_5 at atmospheric pressure. During

the kinetic studies, the samples were shielded from light.

Determination of Decarboxylation Rate A sample was removed from each vial at intervals, and dissolved in phosphate buffer solution (pH 7.0). To determine both PAS and *m*-aminophenol simultaneously, a spectrophotometric method²⁾ was modified. The extent of decomposition was calculated from the combination of absorbances at two wavelengths (300/280 nm, 280/265 nm, 265/230 nm and 230/300 nm), and the data of the four combinations were averaged.

Preparation of α -CD Inclusion Compound Crystal The coprecipitation method⁹⁾ was applied to obtain crystals of the inclusion compound of α -CD and PAS. The mixture of PAS (300 mg) and α -CD (2.0 g) was added to 10 ml of water and the solution was vigorously agitated for 1 h at 50 °C. After filtration of the solution, the filtrate was gradually cooled and allowed to stand for 8 h in an ice bath. The precipitate was filtered off, washed with ethyl ether, and dried *in vacuo*. The stoichiometry of the inclusion compound was determined by the spectrophotometric measurement of PAS.

Results and Discussion

Pullulan-PAS System Pullulan is a linear polysaccharide (molecular weight: 50000–100000) constructed from $\alpha(1 \rightarrow 6)$ -linked maltotriose units.¹⁰⁾ Pullulan was used as a freeze-drying additive because of its high water solubility and inability to form an inclusion complex. Figure 1

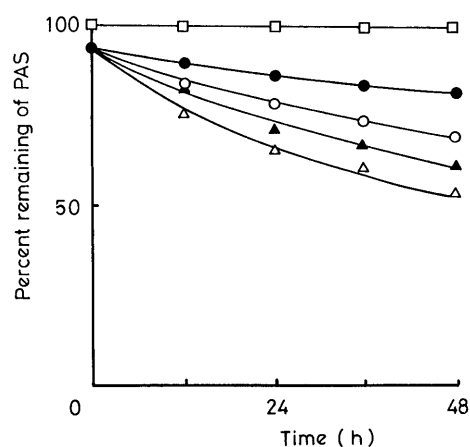


Fig. 1. Degradation of PAS Freeze-Dried with Various Amounts of Pullulan at 80 °C and RH 0%

Amount of pullulan added to 25 ml of PAS solution: □, 0 mg; ●, 60 mg; ○, 120 mg; ▲, 180 mg; △, 360 mg. Freezing condition: -13 °C

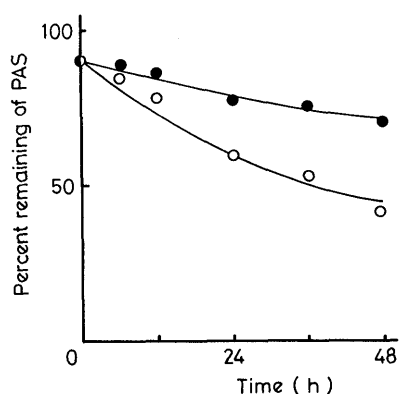


Fig. 2. Effects of Freezing Conditions on the Degradation of PAS Freeze-Dried with Pullulan (160 mg) at 80 °C and RH 0%

Freezing condition: ●, -13 °C; ○, liquid nitrogen.

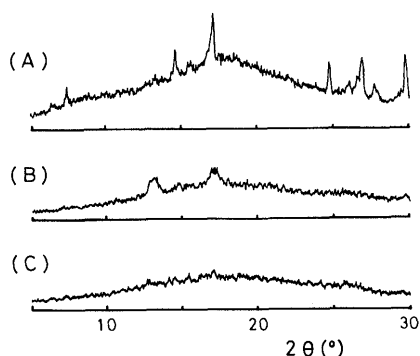


Fig. 3. Powder X-Ray Diffractograms of PAS-Pullulan Mixtures (PAS content 24.1%)

(A) physical mixture, (B) freeze-dried (frozen at -13 °C), (C) freeze-dried (frozen by liquid nitrogen).

presents the degradation curves of PAS freeze-dried with various amounts of pullulan at 80 °C. Freezing of these samples was carried out at -13 °C (thermostat) for 24 h. Freeze-dried PAS containing no additive was obtained in a crystalline state, and was observed to be relatively stable at 80 °C and relative humidity (RH) 0%. It was also observed that the increase of pullulan content caused the acceleration of PAS decomposition and that the reproducibility of the decomposition kinetics was good among different preparations.

The effects of freezing conditions on the degradation rate of PAS in the freeze-dried samples consisting of PAS and pullulan were also investigated. Figure 2 shows the degradation curves of PAS in the 24% PAS and 76% pullulan freeze-dried samples prepared by the different freezing processes, that is, by using liquid nitrogen (rapid freezing) and at -13 °C (slow freezing). The faster degradation was observed in the rapidly frozen sample.

As reported in a previous paper,⁸⁾ a crystalline medicinal became amorphous as a result of binary freeze-drying, and the amorphous medicinal molecules were considered to be dispersed in the hydrogen-bond network formed by the saccharide molecules. We also reported that the amorphous fraction was dependent on the freezing conditions. Powder X-ray diffractograms of the mixtures of 24% PAS and 76% pullulan are shown in Fig. 3. The crystalline diffraction peaks at $2\theta = 7.0, 12.6, 16.5, 24.3, 26.5$ and 29.3° were due

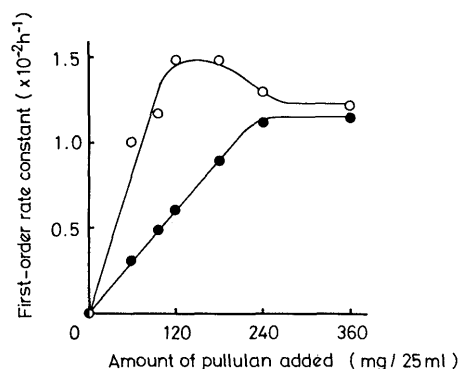


Fig. 4. Apparent First-order Rate Constants of PAS Degradation in Freeze-Dried Samples with Pullulan at 80 °C and RH 0%

Freezing condition: ●, -13 °C; ○, liquid nitrogen.

to PAS crystals, since pullulan was originally amorphous. The intensities of the X-ray diffraction peaks due to PAS crystals were reduced by binary freeze-drying, indicating the existence of amorphous PAS in binary freeze-dried samples. It was observed that the crystallinity of PAS was affected by the freezing conditions. The decomposition rate seemed to be closely related to the amount of amorphous fraction of PAS in freeze-dried samples.

Decarboxylation rate constants were calculated by fitting the results to first-order kinetics. The apparent first-order rate constants of PAS degradation are plotted against pullulan content in Fig. 4. Pullulan content was expressed in mg of pullulan added to 25 ml of aqueous PAS solution (5×10^{-3} M). In the -13 °C freezing systems, the degradation rate constants increased linearly with the amount of pullulan up to 240 mg. It was considered that in this region the amorphous fraction of PAS, which was dispersed in the hydrogen-bond network^{8,11-14)} of pullulan, increased in proportion to the pullulan content. The constancy of the degradation rate in the region above 240 mg of pullulan seemed to indicate that all the PAS exists in the amorphous state. In the liquid nitrogen freezing systems containing less than 240 mg of pullulan, faster degradation rates were observed in comparison with the -13 °C freezing systems. The degradation rates in the region above 240 mg of pullulan were almost the same as those in the -13 °C freezing system. The stability of the PAS molecules in the amorphous state seemed to be similar in the slow freezing system and rapid freezing system. These results suggested that rapid freezing increased the amorphous fraction of the drug in binary freeze-dried samples, as reported in the previous paper.⁸⁾ In the rapid freezing system, the greatest degradation rates were observed at pullulan contents near 150 mg. Although this can not be clearly explained, the PAS molecule might exist in a more readily degradable state in the freeze-dried samples.

α -CD-PAS System α -CD formed a crystalline inclusion compound with PAS by coprecipitation. This inclusion compound had the channel-type crystal structure and the stoichiometry was 2:1 (α -CD: PAS). Figure 5A shows the powder X-ray diffractogram of the inclusion compound. Binary freeze-drying of PAS was carried out by using α -CD as an additive. Figure 5B and C show the powder X-ray diffractograms of the 2:1 (molar ratio of α -CD: PAS) freeze-dried samples prepared under different freezing con-

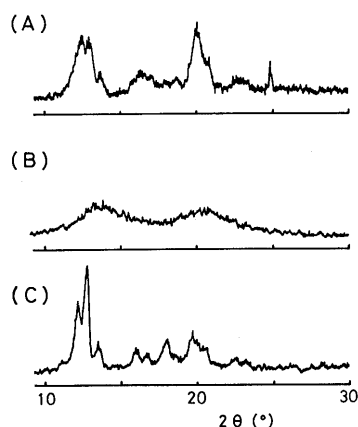


Fig. 5. Powder X-Ray Diffractograms of PAS- α -CD Systems

(A) Inclusion compound (molar ratio of PAS: α -CD = 1:2) prepared by coprecipitation. (B) Freeze-dried sample (1:2) prepared by liquid nitrogen freezing. (C) Freeze-dried sample (1:2) prepared by -13°C freezing.

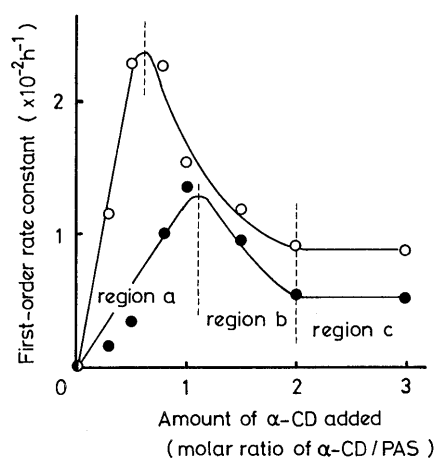


Fig. 6. Apparent First-Order Rate Constants of PAS Degradation in Freeze-Dried Samples with α -CD at 80°C and RH 0%

Freezing condition; $\bullet$, -13°C ; $\circ$, liquid nitrogen.

ditions. It was observed that the rapid freezing provided an amorphous freeze-dried sample while the slow freezing provided the crystalline inclusion compound, similarly to the case of the α -CD-methyl *p*-hydroxybenzoate system.⁸⁾

Decarboxylation kinetics of PAS was investigated in freeze-dried samples with various mixing ratios of α -CD/PAS at 80°C and RH 0%. The apparent first-order rate constants were plotted against the amount of α -CD expressed as the molar ratio of α -CD/PAS (Fig. 6). The degradation rate increased linearly the amount of α -CD in the low α -CD-content region (region a), decreased gradually after reaching a maximum in the following region (region b), then remained constant at molar ratios of more than 2.0 (region c). The freezing conditions were found to affect the slope in region a, the molar ratio giving the fastest degradation rate, and the plateau level in region c. It was considered that the slope in region a was closely related to the value of the amorphous PAS fraction, as shown in the pullulan-PAS system, and in the α -CD-methyl *p*-hydroxybenzoate system.⁸⁾

The inclusion compound crystals of α -CD and PAS prepared by coprecipitation had the stoichiometry of 2:1 (α -CD: PAS), and the powder X-ray diffractograms gave

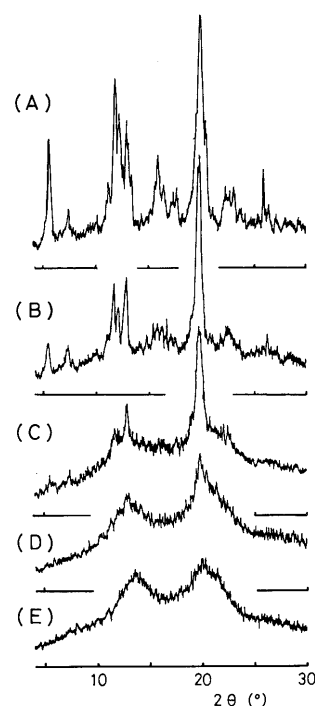


Fig. 7. Changes in Powder X-Ray Diffractogram of α -CD-PAS (2:1) Inclusion Compound by Grinding

Grinding time: (A), 0 min; (B), 1 min; (C), 2 min; (D), 5 min; (E), 10 min.

TABLE I. Crystallinity Dependence of Degradation Rate of PAS

First-order rate constant ($\times 10^{-2} \text{ h}^{-1}$)						
Ground inclusion compound					Freeze-dried sample	
0 ^{a)}	1 ^{a)}	2 ^{a)}	5 ^{a)}	10 ^{a)}	$-13^{\circ}\text{C}^{b)}$	liq. N ₂
0.23	0.37	0.90	1.01	1.01	0.55	0.92

a) Grinding time (min). b) Freezing condition.

similar patterns for the coprecipitate and the crystalline freeze-dried sample of α -CD and PAS (2:1) obtained by the slow freezing. This suggested that all of the PAS molecules were included in the α -CD cavity in region c, since α -CD molecules were present in excess at molar ratios higher than stoichiometric (2.0), accounting for the constancy of the degradation rate in region c. It was, however, found that the plateau levels in region c were different between the crystalline and the amorphous freeze-dried samples. In order to investigate the relationship between the degradability of PAS and the solid state of the freeze-dried samples, the degradation rates of PAS were measured using the inclusion compound with various grinding times. The crystallinity was altered by grinding the inclusion compound crystal with a mortar and pestle. Figure 7 shows the changes of X-ray diffractograms caused by the grinding. Table I shows the apparent first-order rate constants of PAS in ground samples in comparison with those of the freeze-dried samples of 2:1 (α -CD: PAS). It was observed that the PAS became unstable as the crystallinity of the inclusion compound was reduced by the grinding. The results indicated that the difference in the degradation rate in region c between the crystalline and the amorphous freeze-dried samples was attributable to the difference in the crystal-

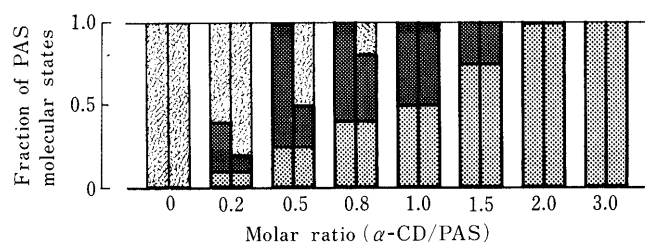


Fig. 8. Effects of α -CD Content and Freezing Conditions on Molecular States of PAS in Binary Freeze-Dried Samples

Molecular state of PAS: , crystalline; , included in α -CD cavity; , dispersed in hydrogen-bond network of α -CD.

linity of the freeze-dried samples.

In the previous paper,⁸⁾ we concluded that in binary freeze-dried samples with α -CD, the drug molecules could exist in the intermolecular hydrogen-bond network formed by α -CDs as well as in the α -CD cavity. Some fraction of PAS in the freeze-dried sample might exist in the dispersing state in the hydrogen-bond network at less than the molar ratio of 2.0 (region a and region b). The decrease of the degradation rate with increasing ratio in region b might be explained by the following assumptions. (1) The degradation of PAS in the hydrogen-bond network took place significantly faster than that in the α -CD cavity. (2) In region b, all PAS was in the amorphous state, and the fraction of PAS in the hydrogen-bond network gradually decreased with increase of α -CD content. These assumptions seem reasonable, indicating that the slow rate of degradation in region c was due to the included state of PAS, and that the PAS molecules preferred to be in the included state thermodynamically rather than in the dispersed state.

The above discussion of the PAS degradation rate profile in α -CD-PAS freeze-drying systems is schematically summarized in Fig. 8. The rapid and slow freezing systems are shown in the left and right columns, respectively, at each molar ratio. This figure shows that in α -CD-PAS freeze-dried samples, there are three molecular states of PAS, which have different degradation features: (A) the crystalline state, almost stable; (B) the included state in the α -CD cavity, moderately unstable; (C) the dispersed state in the hydrogen-bonding network of α -CD, unstable. In the α -CD-

free system, all PAS molecules existed as crystals, whereas in the system containing an excess amount of α -CD, all PAS molecules were included in the α -CD cavity. At intermediate contents of α -CD, the PAS molecules were present as a mixture of two or three states. The amount of the included fraction could be calculated from the assumption of 2:1 (α -CD: PAS) inclusion stoichiometry. When the molar ratios were more than 0.5 (fast freezing) or 1.0 (slow freezing), PAS molecules could not exist as crystals in the system. In region a, we can consider that the sum of the amorphous fractions of PAS [(B) and (C)] is proportional to the molar ratio of α -CD/PAS, according to the previous paper.⁸⁾ It was concluded that freeze-drying with α -CD caused PAS molecules to be included in the α -CD cavity and to be dispersed in the hydrogen-bond network of α -CD, and that the freezing conditions influence the amount of PAS molecules existing in the intermolecular hydrogen-bond network of α -CD.

Acknowledgment This work was supported in part by a grant from the Uehara Memorial Foundation.

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