Effects of Saccharide on the Decomposition of Cephalothin Sodium and Benzylpenicillin Potassium in Freeze-Dried Preparations

Toshio OGUCHI, Jun YAMASHITA,[1a] Etsuo YONEMOCHI, Keiji YAMAMOTO* and Yoshinobu NAKAI[1b]

Faculty of Pharmaceutical Sciences, Chiba University, 1–33 Yayoicho, Chiba 260, Japan. *Received October 7, 1991*

Freeze-dried samples of benzylpenicillin potassium or cephalothin sodium with a variety of saccharides were prepared. Effects of the saccharides on the stability of the antibiotics were investigated in the binary freeze-dried samples. Addition of a saccharide accelerated the solid-state decomposition of antibiotics except for the case of cephalothin sodium–maltose and –lactose systems, and the decomposition rate was enhanced with an increase of the chain length of saccharide molecules. No significant difference in water content was found among the systems containing different kinds of saccharide. It was suggested that the stability of cephalothin sodium and benzylpenicillin potassium in the binary freeze-dried samples was affected by the molecular interactions with saccharide molecules.

Keywords freeze-dry; antibiotics; cephalothin; benzylpenicillin; decomposition; amorphous; molecular interaction; solid dispersion

Introduction

Stability studies of antibiotics in freeze-dried samples[2] as well as in solution[3,4] have been of interest, since antibiotics are occasionally supplied in freeze-dried preparations. Freeze-dried β-lactam antibiotics were usually found in an amorphous state and easily decomposed.[5] Thus techniques to crystallize the antibiotics during the freeze-drying process have been developed to meet practical requirements.[6–8]

Several researchers reported that the crystallinity and stability of freeze-dried drugs were significantly affected by the presence of saccharide added as an excipient. These effects were explained by means of molecular interaction with drugs,[9] adsorption of water vapor[10] or change in the state of crystal water of a drug.[11]

We earlier studied molecular interactions between drug and saccharide in a variety of solid dispersions, and previously discussed the relationship between the molecular interaction and the chain-length of saccharide using maltose, maltotriose, maltopentaose and maltohexaose.[12,13] As the saccharide chain lengthened, the intermolecular hydrogen-bond network formed by the saccharide became more rigid and the drug molecules were dispersed more stably in it. In the present work, we investigated the decomposition feature of cephalothin sodium and benzylpenicillin potassium in the binary freeze-dried sample and the effect of the chain length of saccharide on the stability to obtain information regarding unstabilizable factors.

Experimental

Materials Cephalothin sodium and benzylpenicillin potassium were provided by Meiji Seika Kaisha, Ltd. (Tokyo, Japan). Maltose, lactose, maltotriose and maltopentaose were purchased from Nacalai Tesque. All reagents were used without further purification.

Freeze-Drying Procedure The freeze-drying procedures were the same as those reported previously.[12] Various amounts of saccharide (0—550 mg) were dissolved in an aqueous antibiotic solution (1×10^{-2} M, 25 ml). After filtration, these solutions were frozen with liquid nitrogen and then lyophilized with a Neo Cool DC 55-B freeze-dryer (Yamato). The freeze-dried samples were stored in a desiccator with P_2O_5.

Kinetic Study Each freeze-dried sample in a vial-tube was kept in a desiccator humidified at 50 °C. Saturated salt solutions were used to maintain relative humidities (RH) of 11.4 and 16.7%.

Determination of Decomposition Rate A sample was removed from each vial at intervals, and the decomposed portion was determined by iodometric methods based on the quantitative reduction of iodine by β-lactam ring cleavage products but not by β-lactams. Iodine colorimetric method[14] and micro-iodometric method[15] were applied for cephalothin sodium systems and for the benzylpenicillin potassium systems, respectively.

Powder X-Ray Diffractometry A Rigaku Denki 2027 diffractometer was used to determine the crystallinity of freeze-dried samples.

Determination of Water Content Water content of each sample was determined by the Karl–Fischer method using a Hiranuma AQ-3C aquacounter.

Results and Discussion

Cephalothin Sodium (CET) Binary freeze-dried samples were prepared in various ratios of CET to saccharide. All of the samples were obtained in an amorphous state, while the supplied CET was in a highly crystalline state. Figure 1 shows the decomposition curves of the freeze-dried CET with or without maltopentaose (molar ratio; 1.2 of maltopentaose/CET) and the intact CET at 50 °C, RH 16.7%. The results of powder X-ray diffractometry indicated that the samples remained in an amorphous state even after 20 d of storage. Freeze-dried CET showed greater decomposition rate than the intact CET because of its lower crystallinity, and the addition of maltopentaose caused further acceleration of CET decomposition. Figure

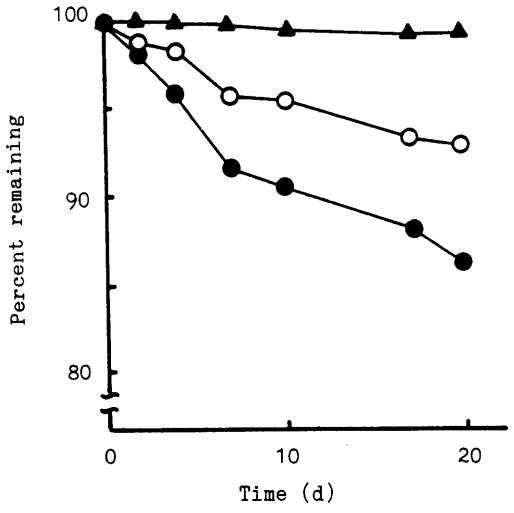

Fig. 1. Decomposition Curves of Freeze-Dried CET in the Presence and Absence of Maltopentaose at 50 °C, 16.7% RH

○, freeze-dried without maltopentaose; ●, freeze-dried with maltopentaose (molar ratio; 1.2 of maltopentaose/CET); ▲, intact CET.

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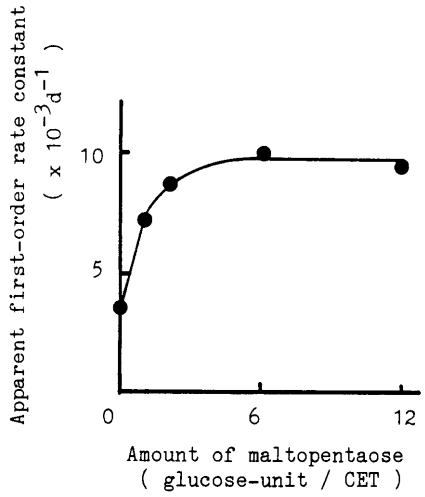

Fig. 2. Effect of Maltopentaose Amount on the Decomposition Rate of CET in a Binary Freeze-Dried Sample at 50 °C, 16.7% RH

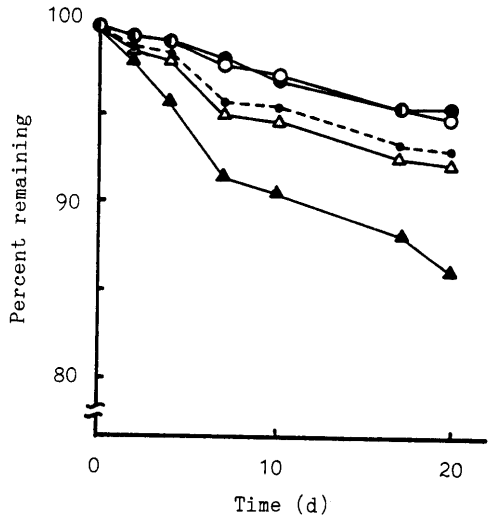

Fig. 3. Decomposition Curves of Freeze-Dried CET in the Presence of Various Saccharides at 50 °C, 16.7% RH

Molar ratio; 1 : 6 of CET: glucose-units of saccharide. Saccharide: —●—, lactose; —○—, maltose; —△—, maltotriose; —▲—, maltopentaose; ---●---, none.

2 shows the dependency of CET decomposition rate on the amount of maltopentaose added. Decomposition rate constants were calculated by fitting the results to first-order kinetics. Maltopentaose content was expressed in a molar ratio of the glucose units to CET, *e.g.* the value of 5.0 in the abscissa corresponds to the equimolar of maltopentaose and CET. The apparent first-order rate constants increased with the amount of maltopentaose added up to the ratio of *ca.* 6.0, and then became constant.

Decomposition curves of CET in freeze-dried samples containing various kinds of saccharides (CET : glucose units = 1 : 6) are shown in Fig. 3. The results indicated that CET decomposition rate was dependent on the kind of saccharide added. The addition of maltose or lactose decelerated the decomposition of CET in comparison with the CET unary freeze-dried system, while the addition of maltotriose and maltopentaose accelerated the decomposition. Further, the decomposition of CET became more rapid as the saccharide chain lengthened, even though the added amounts of saccharides by weight were approx-

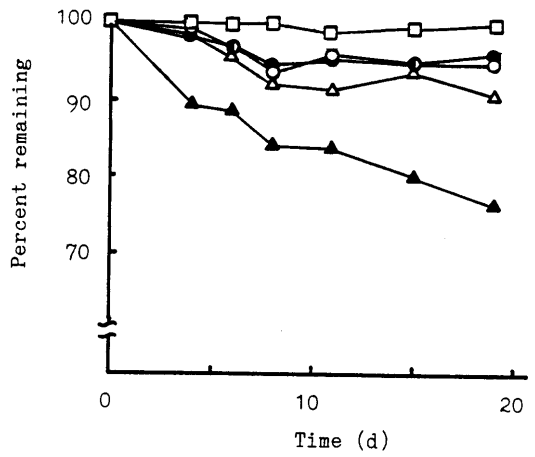

Fig. 4. Decomposition Curves of Freeze-Dried PCG in the Presence of Various Saccharides at 50 °C, 11.4% RH

Molar ratio; 1 : 6 of PCG: glucose-units of saccharide. Saccharide: —●—, lactose; —○—, maltose; —△—, maltotriose; —▲—, maltopentaose; —□—, none.

TABLE I. Apparent First-Order Rate Constant of PCG Decomposition and Water Content in a Binary Freeze-Dried Sample Stored at 50 °C, 11.4% RH

Saccharide	Decomposition rate constant ($\times 10^{-3} d^{-1}$)	Water content (%)
Lactose	1.60	3.7
Maltose	2.70	3.7
Maltotriose	4.71	4.2
Maltopentaose	12.5	5.4

Molar ratio; 1 : 6 of PCG: glucose-units of saccharide.

imately equal among the systems.

It is well known that moisture in a solid sample usually exerts significant influence on the hydrolysis of a drug. In the present cases, however, moisture was not an important factor to explain the difference in decomposition feature, since no significant difference in water content was found among the systems (5.5—6.5%). It was therefore suggested that the decomposition of CET in the binary freeze-dried samples was also governed by other factors, such as molecular interaction between CET and saccharides.

In previous papers,[9,12] we reported that the freezing condition affected the crystallinity and the molecular state of a drug in the binary freeze-dried samples. Using a slow freezing condition (frozen at −16 °C), binary freeze-dried samples of CET and saccharides were prepared and the decomposition rates of CET determined. All samples were obtained in amorphous state and the decomposition features were almost the same as those of the liquid nitrogen freezing systems. It appeared that the molecular state of CET, related to the decomposition features, was little affected by the freezing condition.

Benzylpenicillin Potassium (PCG) The stability of PCG in the binary freeze-dried samples has also been investigated. All of the freeze-dried samples were obtained in an amorphous state. Figure 4 shows the decomposition curves of freeze-dried PCG with several saccharides at 50 °C, RH 11.4%. Freeze-dried PCG without saccharide was found to crystallize during storage from powder X-ray diffractometry, and was stable against decomposition. On the other

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hand, the freeze-dried samples containing saccharide were in an amorphous state after 20 d storage. Similarly to the CET systems, greater decomposition rate was observed with increasing chain length of saccharide added. The apparent first-order rate constants and the water contents are listed in Table I. Though this suggested a correlation between the water content and the rate constant, the variation of water content was not great and was within experimental error. Thus, we found it difficult to explain the difference in the decomposition rate by the water content in the freeze-dried samples.

In conclusion, the stability of freeze-dried CET and PCG was affected by the addition of saccharide. Decomposition rates were accelerated with increasing chain length of saccharide added. Possible factors affecting the drug hydrolysis in the molecular dispersion are considered to be; (1) catalytic effect of additives, (2) water-vapor adsorption by additives, and (3) change in drug molecular state by interaction with additives. In the present case, factor (1) was believed unlikely because the functional groups existing in the dispersion were almost the same among the systems containing different saccharides. Factor (2), relating to the water content of samples, may somewhat affect the difference in decomposition rate. However, there seems to be another more important factor causing the difference since the variation of water content was not great among the systems. We previously studied the molecular interaction of a drug and a saccharide in binary freeze-dried systems and reported that the hydrogen-bond network, in which the drug molecules also formed hydrogen-bonding with hydroxy groups of a saccharide, became more rigid and the retention of drugs against sublimation increased as the saccharide chain lengthened.[12] The results of this latest work could, therefore, be explained by the interaction of CET and PCG molecules with saccharide molecules.

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References and Notes

1) Present address: *a) Hospital Pharmacy, College Hospital, Yamanashi Medical College, 1110 Shimokatoh, Tamahomachi, Nakakoma-gun, Yamanashi 409–38, Japan*; *b) Tsumura & Co., 3586 Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki 300–11, Japan.*
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