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Moisture Adsorption-desorption Effect on the Structure of Inclusion Complex of 6-Chloro-2-pyridylmethyl Nitrate and β-Cyclodextrin

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Abstract—A new anti-anginal drug, 6-chloro-2-pyridylmethyl nitrate (FR46171), was found to form a complex with β-cyclodextrin (β-CyD), molecular ratio 1:1. The FR46171/β-CyD complex thus prepared showed a moisture adsorption-desorption hysteresis characteristic of hydrophilic polymers. The moisture adsorption-desorption isotherm and differential scanning calorimetry indicated that the moisture adsorbed FR46171/β-CyD complex includes 13–15 mol of water while the moisture desorbed complex includes 5 mol of water. X-ray diffraction patterns of these samples confirmed their different structures. The scanning electron photomicrographs and the surface areas (BET) suggested that the moisture adsorption-desorption hysteresis observed in FR46171/β-CyD complex can be attributed to reversible hydrogen bonding between water molecules and hydroxyl groups.

Cyclodextrins interact with drug molecules to form inclusion complexes that may confer desirable properties on drug molecules, such as increased stability to temperature or hydrolysis, better solubility and masking of unpleasant tastes or odours (Nambu et al 1978; Uekama et al 1979, 1980).

6-Chloro-2-pyridylmethyl nitrate (FR46171; Fujisawa Pharmaceutical Co. Ltd) has been developed as an anti-anginal drug (Ohtsuka et al 1985) and the inclusion complex with β-cyclodextrin was prepared with the aim of overcoming anticipated problems with its instability to temperature and its low boiling point (Kitamura et al 1987).

The physicochemical properties of the inclusion complex (FR46171/β-CyD complex) showed a moisture adsorption-desorption hysteresis characteristic of hydrophilic polymers (Smith 1947). It is thought that cyclodextrin complexes have a small number of water molecules included in the crystalline structure constituting a complicated network of hydrogen bonds between host molecules and guest molecules. For example, the complex of α-cyclodextrin with benzaldehyde includes six molecules of water and these water molecules have been shown to stabilize the packing of its channel-type structure (Harata et al 1981).

In this paper the effect of moisture adsorption-desorption on the structure of the FR46171/β-CyD complex is investigated as water molecules are considered to be important in the crystal structure of cyclodextrin complexes.

Materials and Methods

Samples

FR46171 ($C_6H_5N_2O_3Cl$, mol. wt: 188·57) was prepared as described by Ueda et al (1984). β-Cyclodextrin (Tokyo Kasei; Lot No. AS01) was used without purification. FR46171/β-CyD complex was prepared as follows. A solution of 60 mg FR46171 in 180 mL acetone, was added dropwise to β-cyclodextrin (430 mg) in water (9 L) at 50°C. The mixed solution was continuously stirred overnight at room temperature (20°C); the white solid FR46171/β-CyD complex was filtered, washed with cold water, and dried under vacuum for 24 h at 35°C. The water content of the crystalline complex obtained was determined by Karl-Fischer's method as 6·05%. For elemental analyses of C, H and N a Yanaco CHN Coder MT-3 was used.

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Adsorption-desorption study

Relative humidity chambers were prepared by filling desiccating jars with appropriate salt solutions (range 0–100% RH at 25°C). The adsorption isotherm was determined by placing samples in the chambers and weighing them at intervals until constant weight was attained. The desorption isotherm was determined by equilibrating the samples in a 100% relative humidity chamber.

Scanning electron microscopy

The morphology of the FR46171/β-CyD complex was investigated by means of a scanning electron microscope (Hitachi S-650).

X-ray diffraction

The X-ray diffraction patterns were obtained with an X-ray diffractometer (Rigaku Denki, target Cu; filter Ni, voltage 30 kV, current 10 mA).

Thermal analysis

FR46171/β-CyD complex and an appropriate amount of water were accurately weighed and transferred to aluminium pans. The pans were crimped and allowed to stand at room temperature for 12 h then cooled to −80°C with liquid nitrogen at a cooling rate of 5°C min^{-1}. Differential scanning calorimetry (Seiko Densi Kogyo, DSC 20) was carried out at a heating rate of 5°C min^{-1}. The "free" and "bound" water in the samples was calculated from the enthalpy of fusion of free water referring to the known weight of added and adsorbed water in the samples (Hatakeyama 1979).

Results and Discussion

Inclusion complex formation of FR46171 with β-cyclodextrin

Elemental analyses of FR46171/β-CyD complex gave results consistent with a 1:1 complex $C_6H_5N_2O_3Cl/C_{42}H_{70}O_{35}$ as follows:

	C	H	N	Cl
Calculated (%)	43·56	5·71	2·12	2·68
Found (%)	43·27	5·86	2·09	2·98

Adsorption and desorption of water vapour

The water in β-cyclodextrin and FR46171/β-CyD complex reached a constant value within a day and the moisture content did not change over 2 weeks. Moisture adsorption-desorption isotherms for β-cyclodextrin and the FR46171/β-CyD complex are shown in Figs 1 and 2, respectively.

FR46171/β-CyD complex exhibits Type II adsorption as classified by Brunauer (1943) and the typical closed hysteresis loop of the moisture adsorption-desorption isotherms (Umprayn & Mendes 1987). On the other hand, β-cyclodextrin shows type I adsorption and an open hysteresis loop of moisture adsorption-desorption isotherms (Brunauer 1943; Umprayn & Mendes 1987). The closed hysteresis for FR46171/β-CyD complex may be attributed to a capillary pore in its structure and the open hysteresis loop of β-cyclodextrin may be explained by "ink bottle" pores (narrow neck pores) in the structure (Martin et al 1983).

Characterization of water molecules in β-cyclodextrin and FR46171/β-CyD complex

In the moisture adsorption-desorption isotherm of FR46171/β-CyD complex, the maximum equilibrium amount of water was found to be about 15% (Fig. 2). The amounts of "bound" and "free" water in hydrophilic polymers can be determined by differential scanning calorimetry (DSC). Thus, the equilibrium amount of water included in FR46171/β-CyD complex was also studied using DSC by adding different amounts of pure water to the complex, and Table 1 shows the calculated results of "free" and "bound" water in those samples. Fig. 3 shows the DSC

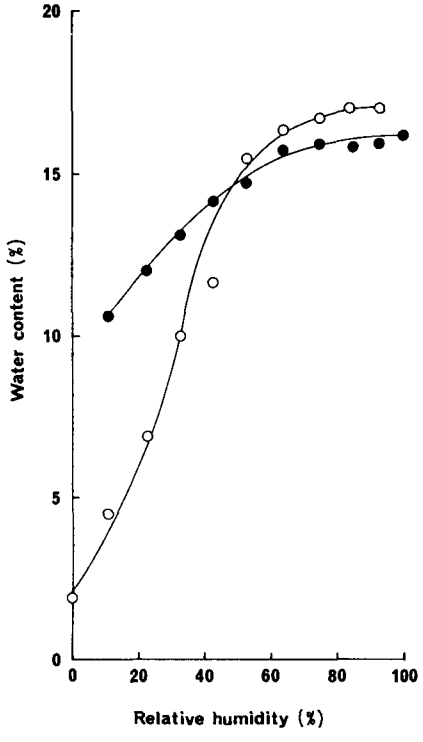

FIG. 1. Adsorption and desorption isotherms for water vapour on β-cyclodextrin at 25°C. ○, adsorption process. ●, desorption process.

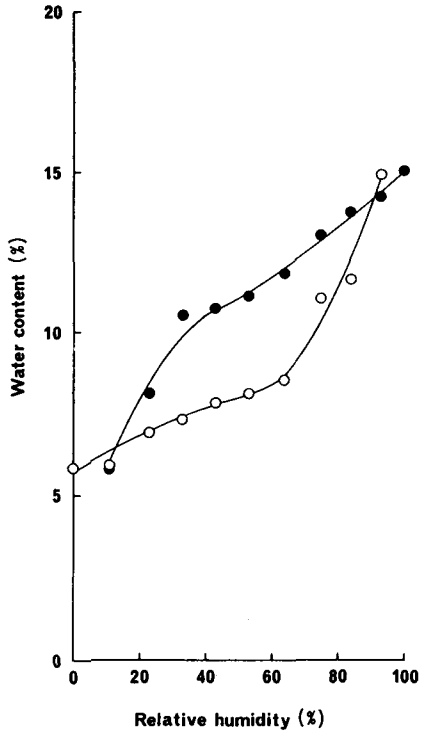

FIG. 2. Adsorption and desorption isotherms for water vapour on FR46171/β-CyD complex at 25°C. ○, adsorption process. ●, desorption process.

Table 1. Amounts of "free" and "bound" water in FR46171/β-CyD complex.

Sample (mg)	Added water (mg)[a]	"Free" water (mg)[b]	"Bound" water (mg)[c]	% of "bound" water in β-CyD complex[d]
3·41	9·28	8·82	0·67	17·3
6·37	4·83	3·85	1·37	18·6
7·47	2·70	1·92	1·23	14·9
—	—	—	—	x: 16·9

[a] water in FR46171/β-CyD complex, 6·05%.

[b] "free" water (mg) =

$$\frac{\text{enthalpy of fusion for water in sample (mJ)}}{\text{enthalpy of fusion for pure water (333·42 mJ mg}^{-1})}$$

[c] "bound" water (mg) =

[sample weight × 0·0605 + added water] − ["free" water]

[d] % of "bound" water in FR46171/β-CyD =

$$\frac{\text{"bound" water} \times 100}{[\text{sample weight} - (\text{sample weight} \times 0{\cdot}0605)] + \text{"bound" water}}$$

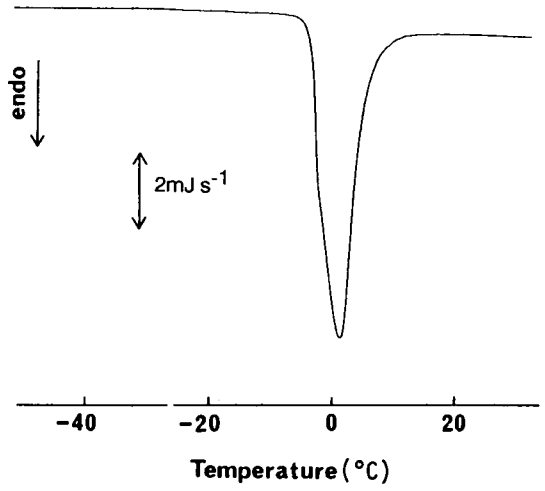

FIG. 3. DSC curve of FR46171/β-CyD. 2·7 mg of water was added to 7·47 mg of FR46171/β-CyD.

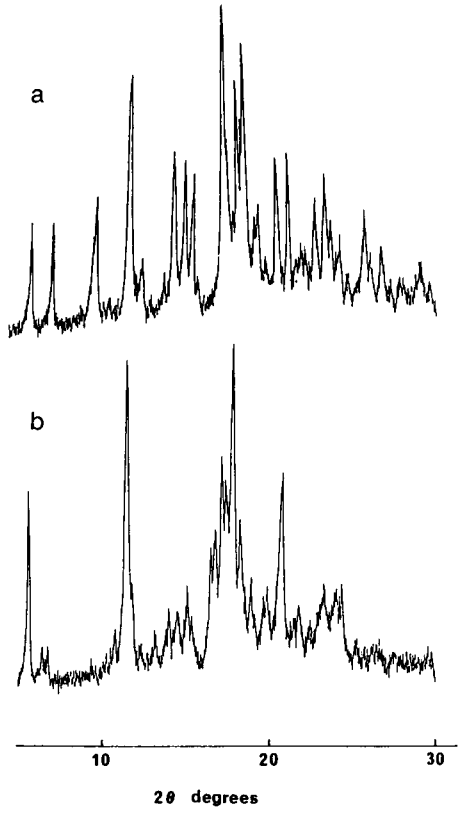

FIG. 4. Powder X-ray diffraction patterns of FR46171/β-CyD complex a, stored at 93% RH for 2 weeks, b, stored at 11% RH for 2 weeks.

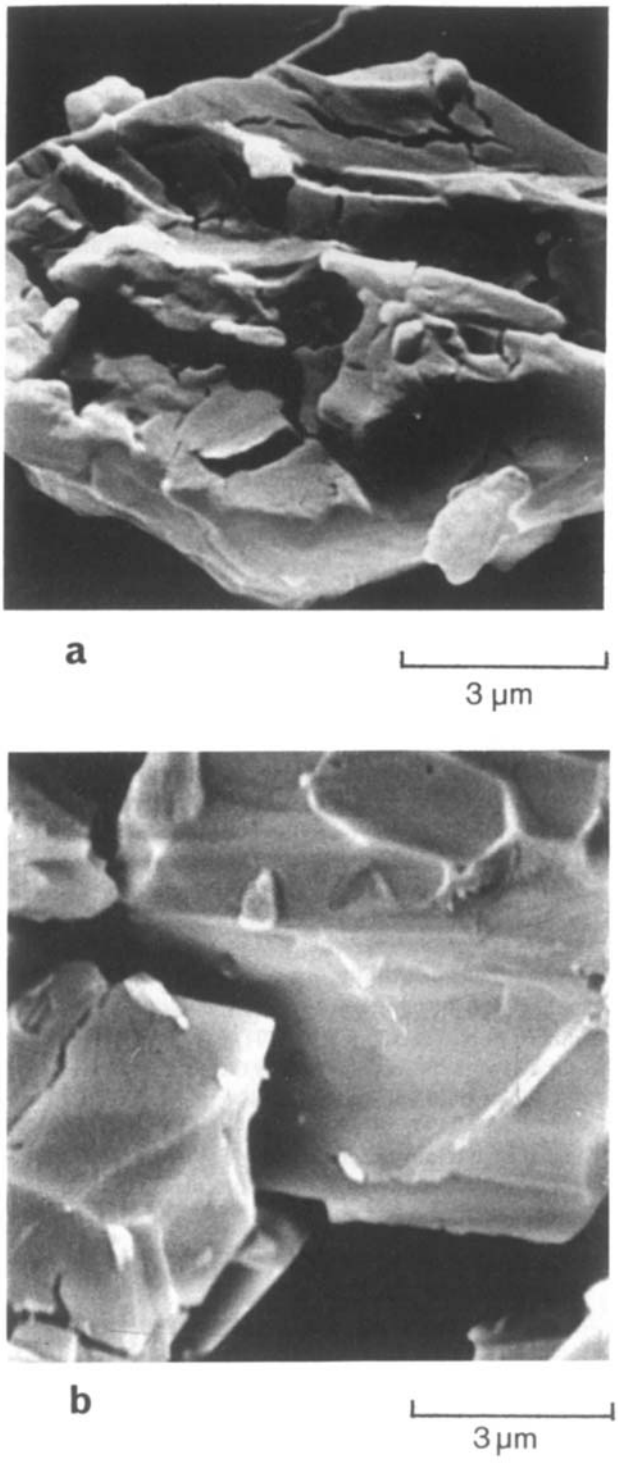

FIG. 5. Scanning electron photomicrographs of FR46171/β-CyD complex a, stored at 93% RH for 2 weeks, b, stored at 11% RH for 2 weeks.

curve of 7·47 mg FR46171/β-CyD complex to which 2·70 mg of water was added before the test.

As shown in Table 1, the "bound" water was independent of the water added, and was equal to the maximum equilibrium amount of water in FR46171/β-CyD complex. These values obtained by DSC (17%) and by moisture adsorption-desorption (15%) also indicated that FR46171/β-CyD complex was able to include 13–15 mol of water in its structure under humid conditions. On the other hand, the equilibrium water content of FR46171/β-CyD complex under dry conditions was about 6% (Fig. 2) corresponding to 5 mol of water. Thus, the crystal structure of FR46171/β-CyD complex stored under humid conditions is considered to be different from the complex stored under dry conditions.

Adsorption and desorption effect on the crystal structure of FR46171/β-CyD complex

Fig. 4 shows powder X-ray diffraction patterns of FR46171/β-CyD complex stored at 93 and 11% RH.

The different X-ray patterns suggest different crystal structures of FR46171/β-CyD complex stored under humid and dry conditions.

Scanning electron photomicrographs of FR46171/β-CyD complex stored at 93 and 11% RH for two weeks are shown in Fig. 5.

The surface of the moisture desorbed FR46171/β-CyD complex was found to be smooth and without pores (magnification 10 000 ×). On the other hand, the surface of the moisture adsorbed FR46171/β-CyD complex was different and some cracks were observed (Fig. 5a).

Although the application of the BET-equation to moisture adsorption and desorption isotherms remains controversial, Das et al (1972) revealed that the BET analysis of the sorption isotherm of starch was in close agreement with the value calculated by the method of Harkins & Jura (1944). In our study, using BET to determine surface area, the calculated values of the moisture adsorbed and desorbed FR46171/β-CyD complex were 22·4 and 15·4 $m^2 g^{-1}$, respectively. Scanning electron microscopy and BET analysis therefore indicate that the surface area of the complex increased upon swelling.

Wurster et al (1982) obtained similar hysteresis loops in the study of adsorption-desorption of starch and some hydrophilic polymers. We propose the following explanation for the characteristic moisture adsorption and desorption loop of FR46171/β-CyD complex. During the drying process hydrogen bonds are formed between some of the hydroxyl-groups which had just become free by the removal of water. On the other hand, during the adsorption process, water vapour is initially adsorbed on those hydroxyl-groups which had been free in the dried FR46171/β-CyD complex. Then, as the adsorption process proceeds the interhydroxyl hydrogen bonds would be broken and the new hydrogen bonds formed between hydroxyl-groups and water molecules. Thus, at high relative humidity, more sites are available for the interaction between water molecules and hydroxyl-groups in FR46171/β-CyD complex during the desorption process than during the adsorption process.

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