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Inclusion Compounds of Non-Steroidal Antiinflammatory and Other Slightly Water Soluble Drugs with α- and β-Cyclodextrins in Powdered Form[1,2]

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Investigations were made to prepare the inclusion compounds of the non-steroidal antiinflammatory drugs and additionally other slightly water soluble drugs with α-cyclodextrin (α-CD) and β-cyclodextrin (β-CD) in solid powdered form by the freeze-drying and the coprecipitation methods.

The freeze-drying method was successful in obtaining the inclusion compounds of all the test drugs with β-CD and also some of them with α-CD by obtaining their aqueous solutions with addition of aqueous ammonia before the process, with a very good yield compared with the usual coprecipitation method.

The drugs included by the coprecipitation method seemed to have fittable molecular sizes. The result showed that the coprecipitation method might be originally inferior to the freeze-drying method in obtaining the inclusion compounds of drugs in powdered form. It was shown by X-ray diffractometry that the inclusion compounds obtained by the freeze-drying method were amorphous. Clear differences in infrared (IR) absorption spectroscopy and differential calorimetry were observed between the inclusion compounds and the physical mixtures of drug/β-CD.

β-Cyclodextrin (β-CD) is known to form inclusion compounds with many kinds of compounds and has widely been used in various fields,[4] as well as in pharmaceutical field.[5,6] Non-steroidal antiinflammatory drugs, which are generally very slightly soluble in water and also sometimes cause adverse reactions due to a stimulant property to stomach upon oral administration, are included in α-cyclodextrin (α-CD) and β-CD in aqueous solution.[1]

From the pharmaceutical point of view, if an inclusion compound with β-CD is provided in solid powdered form, it may be more widely used, especially being convenient for oral administration. However, there have been few reports concerning the inclusion compounds obtained in solid powdered form.[7]

The present study was attempted to obtain the inclusion compounds of the non-steroidal antiinflammatory drugs and additionally other slightly water soluble drugs with α- and β-CD in solid powdered form. As a result, the freeze-drying method was successful in obtaining the inclusion compounds of all the test drugs with β-CD and also of some of them with α-CD, with a very good yield compared with the usual coprecipitation method.

1) This paper forms Part IV of "Pharmaceutical Interactions in Dosage Forms and Processing." The preceding paper, Part III: Y. Hamada, N. Nambu, and T. Nagai, *Chem. Pharm. Bull.* (Tokyo), **23**, 1205 (1975).
2) A part of this work was presented at the 94th Annual Meeting of Pharmaceutical Society of Japan, Sendai, April 1974.
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4) K. Takemoto, "Hosetsu Kagoobutsu no Kagaku," Tokyo Kagaku Dojin, Tokyo, 1969.
5) H. Mima, *Yakugaku Zasshi*, **77**, 1196 (1957).
6) K. Koizumi, J. Tatsumi, M. Ohae, H. Kumagai, and T. Hayata, *Yakugaku Zasshi*, **89**, 1594 (1969).
7) *a*) M. Hayashi (Ono Pharmaceutical Co., Ltd.), Japan Patent Sho-47-39057 (1972); *b*) Y. Hatachi (Ono Pharmaceutical Co., Ltd.), Japan Patent Sho-49-26416 (1974); *c*) K. Ota (Ono Pharmaceutical Co., Ltd.), Japan Patent Sho-49-30224 (1974).

Experimental

Materials——β-CD supplied by Teijin Ltd., was used after recrystallization from water. α-CD, anthranilic acid (ANA), *p*-aminobenzoic acid (ABA) and methyl salicylate (MSL) used were of the reagent grade. Very pure compounds of non-steroidal antiinflammatory drugs supplied by the respective companies, which all conformed to the standards, were as follows: azapropazone (APZ) by Nippon Chemipha Co., Ltd., mp 236—238°; indomethacine (IMC) by Sumitomo Chemical Co., Ltd., mp 153—154°: flufenamic acid (FFA) by Sankyo Co., Ltd., mp 133—136°; mefenamic acid (MFA) by Sankyo Co., Ltd., mp 225°; phenylbutazone (PBZ) by Fujisawa Pharmaceutical Co., Ltd., mp 105°; ibufenac (IFC) by Kaken-Yakka Kogyo Co., Ltd., mp 85—87°; ibuprofen (IPF) by Kaken-Yakka Kogyo Co., Ltd., mp 74—75°; ketoprofen (KPF) by Nippon Rhodia Co., Ltd., mp 93°. The other drugs used were barbital J.P. VIII (BTL), phenobarbital J.P. VIII (PBT), sulfisoxazole J.P. VIII (SIZ) and sulfathiazole J.P. VII (STZ).

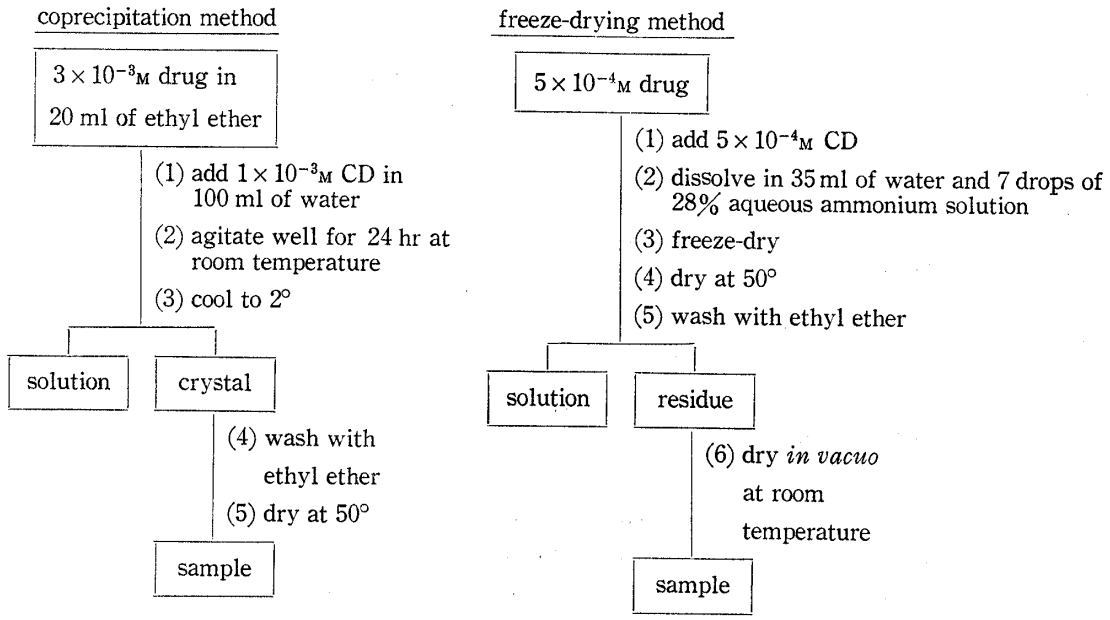

Chart 1. Methods for Preparation of Inclusion Compounds

Preparation of Inclusion Compounds——The inclusion compounds were prepared by the two methods, as shown in Chart 1. According to the coprecipitation method, which is most general to preparing inclusion compounds,[5] CD[8] in water and a drug in ethyl ether were mixed with molecular ratio 1: 1. According to the freeze-drying method, CD and a drug with molecular ratio 1: 1 were dissolved in aqueous ammonium solution because the drugs are acidic and generally very slightly soluble in water,[9] and then freeze-dried, no ammonium ion being detected in the products by the qualitative analysis using Nessler's reagent.

Determination of the Amount Included by CD——After the coprecipitated or freeze-dried sample prepared in the way in Chart 1 was dissolved in water, the amount of the drug included by CD was determined according to ultraviolet (UV) absorption method using a Hitachi 323 spectrophotometer. The physical mixtures of the drug and CD with the same molecular ratio as in the preparation of inclusion compounds were washed following the processes below (4) and (5) in Chart 1 for the coprecipitation and for the freeze-drying methods, respectively, and then subjected to the UV spectrometry mentioned above, no drug being detected.

IR Absorption Spectroscopy——This was done using a Shimadzu Model IR-400 infrared spectrophotometer according to the KBr disk method.

Differential Scanning Calorimetry (DSC)——This was done using a Perkin-Elmer Model DSC 1B differential scanning calorimeter, in the sample pan for solid sample at the scanning speed of 8°/min from 298° to 600°K.

X-Ray Diffraction Studies——Powder X-ray diffractometry was carried out using a Rigaku Denki Geigerflex Model D-2 diffractometer by Ni-filtered Cu-Kα radiation.

8) The abbreviation CD without α- or β- is common to both α- and β-CD.
9) It was assertained by the thin-layer chromatography of the products that the drug was not degradated in the procedure.

Results and Discussion

Certainty of the Formation of Inclusion Compounds with β-CD in Solid Powdered Form by the Freeze-Drying Method in Comparison with That by the Coprecipitation Method

The freeze-drying method was successful in obtaining the inclusion compounds of all the test drugs with β-CD with a very good yield compared with the coprecipitation method, as shown in Table I.

It may be possible that also ethyl ether was included by β-CD during the washing process with ethyl ether.[10] In the present study as a preliminary pharmaceutical one, however, the discussion was centered upon the drugs included.

Table I. Amounts of Drugs Included by CD (mole/mole of CD)

Drugs	CD	C.P.[a,b]	F.D.[b,c]	Drugs	CD	C.P.[a,b]	F.D.[b,c]
IMC	β	0	0.92	IPF	β	0.36	0.82
APZ	α	0	0.97	KPF	β	0.39	0.72
APZ	β	0	0.90	ANA	β	0.23	0.95
PBZ	α	0	0.94	BTL	β	0	0.61
PBZ	β	0	0.87	PBT	β	0	0.83
FFA	β	0	0.83	SIZ	β	0	0.95
MFA	β	0	0.96	STZ	β	0	0.98
IFC	β	0.43	0.79	MSL	β	0.73	—[d]

a) C.P.: coprecipitation method
b) Each value is the mean of three experimental runs.
c) F.D.: freeze-drying method
d) Because the suitable aqueous solution was not obtained.

IFC, IPF, KPF, and ANA formed the inclusion compounds with β-CD also by the coprecipitation method. However, the amounts included was less than those by the freeze-drying method. Although a clear explanation was not given for the reason why the drugs other than the above four were not included by β-CD by the coprecipitation method, the molecular size of the drugs was considered to have some relation to the formation of inclusion compounds, as was discussed in the previous paper.[1] The molecular sizes of the drugs were evaluated by the assembled Stuart type molecular models, as shown in Table II, where the longest dimension was taken as the z-axis and the two values in the column of y-axis were given for the molecules

Table II. Molecular Sizes of Drugs Evaluated by the Assembled Stuart Type Molecular Model (Å)

Drugs	x-axis direction	y-axis direction	z-axis direction[a]
ABA	4.5	0.6	7.1
ANA[b]	6.0	1.4	6.0
MSL[b]	5.0	1.6	8.0
IFC[b]	4.6	3.2	11.0
IPF[b]	4.8	3.4 or 4.1[c]	11.0
KPF[b]	6.5	5.1	10.4
FFA	6.1	6.2	10.1
MFA	6.2	5.5 or 6.0[c]	10.0
IMC	8.5	4.5	12.5

a) the longest dimension
b) Inclusion compounds with β-CD were obtained by the coprecipitation method.
c) for the two different conformations

10) K. Takeo and T. Kuge, *Agr. Biol. Chem.*, 36, 2615 (1972).

of two different conformations. Considering the size of the cavity of β-CD is 7—8 Å, the result shown in Table II suggested that ANA, IFC, IPF, and KPF, which were included, might have fittable molecular sizes regarding both *x*- and *y*-axes, and that FFA, MFA, and IMC seemed too large to be included. In this connection, ABA and MSL were subjected to the same coprecipitation method, and then the former was not included, the latter been done with the yield 0.73 mole/mole of β-CD. The reason for this result also might be explained on the consideration that ABA seemed too small while MSL fittable to be included, as shown in Table II. Additionally, as ethyl ether is known to be included by β-CD,[10] the formation of inclusion compounds of drugs might be affected by the large amount of ethyl ether used in the coprecipitation method shown in Chart 1. On the other hand, by the freezing procedure the inclusion compounds of drugs in solution were considered to be easily solidified, as such a solvent as ethyl ether did not exist.

Provement of the Low Possibility of the Formation of Inclusion Compounds with β-CD in Solid Powdered Form by the Coprecipitation Method

The kind of solvent for the drug in the coprecipitation method shown in Chart 1 is known to affect the formation of the inclusion compound with CD.[11] Following Hayashi's patent,[7a] ethanol was used as the solvent at first, but the inclusion compounds were not obtained for all drugs. The solvents themselves such as alcohols, benzene, *n*-heptane, cyclohexane and so on were considered to be included by β-CD,[11] and thus the inclusion compounds of drugs might hardly be obtained from these solvents. In the present study, therefore, according to Cramer,[11a] ethyl ether was used as the solvent to prepare inclusion compounds by the coprecipitation method as already described in Chart 1.

The reproducibility of the experimental result by the coprecipitation method was very high, for example, as shown for IFC in Table III.

Table III. Amounts of IFC Included by β-CD by the Coprecipitation Method in Different Experimental Runs

Experimental (run)	IFC (mg)	Ethyl ether (ml)	β-CD (mg)	Water (ml)	Mole ratio[a]	Mole ratio[b]
1	961.3	20	567.5	30	10	0.46
2	961.3	20	567.5	50	10	0.46
3	480.6	20	567.5	50	5	0.46
4	576.8	20	1135.0	100	3	0.39
5	577.0	20	1135.0	100	3	0.39

a) ratio of IFC/β-CD (mole/mole) before coprecipitation
b) ratio of IFC/β-CD (mole/mole) of the coprecipitated sample

Conclusively, the coprecipitation method, which has been widely tried using ethyl ether, might be originally inferior to the freeze-drying method in obtaining the inclusion compounds of drugs with β-CD in solid powdered form.

Physico-chemical Properties of the Inclusion Compounds with β-CD in Solid Powdered Form Prepared Both by the Freeze-drying Method and by the Coprecipitation Method

Figure 1 shows the IR spectra of the physical mixture and the freeze-dried product, for example, of FFA/β-CD, being different between them. The bands at 1650 and 1700 cm^{-1} due to C=O, which were observed in the physical equimolar mixtures of FFA, MFA, IMC, or APZ with β-CD, shifted to the lower wave number or hided itself by the broadening in the freeze-dried sample, suggesting the existence of some interactions between the drugs and β-CD in the latter. The two bands due to C=O of the coprecipitated inclusion compounds lited in Table

11) *a*) F. Cramer, *Chem. Ber.*, **84**, 851 (1951); *b*) F. Cramer and F.M. Henglein, *ibid.*, **90**, 2561 (1957).

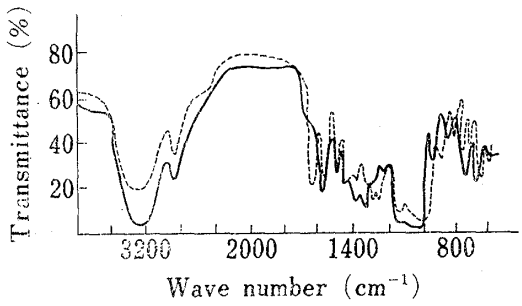

Fig. 1. IR Absorption Spectra of FFA/β-CD According to KBr Disk Method

——: physical mixture of FFA and β-CD
——: freeze-dried FFA/β-CD

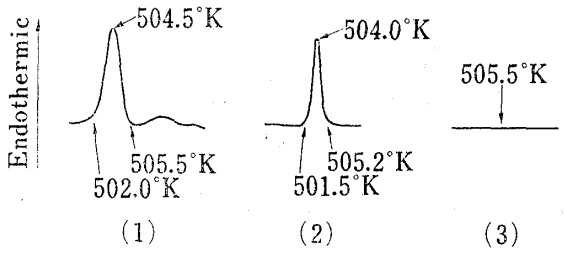

Fig. 2. DSC Curves of MFA/β-CD at Scanning Speed of 8°/min

(1) freeze-dried MFA (0.8 mg)
(2) physical mixture of MFA and β-CD (3 mg)
(3) freeze-dried MFA/β-CD (3 mg)

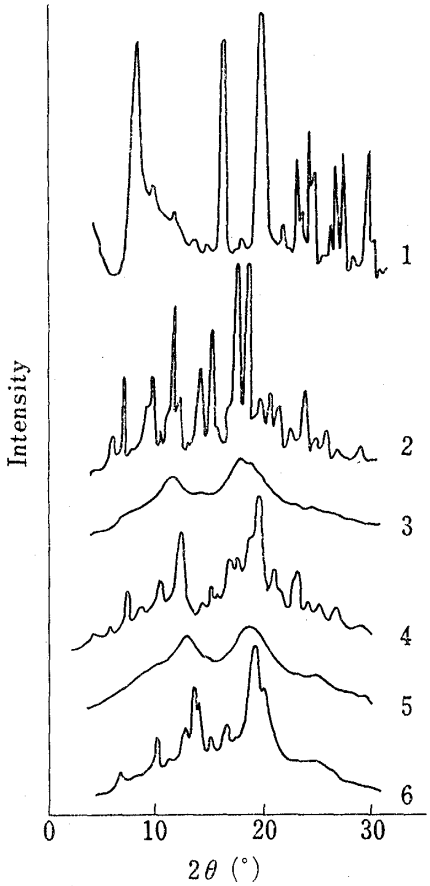

Fig. 3. Powder X-Ray Diffraction Patterns of IFC/β-CD

1. intact IFC
2. coprecipitated IFC/β-CD
3. freeze-dried IFC/β-CD
4. physical mixture of IFC and β-CD both separately freeze-dried
5. freeze-dried β-CD
6. recrystallized β-CD

I also shifted to the lower wave number and broadened in a tendency similar to the above example but not so remarkable. Considering the amount of KPF included by the coprecipitation method was not so much, *i.e.*, 0.39 mole/mole of β-CD, it seemed reasonable thta the change in spectrum was not so remarkable.

Figure 2 shows the DSC curves of MFA/β-CD, for example. The endothermic peak arround 505°K, which was observed in the freeze-dried MFA and the physical mixture of MFA with β-CD, disappeared in the freeze-dried MFA/β-CD in the scanning temperature range. This disappearence of the endothermic peak was observed also in the coprecipitated samples listed in Table I, corresponding to the result that a similar change in IR spectra was observed both in the freeze-dried and the coprecipitated samples.

Figure 3 shows the X-ray diffraction patterns of IFC/β-CD, for example, indicating that the mixture transformed to an amorphous one by freeze-drying. The two peaks of the broad diffraction pattern of the freeze-dried samples appeared at the interplanar distances arround 7.2—7.7 and 4.8—5.0 Å, as shown in Table IV. The freeze-dried β-CD showed the two peaks of diffraction pattern at the interplanar distances of 7.0 and 4.8 Å, as might be considered due to the cylindrical structure of β-CD molecule compared with the small interplanar distance in the case of α-CD shown in Table V. In this connection, thoroughly dried samples of inclusion compounds of amylose, α-CD and β-CD have been reported to show the two broad peaks in X-

Table IV. Powder X-Ray Diffractions of Drug/β-CD Expressed by Interplanar Distance (Å) and Relative Diffraction Intensity (in Parenthesis)

Drugs	Drugs only	Coprecipitation method	Freeze-drying method[a]
β-CD		4.92(1.00), 6.91(0.77), 4.72(0.77)	4.48(1.00), 6.91(0.91)
Antiinflammatory drugs			
IMC	7.69, 5.34, 4.09		4.79(1.00), 8.84(0.96)
APZ	8.42, 7.75, 5.71		4.82(0.96), 7.25(1.00)
PBZ	12.44, 11.18, 4.37		4.79(1.00), 7.25(0.98)
FFA	3.81(1.00), 4.64(0.99), 6.19(0.93)		4.87(1.00), 7.19(0.74)
MFA	5.68, 4.19, 3.41		4.92(1.00), 7.49(0.72)
IFC	4.44, 5.40, 10.52	4.74(1.00), 5.03(0.95), 7.37(0.79)	5.01(1.00), 7.69(0.79)
IPF	7.96, 6.15, 4.44	5.12(1.00), 4.95(0.99), 7.62(0.93)	4.98(1.00), 7.37(0.75)
KPF	3.91, 4.87, 14.02	4.90(1.00), 6.96(0.73), 4.67(0.63)	4.92(1.00), 7.49(0.71)
Other drugs			
ANA	5.43, 4.77, 3.67	4.90(1.00), 4.77(0.98), 6.96(0.94)	4.92(1.00), 7.13(0.88)
BTL	7.82, 5.43, 5.12		5.03(1.00), 7.62(0.68)
PBT	5.94(1.00), 3.93(0.93), 5.50(0.73)		5.06(1.00), 7.75(0.58)
SIZ	3.88(1.00), 7.25(0.92), 5.09(0.88)		4.92(1.00), 7.31(0.80)
STZ	4.06(1.00), 5.82(0.77), 3.52(0.75)		4.48(1.00), 7.37(0.70)

a) Each value is given by the symmetrical vertical line of the maximal part of the broad diffraction peak obtained with a good reproducibility.

Table V. Powder X-Ray Diffractions of Drug/α-CD Expressed by Interplanar Distance (Å) and Relative Diffraction Intensity (in Parenthesis)

Drugs	Drugs only	Coprecipitation method	Freeze-drying method[a]
α-CD	—	7.37, 4.09, 6.28	6.32(1.00), 4.31(0.83)
APZ	8.42, 7.75, 5.71	—	6.65(1.00), 4.33(0.89)
PBZ	11.18, 4.37, 4.27	—	6.51(1.00), 4.37(0.89)

a) Each value is given by the symmetrical vertical line of the maximal part of the broad diffraction peak obtained with a good reproducibility

ray diffraction patterns,[12] which are given at almost the same interplanar distances as in this study.

The inclusion of drugs seemed to make the cavity of β-CD a little larger. Since the X-ray diffraction pattern of the cylindrically structured thiourea of the inclusion compound is not affected by the kind of the guest molecules,[5] the cylindrical structure of β-CD of the inclusion compound might be kept as it was intact.

Conclusively, clear differences in IR absorption spectra, DSC curves and X-ray diffraction patterns between the freeze-dried sample and the physical mixture of drug/β-CD indicated that the interaction of the drug with β-CD, *i.e.*, the formation of inclusion compound took place by the freeze-drying. The freeze-drying method is often applied to obtaining a drug in amorphous state,[13] which is very soluble to give a high bioavailability. However, this method is not

12) K. Takeo and T. Kuge, *Agr. Biol. Chem.*, **33**, 1174 (1969).
13) T. Kono, N. Hotta, and H. Tomioka, 90th Annual Meeting of Pharmaceutical Society of Japan, Sapporo, April 1970, 9B9-5.

always useful in obtaining a drug in amorphous, if it is applied to the simple solution of the drug. For example, APZ alone among the drugs used was transformed to an amorphous powder by the freeze-drying of the intact drug. On the other hand, all the drugs used were transformed to amorphous state by the freeze-drying with addition of β-CD. Therefore, the addition of β-CD in the way described in this paper may afford a reliable and useful means for obtaining various drugs in amorphous state.

Formation of the Inclusion Compound of Some Drugs with α-CD in Solid Powdered Form by the Freeze-drying Method

Since APZ and PBZ were shown in the previous paper to form the inclusion compounds with α-CD in aqueous solution,[1] they were subjected to the freeze-drying method in the present study, and the results similar to those of drug/β-CD were obtained. The amount included by α-CD was 0.97 and 0.94 mole/mole of α-CD for APZ and PBZ, respectively. No peak was observed at 522.5°K, and 375.0°K for APZ and PBZ, respectively, in DSC curves of the samples. X-ray diffraction patterns showed that they were amorphous and the interplanar distances of the inclusion compounds corresponding to the two peaks of the broad pattern were a little larger than those of α-CD itself as shown in Table V.

Finally, it was well confirmed that the freeze-drying method is useful to obtaining the inclusion compounds of drugs with α- or β-CD, if the aqueous solution of the drugs are obtained as was done with an addition of a small amount of ammonia before the freeze-drying in this study.

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