[Chem. Pharm. Bull. 23(6)1205—1211(1975)] UDC 547.458.6'751.03 : 615.276.011.4

Interactions of α- and β-Cyclodextrin with Several Non-Steroidal Antiinflammatory Drugs in Aqueous Solution[1,2]

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(Received September 19, 1974)

The interactions of α-cyclodextrin (α-CD) and β-cyclodextrin (β-CD) with several non-steroidal antiinflammatory drugs were studied, observing the effects of α- and β-CD on the solubility and the stability of the drugs in aqueous solution in comparison with that of glucose. Moreover, the combined effects of urea and sodium chloride with β-CD on the solubility of drugs were discussed.

The solubility of all kinds of drugs was found to increase with the addition of β-CD, while not with glucose. The increase of solubility with β-CD was considered due mainly to the formation of inclusion compounds. From the solubility data, the apparent stability constant K of the formation of inclusion compound were calculated, and moreover the functional groups included by β-CD were estimated for the respective drugs. The dissolution rate of drug increased with β-CD, while not with glucose, as was similar in tendency to the solubility data.

β-CD accelerated the degradation of azapropazone in aqueous solution while retarded that of phenylbutazone, and glucose had no effect on the stability of drugs, showing that a formations of inclusion compound with β-CD may make some drugs stable and others labile.

The addition of urea was considered to promote the inclusion of drug with β-CD, while sodium chloride gave the opposite effect, and an elevation of temperature gave the promoting effect.

β-Cyclodextrin (β-CD) is known to form inclusion compounds with many kinds of compounds and has widely been used in various fields.[4] In pharmaceutical field also, applications of such inclusion compounds have already been studied.[5,6] General applications of inclusion compound with β-CD to pharmaceutical preparations are concerned with the solubilization of a very slightly soluble drug,[7–9] the stabilization of a labile drug, the formulation of a solid preparation from a liquid drug, the improvement of a drug which is stimulant or irritant to stomach, and so on.

The present study was attempted to investigate the interactions of α-cyclodextrin (α-CD) and β-CD with non-steroidal antiinflammatory drugs, which are generally very slightly soluble in water and also sometimes cause adverse reactions due to its stimulant property to stomach

1) This paper forms Part III of "Pharmaceutical Interactions in Dosage Forms and Processing." The preceding paper, Part II: Y. Machida and T. Nagai, *Chem. Pharm. Bull.* (Tokyo), **23**, 1003 (1975).
2) A part of this work was presented at the 93rd Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April 1973.
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4) K. Takemoto, "Hosetsu Kagoobutsu no Kagaku," Tokyo Kagaku Dojin, Tokyo, 1969.
5) *a*) H. Mima, *Yakugaku Zasshi*, **77**, 1196 (1957); *b*) *Idem, ibid.*, **78**, 993 (1958); *c*) *Idem, ibid.*, **79**, 644 (1959); *d*) *Idem, ibid.*, **79**, 649 (1959); *e*) *Idem, ibid.*, **79**, 856 (1959); *f*) *Idem, ibid.*, **79**, 891 (1959).
6) *a*) K. Koizumi, J. Tatsumi, M. Ohae, H. Kumagai, and T. Hayata, *Yakugaku Zasshi*, **89**, 1594 (1969); *b*) K. Koizumi and K. Fujimura, *ibid.*, **92**, 32 (1972).
7) J. Cohen and J. L. Lach, *J, Pharm. Sci.*, **52**, 132 (1963).
8) J.L. Lach and J. Cohen, *J. Pharm. Sci.*, **52**, 137 (1963).
9) V.S. Venturella, J.A. Bianculli, and R.W. Sager, *J. Pharm. Sci.*, **53**, 142 (1964).

after oral administration, observing the effects of α- and β-CD on the solubility and the stability of the drugs in aqueous solution in comparison with that of glucose. Moreover, the effect of another component such as urea or sodium chloride on the interaction of α- or β-CD with drugs was discussed.

Experimental

Materials——β-CD supplied by Teijin Ltd., was used after recrystallization from water. α-CD and benzidine 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°.

Procedure for the Determination of Solubility——Unless otherwise stated, an excess amount of non-steroidal antiinflammatory drugs (400 or 600 mg) was added in 100 ml of $1—5\times10^{-3}$ M aqueous solution of α-CD, β-CD or glucose, shaken in a Taiyo M-1 type constant temperature incubator for 50 hr,[10] or vigorously agitated by a stirrer for 24 hr[10] at the respective experimental temperatures, and the mixture was filtered rapidly through a glass filter.[11] The concentration of drugs in the filtrate was determined according to UV absorption method using a Hitachi 323 spectrophotometer.

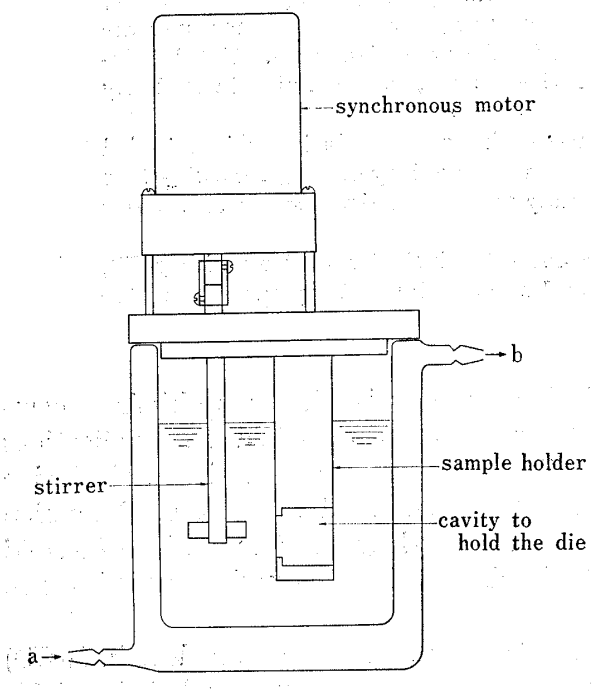


Fig. 1. Apparatus for the Determination of Dissolution Rate

Procedure for the Determination of the Dissolution Rate of Drugs——The dissolution rate was determined by a stationary disk method, using the apparatus shown in Fig. 1.[12] 0.5 g of drug powder was compressed in a cylindrical die of 1.0 cm inner diameter and 3.0 cm overall diameter by a Shimadzu hydraulic press for KBr tablets for infra-red spectroscopy, and the disk was not ejected. The die wearing the compressed disk was put in the cavity of sample holder, and the dissolution of drug from the one face of the disk was measured in the solution at a given temperature controlled by circulating constant temperature water by the way from (a) to (b), under the following conditions: 300 ml of test solution; 600 rpm of rotating velocity of the stirrer; 1.0 cm diameter of the disk of the drug. 1 ml of the solution was sampled out at appropriate time intervals, the resultant want of volume was compensated by adding the same test solution of the same temperature as that before the beginning of the dissolution.

Procedure for the Determination of the Stability of Drugs in Aqueous Solution——A given amounts of α-CD, β-CD or glucose was dissolved in an aqueous solution of each drug of a given concentration and the solution was shaken at 60°. For the study of the stability of drug against light, the solution described above was kept standing in a Nethler tube at 15500 lux under sunlight lamp D400 at 15° for an appropriate period in an Osaka Reiki "Tem-Com" weather maker. The change of UV absorption spectra was determined at appropriate time intervals using a Hitachi 323 spectrophotometer.

Results and Discussion

Effects of α- and β-CD on the Solubility of Drugs in Comparison with That of Glucose

The solubility of all kinds of drugs was found to increase with the addition of β-CD, while not with glucose. The additions of α- and β-CD showed almost the same increasing tendency

10) This was satisfactorily long to attain to equilibrium.
11) The glass filter was preliminarily warmed at the same temperature as the solution which is to be filtered.
12) Devised for the research entitled "Showa 47 Nendo Kosei Kagaku Kenkyu" by the Grant from Ministry of Welfare of Japan to T. Nagai, following the report of G. Milosovich, *J. Pharm. Sci.*, 53, 484 (1964).

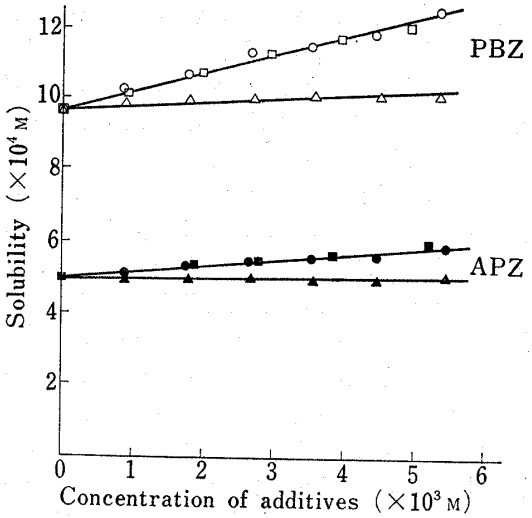


Fig. 2. Effects of α-CD, β-CD and Glucose on the Solubility of APZ and PBZ at 35°

□—□ ■—■ α-CD, ○—○ ●—● β-CD, △—△ ▲—▲ glucose

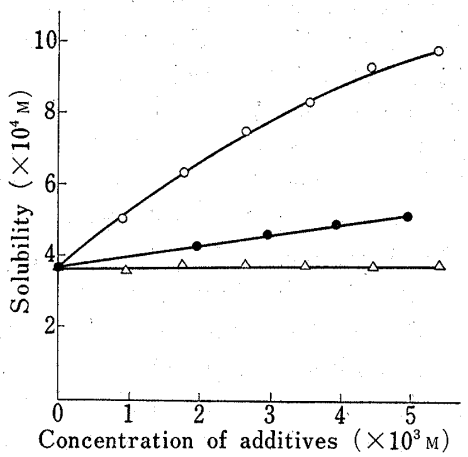


Fig. 3. Effects of α-CD, β-CD and Glucose on the Solubility of IMC at 35°

●—● : α-CD, ○—○ : β-CD, △—△ glucose

of solubility for APZ and PBZ, as shown in Fig. 2. The effect of β-CD on the solubility of IMC was greater than that of α-CD, as shown in Fig. 3. For FFA and MFA, the solubility increased with an increase of the concentration of β-CD, while not with the addition of α-CD and glucose, as shown in Fig. 4.

Since the above results were considered to depend on the molecular size of drugs, a similar examination was done using benzidine which has as large a molecular size as the cavity size of β-CD. The solubility of benzidine increased remarkably with the addition of β-CD and a little with glucose, while not with α-CD, as shown in Fig. 5. Further examination should be made for a clear explanation of the increase of solubility of benzidine with the addition of glucose.

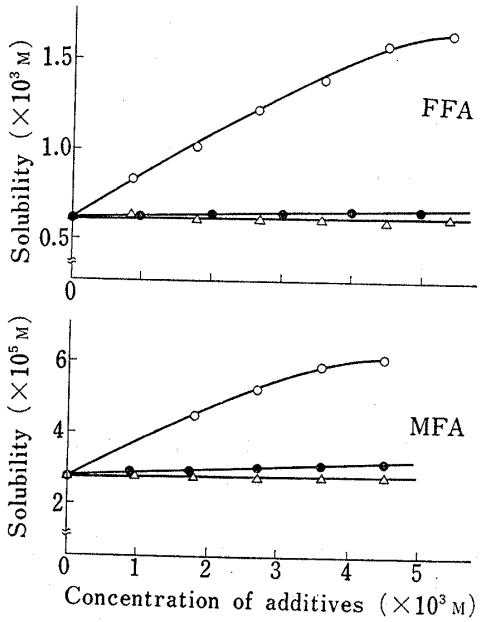


Fig. 4. Effects of α-CD, β-CD and Glucose on the Solubility of FFA and MFA at 35°

●—● : α-CD, ○—○ : β-CD, △—△ glucose

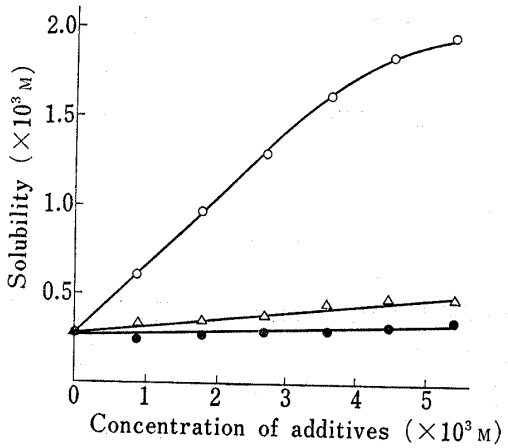


Fig. 5. Effects of α-CD, β-CD and Glucose on the Solubility of Benzidine at 25°

●—● : α-CD, ○—○ : β-CD, △—△ : glucose

The increase of the solubility of drugs with the additions of α- and β-CD thus observed was considered due mainly to the formation of inclusion compounds. Of course, the effect of some interaction of drug molecule with the respective hydroxy groups of α- and β-CD should be taken into consideration on the increase of solubility of drugs, though it might be very little compared with that of the formation of inclusion compounds on the consideration of a little increase of the solubility of benzidine with the addition of glucose mentioned above.

Table I shows the apparent stability constant K of the formation of inclusion compound, which was calculated according to the equation: $K=R/S_0(1-R)$, where R means the slope of the solubility curve and S_0 the solubility in the solution containing no additive. The orders of K were as follows: one power of ten for APZ with both α- and β-CD; two powers of ten for IMC and PBZ with both α- and β-CD, and for FFA and MFA with β-CD; three powers of ten for benzidine with β-CD. In the cases of drugs with glucose, the values were zero except for benzidine. The value of K for benzidine with β-CD was greater than those of other drugs, as the whole molecule of benzidine was considered to be included by β-CD. In the other cases, the respective molecules were considered to be partly included by α- or β-CD. Evaluating the respective sizes of the parts of drug molecules by the assembled Stuart type molecular models, the functional groups shown in Table II were considered to be included by β-CD.

Table I. Solubility and Apparent Stability Constant K of the Formation of Inclusion Compound with α-CD, β-CD and Glucose

Drugs	Solubility (M)	Additives	K (M^{-1})
APZ	4.9×10^{-4}	α-CD	3.6×10
		β-CD	3.6×10
		glucose	0
IMC	3.5×10^{-4}	α-CD	1.1×10^{2}
		β-CD	5.3×10^{2}
		glucose	0
FFA	6.2×10^{-4}	α-CD	6.0
		β-CD	4.4×10^{2}
		glucose	0
MFA	2.8×10^{-5}	α-CD	0
		β-CD	3.2×10^{2}
		glucose	0
PBZ	1.0×10^{-3}	α-CD	7.0×10^{2}
		β-CD	7.0×10^{2}
		glucose	0
Benzidine	2.7×10^{-4}	α-CD	3.0
		β-CD	2.3×10^{3}
		glucose[a]	1.9×10^{2}

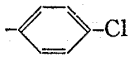
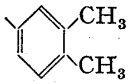
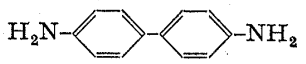
a) due to an interaction differed from a formation of inclusion compound

Effects of β-CD and Glucose on the Dissolution Rate of Drugs

As the dissolution rates of salicylic acid and phenobarbital are known to increase with the addition of β-CD,[13] the examination in the present study was done using APZ, IMC, FFA and PBZ. Fig. 6 shows the result for FFA, which was the most suitable example because the solubility was affected by only β-CD among the three additives and also its increase was great, as shown in Fig. 4. For the other drugs also, the dissolution rate increased with the addition of β-CD, while not with glucose. This effect of β-CD on the dissolution rate was similar in tendency to that on the solubility. Therefore, the increase of dissolution rate

13) K.-H. Frömming and I. Weyermann, *Arch. Pharm.*, **305**, 290 (1972).

Table II. Fuctional Groups Considered to be Included by β-CD and Their Sizes

Drugs	Functional groups	Size (Å)
APZ	$-C_3H_7$	5.0
IMC		7.0
FFA	$-CF_3$	6.5
MFA		7.0
PBZ	$-C_4H_9$	6.0
Benzidine		7.0

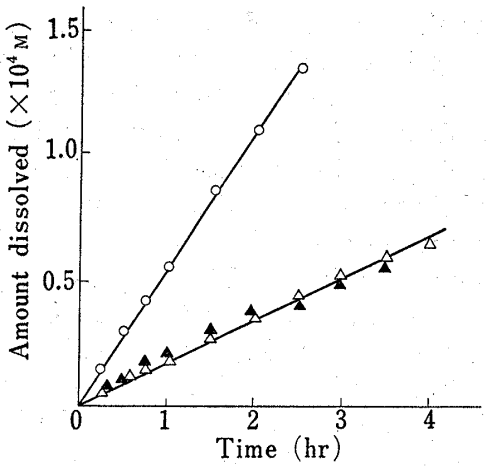


Fig. 6. Dissolution Curves of FFA in Aqueous Solution at pH 6.0 at 35°

○—○ : 9×10^{-3}M β-CD
△—△ : 6.3×10^{-2}M glucose
▲—▲ : no additive

seemed due mainly to that of solubility.[14] Further investigations, however, should be made in order to know how β-CD gives effect on the saturated concentration and the diffusion coefficient in Noyes-Nernst equation.[14] Practically, it is expected that an addition of β-CD in pharmaceutical formulations may enhance the bioavailability of drug.

Effects of α- and β-CD on the Stability of Drugs in Aqueous Solution

It is known that β-CD has an inhibitory effect on the degradation of such drugs as benzocaine,[15] procaine,[6] atropine[6] and aspirin.[6] In the present study, β-CD accelerated the degradation of APZ in aqueous solution in the light at pH 6.0, as shown in Fig. 7, though the plateaus of the curves were found in the initial stage. These plateaus appeared with a high reproducibility, as may be due to the interactions among the drug, the degradation products and β-CD, but should be investigated further in detail. At pH 8.0, the degradation of APZ

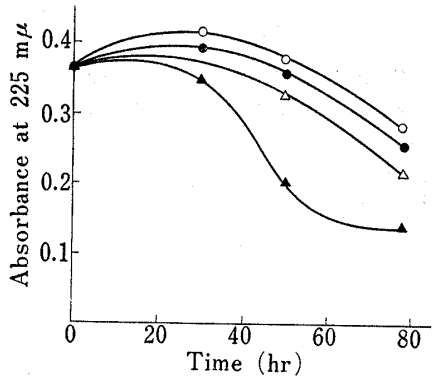


Fig. 7. Effect of β-CD on the Stability of 1.8×10^{-4}M APZ Aqueous Solution against Light at pH 6.0 at 15°

○—○: containing no β-CD, ●—●: 7×10^{-5}M β-CD, △—△: 3×10^{-4}M β-CD, ▲—▲: 1×10^{-3}M β-CD

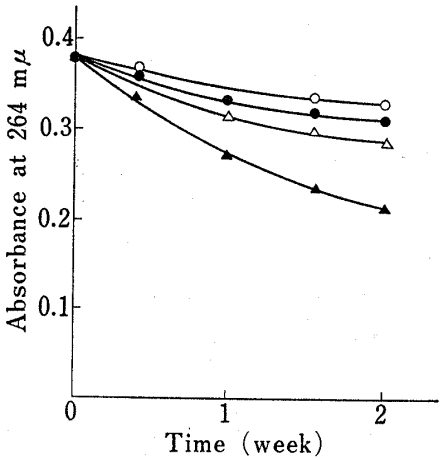


Fig. 8. Effect of β-CD on the Stability of 2.0×10^{-5}M PBZ Aqueous Solution at pH 5.5 at 30°

▲—▲ : containing no β-CD,
△—△ : 1.5×10^{-3}M β-CD,
●—● : 3.0×10^{-3}M β-CD,
○—○ : 4.5×10^{-3}M β-CD

14) H. Nogami, T. Nagai, and A. Suzuki, *Chem. Pharm. Bull.* (Tokyo), **14**, 329 (1966).
15) J.L. Lach and F.F. Chin, *J. Pharm. Sci.*, **53**, 924 (1964).

was also accelerated with the addition of β-CD in a similar way to the case at pH 6.0, though such a kind of plateau as shown in Fig. 7 was not found in this case.

In the case of PBZ, which is shown in Fig. 2 to be similar in solubility property to APZ, the degradation at pH 5.5 was inhibited with the addition of β-CD, as shown in Fig. 8. At pH 8.0, PBZ was so stable that it did not degradate in the present experimental conditions and thus no effect of β-CD was given. Fig. 9 shows that the degradation of PBZ was inhibited by both α- and β-CD, while was accelerated by glucose. This result showed that the same groups were included both by α- and β-CD, corresponding to the result shown in Fig. 2 that both α- and β-CD gave the same effect on the solubility of PBZ. Glucose gave no effect on the solubility of PBZ, while did an accelerating effect on the degradation. Additionally, the hydrolysis of IMC was accelerated just a little with the addition of α-CD and remarkably with glucose, while was inhibited with β-CD. This result seemed due to the situation that β-CD could include the whole functional group of IMC shown in Table II, while α-CD could not, corresponding to the result shown in Fig. 3. Therefore, a formation of inclusion compound with α- or β-CD may make some drugs stable and others labile. A clear explanation for the acceleration of a degradation of the drug with the addition of glucose was not given, but there was considered to be no relation to the situation that glucose does not form any inclusion compound.

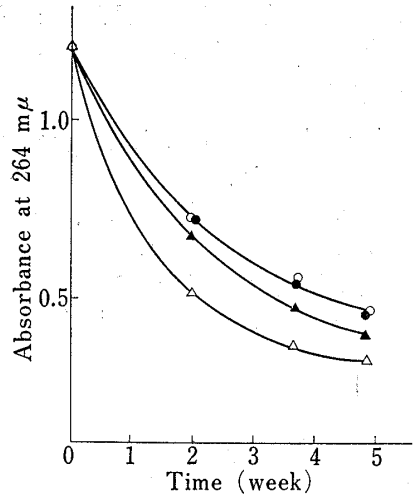


Fig. 9. Effects of α-CD, β-CD and Glucose on the Stability of 2.0×10^{-5}M PBZ Aqueous Solution at pH 6.0 at 60°

●—● : 5.4×10^{-3}M α-CD,
○—○ : 5.4×10^{-3}M β-CD,
△—△ : 3.24×10^{-2}M glucose,
▲—▲ : no additive

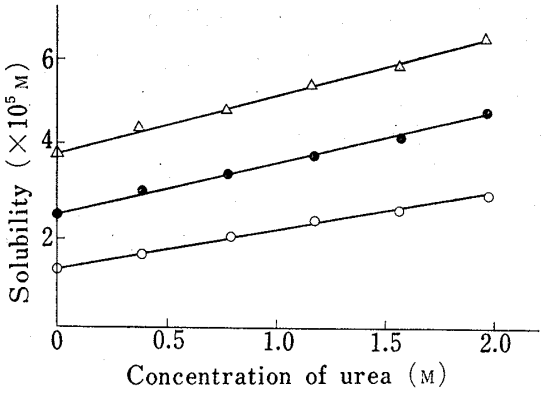


Fig. 10. Combined Effect of Urea with β-CD on the Solubility of MFA in Aqueous Solution at pH 6.0 at 25°

○—○ : containing no β-CD,
●—● : 2.7×10^{-3}M β-CD,
△—△ : 5.4×10^{-3}M β-CD

Effects of Additional Components and of Temperature on the Interaction of β-CD and Drugs

As urea is known to have effect on the water structure in aqueous media resulting a "destruction of iceberg" and thus acting as a solubilizer for many kinds of organic compounds,[16] it was subjected in the present study to an examination of its combined effect with β-CD on the solubility of drug, using MFA which was the most slightly soluble among the present samples. The solubility increased with the additions of both urea and β-CD as shown in Fig. 10, indicating that some potentiation took place. The slope of the solubility curve in Fig. 10

16) *a*) D.B. Wetlaufer, S.K. Malik, L. Stoller, and R.L. Coffin, *J. Am. Chem. Soc.*, **86**, 508 (1964); *b*) H. Nogami, T. Nagai, and H. Umeyama, *Chem. Pharm. Bull.* (Tokyo), **18**, 328 (1970).

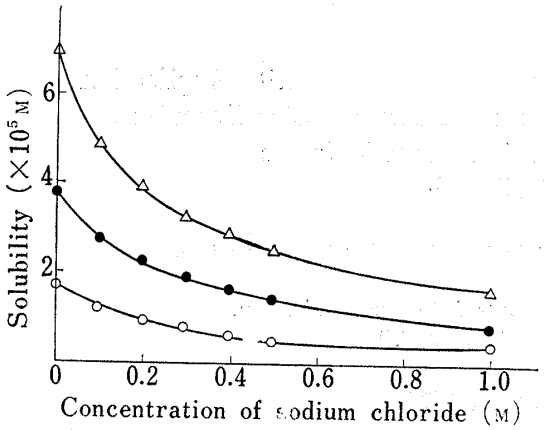


Fig. 11. Combined Effect of Sodium Chloride with β-CD on the Solubility of MFA in Aqueous Solution at pH 6.0 at 30°

○—○ : containing no β-CD,
●—● : 3.6×10^{-3}M β-CD,
△—△ : 7.2×10^{-3}M β-CD

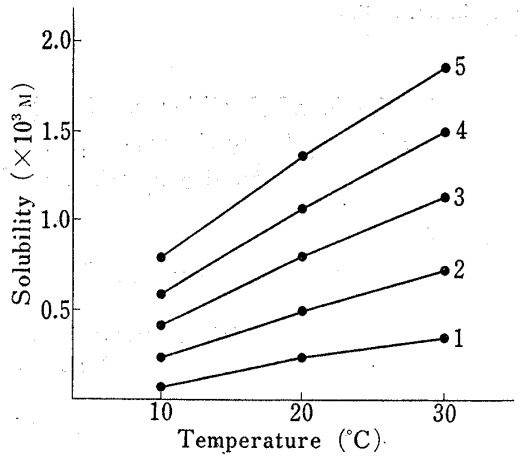


Fig. 12. Combined Effect of Temperature with β-CD on the Solubility of Benzidine in Aqueous Solution

1. containing no β-CD, 2. 0.9×10^{-3}M β-CD,
3. 1.8×10^{-3}M β-CD, 4. 2.7×10^{-3}M β-CD,
5. 3.6×10^{-3}M β-CD

increased with concentration of β-CD as follows: 0.8×10^{-5} for urea only; 1.1×10^{-5} for the addition of 2.7×10^{-3}M β-CD; 1.3×10^{-5} for the addition of 3.4×10^{-3}M β-CD. Therefore, the inclusion of drug with β-CD was considered to be promoted with the addition of urea.

On the other hand, as sodium chloride has the salting-out effect on various kinds of organic compounds, its combined effect with β-CD on the solubility of MFA was examined, as shown in Fig. 11. The slope of the solubility curve clearly decreased with the increase of β-CD. Therefore, sodium chloride was considered to give the effect opposite to urea on the inclusion of drug with β-CD.

Regarding the combined effect of β-CD and temperature, the solubility of benzidine, which was remarkably affected by β-CD, increased with the elevation of temperature, accompanying a clear increase of the slope of the solubility curve with the increase of β-CD, as shown in Fig. 12. This increase of the slope of the solubility curve seemed to have relation to a destruction of iceberg with an elevation of temperature.[17]

The above three results suggested that the formation of inclusion compound might depend also on the nature of water arround the solute molecules, *i.e.*, both drug and β-CD, in addition to the size of drug molecule mentioned already. Here, the nature of water arround the solutes might be influenced by such an additional component as urea or sodium chloride and also by temperature, resulting in promotion and in inhibition of the formation of inclusion compound by an iceberg crushing factor and by a salting-out one, respectively.

Acknowledgement The authors are very grateful to Teijin Ltd. for a grant to T.N., and to Nippon Chemipha Co., Ltd., Sumitomo Chemical Co., Ltd., Sankyo Co., Ltd., and Fujisawa Pharmaceutical Co., Ltd. for supplying the materials. Thanks are also given to Miss Kazuyo Takeuchi and Miss Sakiko Honda for their assistance in the experimental work.

17) K. Shinoda and M. Fujihira, *Bull. Chem. Soc. Japan*, **41**, 2612 (1968).