Inclusion Complex of Cinnarizine with β-Cyclodextrin in Aqueous Solution and in Solid State

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ABSTRACT. Inclusion complex formation of cinnarizine(CN) with β-cyclodextrin(β-CD) in aqueous solution and in solid state was confirmed by the solubility method, powder X-ray diffractometry, differential scanning calorimetry(DSC) and proton nuclear magnetic resonance(^{1}H-NMR) spectroscopy. The apparent stability constant, K', of the complex in water at 20°C was estimated as $6.2 \times 10^3 M^{-1}$. The stoichiometry of the complex was given as the ratio 1:2 of CN to β-CD. The dissolution rate of CN/β-CD complex which could be prepared three different methods, coprecipitation method, neutralization method and spray-drying method, was much more rapid than intact CN, i.e., about 30 times or more. The degradation of CN in acidic solution was found to be of pseudo first-order reaction. The pseudo first-order rate constant with β-CD decrease with an increase in concentration of β-CD at pH 1.20. The inclusion complex prepared by spray-drying method was very stable under heating conditions and under high humid conditions. There was no difference in the bioavailability of CN between oral administration of β-CD complex and that of CN alone. The absorption of CN decreased significantly when CN administered with $NaHCO_3$. However, there was observed no decrease in the case of CN/β-CD inclusion complex.

Cinnarizine(CN), [1-(diphenylmethyl)-4-(3-phenyl-2-propenyl)-piperazine], one of cerebral blood flow improvers, is widely used orally for treatment of cerebral apoplexy, cerebral arteriosclerosis and post traumatic cerebral symptom. However, CN, a weak base of pKa 7.47(1), is slightly soluble in water, as the solubility is less than 10 mg/ml at 37°C. Accordingly, it was less absorbed in patients of achlorhydria. This study, therefore, was attempted to apply cyclodextrin complexation to an enhancement of the solubility, dissolution rate, and bioavailability of CN.

Inclusion complex formation of CN with β-CD in aqueous solution and in solid state was ascertained by solubility method, powder X-ray diffractometry, differential scanning calorimetry(DSC) and proton nuclear magnetic resonance(^{1}H-NMR)(2). Complex of CN with β-CD in

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solid state was prepared by three different methods i.e., coprecipitation method, neutralization method(2) and spray-drying method(3). The effect of β-CD on the stability of CN in aqueous solution(pH 1.20) was investigated at 60°C, 70°C, 80°C and 90°C(4,5). The stability of CN/β-CD complex in solid was investigated at 70°C for 1 week and at 40 °C 75% R.H. for 4 weeks(3). The dissolution rate of CN/β-CD complex was determined in pH 5.0 buffer solution using the paddle method of Japanese Pharmacopoeia X(2). The bioavailability study was carried out in beagle dogs after fasting, determining the plasma concentration of CN by HPLC method(6,7).

Results and Discussion

1. Evidence of Inclusion Complex Formation(2)

Complex formation of CN with β-CD in aqueous solution was studied by solubility method and ^{1}H-NMR. Figure 1 shows an equilibrium phase solubility diagram obtained for the CN/β-CD system in water. The solubility of CN increased about five times in the presence of β-CD, showing the features of a Bs type phase diagram. In the higher concentration range of β-CD, a solid complex was precipitated. The stoichiometry of the complex was found to be 1:2 (CN:β-CD) from the data in the plateau region of the solubility diagram. The apparent stability constant, K', of the complex was estimated to be $K'=6.2\times10^3$ M^{-1} from the initial straight line portion of the solubility diagram. This large stability constant may be a result of substantial 1:2 inclusion complex formation.

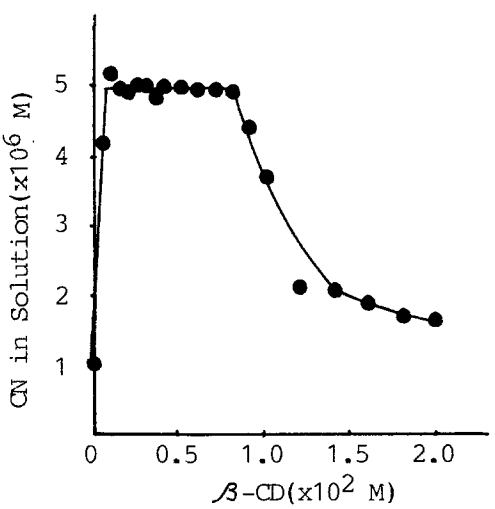


Figure 1. Solubility of CN as a Function of β-CD Concentration in Water at 20°C.

Figure 2 shows the effect of β-CD on the ^{1}H-NMR spectrum of CN in D_2O. The chemical shifts of the phenyl signals at δ 7.6-7.3 could not be determined exactly because of the multiplet structure. Furthermore, the signals of the H_2-protons (piperazine ring) overlapped with signals due to β-CD. It was apparent that the H_1-proton signal was remarkably shifted to higher field in the presence of β-CD, suggesting that the ring-currents of the diphenyl group in the drug were affected by the binding to β-CD. The lowfield shift of the H_3-proton might be induced by decreased freedom of rotation of the 3-phenyl-2-propenyl group due to the interaction with β-CD. The phenyl groups on both sides of the CN molecule might interact with β-CD at a molar ratio of 1:2 (CN:β-CD). This speculation was considered to be cosistent with the results obtained by the solubility method.

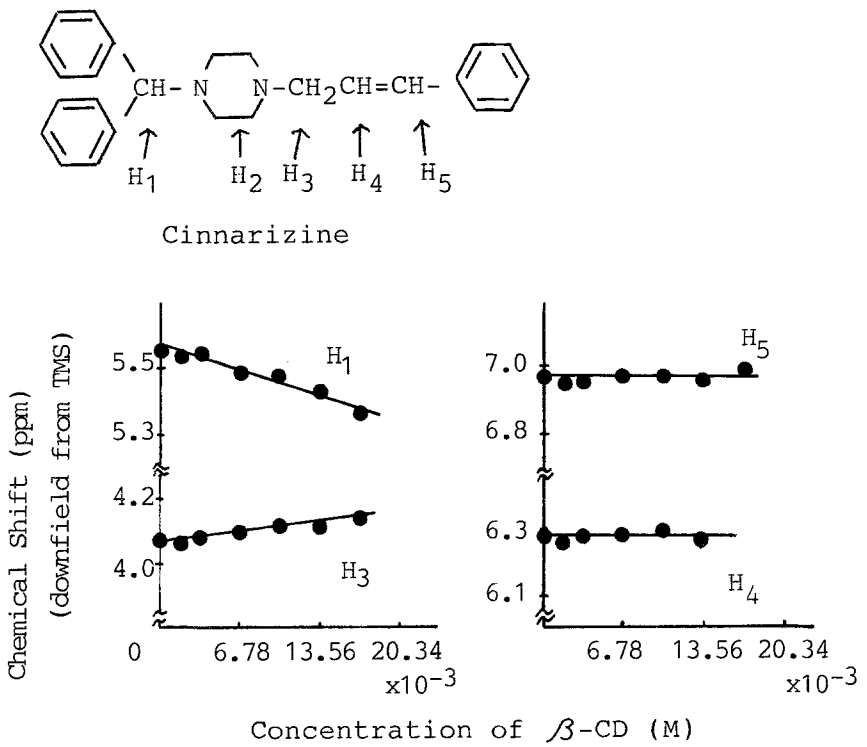


Figure 2. Variation of ^{1}H Chemical Shifts of 6.78×10^{-3} M Cinnarizine with Concentration of β-Cyclodextrin.

2. Method for Preparation of Inclusion Complex on a Manufacturing Scale(2,3)

The ordinary method(8), e.g., the coprecipitation method based on phase solubility and the kneading method, are not appropriate to obtain CN/β-CD complex with satisfactory efficiency and yield. However, the inclusion complex could be prepared easily by the neutralizing

procedure. The preparation methods are shown in Figure 3. The stoichiometry of the complex prepared by neutralization method was then analyzed chemically, and was found to be 1:2 (CN:β-CD) as shown in Table I. The principal advantages of neutralization method and spray-drying method can be summarized as follows; (1) the unnecessity of organic solvent, (2) a good yield in a short operating time and (3) suitability for extension to manufacturing scale. Further, neutralization method is suitable for a preparation of complex with higher purity in short operation time. Spray-drying method is suitable for a preparation in larger scale.

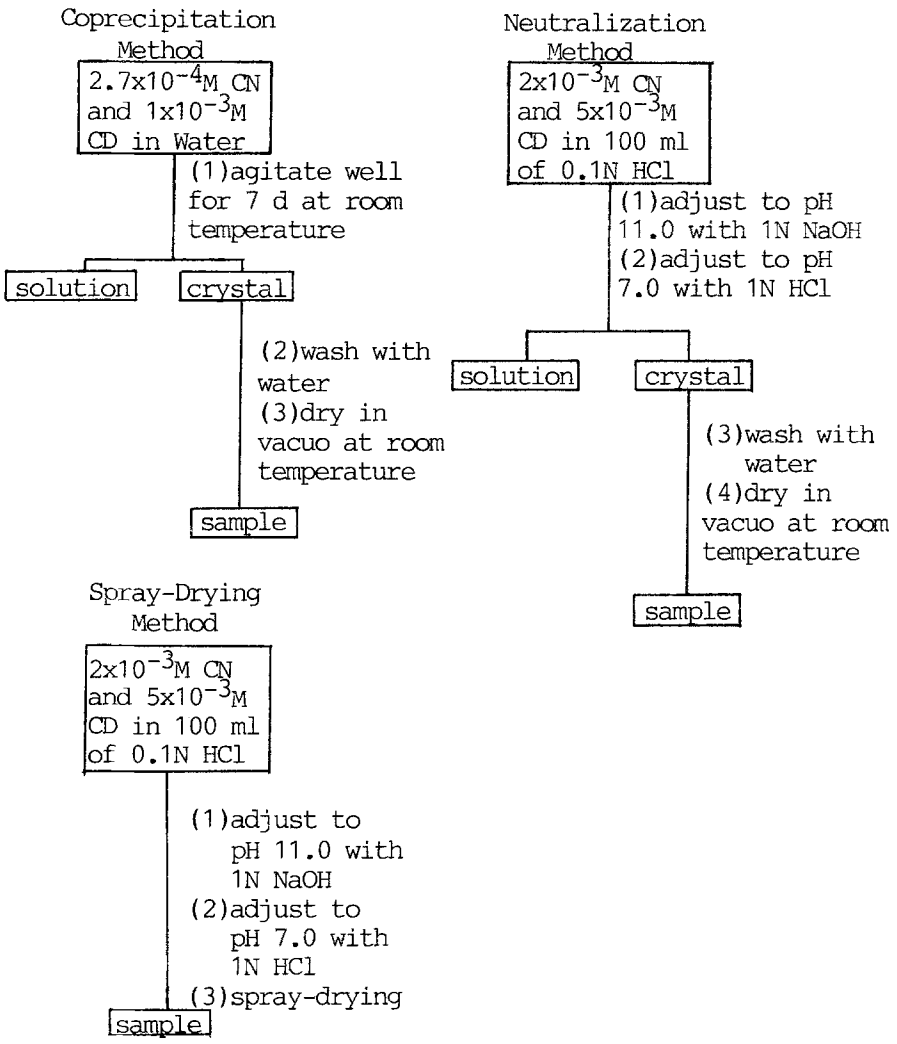
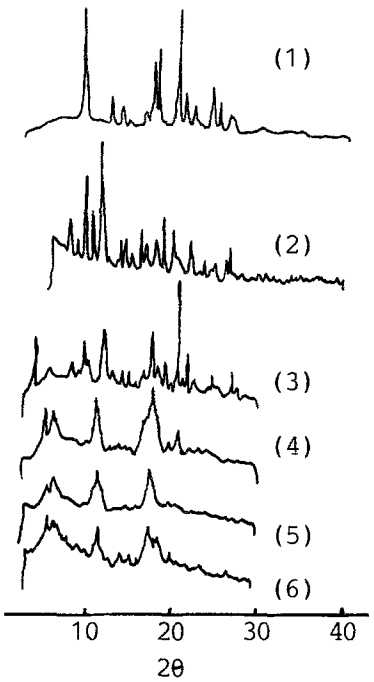


Figure 3. Method for Mass Preparation of Inclusion Complex



(1) intact cinnarizine.
(2) recrystallized β-cyclodextrin.
(3) physical mixture of cinnarizine and β-cyclodextrin.
(4) inclusion complex coprecipitation method.
(5) inclusion complex neutralization method.
(6) inclusion complex spray-drying method.

Figure 4. Powder X-ray Diffraction Patterns of CN/β-CD.

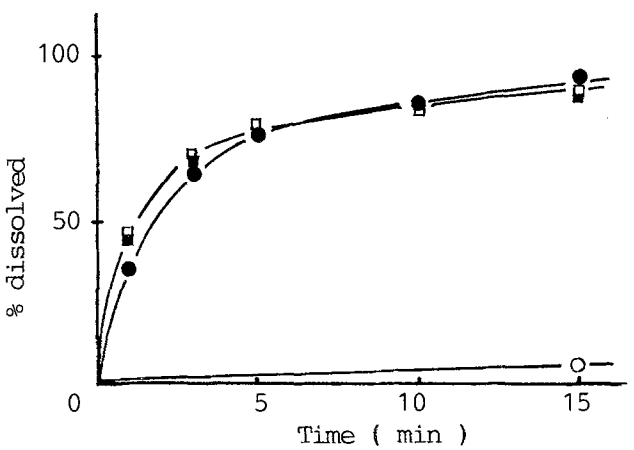


Figure 5. Dissolution Rate Curves of Cinnarizine Complexes in pH 5.0 Buffer Solution.
(●) : spray-drying method. (□) : neutralization method.
(■) : coprecipitation method. (○) : intact cinnarizine.

TABLE I Purity of Inclusion Compounds Prepared by Three Methods.

	Content of CN	Content of Inclusion Compound	Molar * Ratio
Coprecipitation Method	14.11%	101.2%	1 : 1.98
Neutralization Method	14.03%	100.4%	1 : 1.99
Spray-Drying Method	11.35%	81.3%	-

Each value is the mean of three experimental runs.
* Molar Ratio ; CN : β-CD.

3. Dissolution Behavior of CN/β-CD Complex(2,3)

Interaction of CN with β-CD in solid phase was confirmed by DSC and powder X-ray diffractometry.

Figure 4 shows the powder X-ray diffraction patterns of the complexes prepared by three different methods in comparison with that of a physical mixture at the same molar ratio. The diffraction pattern of the physical mixture was found to be a simple superposition of those of the components, while that of the complex was apparently different, corresponding to a new solid phase. In addition, there was no difference in diffraction patterns among the complexes. These results indicate that CN interacted with β-CD to form an inclusion complex.

In the result of DSC, the endothermic peak at around 120°C, which was observed for intact CN and the physical mixture of CN with β-CD, disappeared in the CN/β-CD inclusion complex. This result corresponded to the change in X-ray diffraction patterns.

The relative rates of dissolution of CN and its β-CD complex in powder form are shown in Figure 5. It is evident that CN/β-CD complex dissolved much more rapidly than intact CN. The dissolution rates of CN/β-CD complex prepared by three different methods were essentially the same, and the dissolution rate of CN/β-CD complex was 30 times or more larger than that of intact CN. The enhancement of the dissolution characteristics of CN by β-CD inclusion complexation may be due to improvements in solubility and wettability. The above results suggested that complex formation with β-CD may enhance the bioavailability of CN.

4. Effect of β-CD on the Stability of CN in Aqueous Solution and in Solid State(3,4,5)

The stability study in aqueous solution was an attempt to investigate the effect of β-CD on the degradation rate of CN in acidic solution

under heating conditions, confirmed that there may be no problem concerning the degradation of CN in the process of neutralization on spray-drying method in preparation of CN/β-CD inclusion complex. The degradation of CN with or without β-CD was found to be pseudo first-order reaction. When β-CD was in the reaction system, the apparent first-order rate constant, k, was smaller than that of CN alone at four different temperatures as shown in Table II. It was indicated that the addition of β-CD in the reaction system stabilized CN and the degree of stabilizing effect increased with increasing concentration of β-CD in the system. This result indicates that there is no strict problem in dissolving CN in acidic solution with β-CD when preparing CN/β-CD complex by use of neutralization method and spray-drying method.

TABLE II Rate Constants for Apparent First-Order Degradation of Cinnarizine.

	k (min^{-1})				
		Molar Ratio (CN:β-CD)[a]			
°C	CN	1:1	1:2	1:5	1:9.4
60	1.85×10^{-5}	-	1.07×10^{-5}	-	1.82×10^{-6}
70	1.05×10^{-4}	-	8.56×10^{-5}	-	4.99×10^{-5}
80	4.07×10^{-4}	3.88×10^{-4}	3.46×10^{-4}	2.80×10^{-4}	2.60×10^{-4}
90	1.94×10^{-3}	-	1.68×10^{-3}	-	1.26×10^{-3}

a) molar ratio of initial concentration of CN and β-CD in the solution.

Stability of the CN/β-CD inclusion complex prepared by spray-drying method was examined under heating conditions and under high humid conditions. The result is shown in Table III. The inclusion complex was found to be very stable. The dissolution rate of the samples stored under high humid conditions, is more rapid than initial value. This reason is considered to be due to the increase of the water content in the samples.

5. Bioavailability of CN/β-CD Complex(6,7)

Figure 6 shows the mean plasma levels of CN after the oral administration of CN and its β-CD complex to dogs. When the equivalent dose to 50 mg of CN was administered to dogs, the CN/β-CD complex gave the maximum plasma level of 166.9±22.4 ng/ml (mean±S.E.) at 0.5 h, which was 8.6 times as high as that of CN alone. This initial increase in drug absorption might be due to a high dissolution rate of the complex as previously described. However, there was no statistically significant difference in AUC_{0-8} between CN and CN/β-CD complex which were 267.2± 102.9 ng h/ml and 374.2±97.2 ng h/ml, respectively. This result indicates that the enhancement of dissolution rate of the complex doesn't affect the bioavailability of CN. This phenomenon seemed very

peculiar because generally the bioavailability is enhanced with increasing dissolution rate of drug caused by complexation with CDs, e.g. phenytoin(8), digoxin(9), acetohexamide(10) and nonsteroidal antiinflammatories(11). The reason of no enhancement of bioavailability after administration of CN/β-CD complex seemed to be a large stability constant of complex which is estimated to be apparently 6.2×10^3 M^{-1} in water at 20°C. In the case of complex having a large stability constant, the dissolved complex cannot dissociate enough, and so the concentration of free drug seemed not to be increased in spite of apparently high concentration of drug. From the above reason, it is assumed that the absorption of CN in gastro-intestinal tract after administration of CN/β-CD complex couldn't exceed the absorption after administration of CN alone.

TABLE III Stability of the Solid Complex of Cinnarizine with β-CD Prepared by Spray-Drying Method

		Content of CN	Degradation Products	Appearence	Dissolution Rate[a]
Initial Value		11.35%	n.d.[b]	control	6.2
70°C	3 d	11.83%	n.d.	(-)[c]	6.2
	7 d	11.84%	n.d.	(-)	6.2
40°C 75% R.H.	1 W	11.80%	n.d.	(-)	0.5
	2 W	11.89%	n.d.	(-)	0.5
	3 W	11.73%	n.d.	(-)	0.5
	4 W	11.84%	n.d.	(-)	0.5

a) $T_{80\%}$, min. b) n.d. : not detected.
c) (-) : unchanged.

6. Effect of $NaHCO_3$ and Citric Acid on the Bioavailability of CN(7)

Figure 7 shows the effect of $NaHCO_3$ on the absorption of intact CN and its β-CD complex. When CN was administered to dogs with $NaHCO_3$, the plasma level of CN hardly increased. However, in the case of CN/β-CD complex, the plasma levels of CN was changed by simultaneous administration of $NaHCO_3$. These results seemed to indicate clearly the difference of the dissolution rate of CN in pH 5.0 as shown in Figure 5, because the acid in stomach is neutralized by $NaHCO_3$.

Figure 8 shows the result of experiment concerning the effect of citric acid on the absorption of CN. It had been reported that the bioavailability of CN was enhanced by simultaneous administration of citric acid(12). However, a difference in absorption of CN as CN alone or CN/β-CD complex with and without administration of citric acid was not observed.

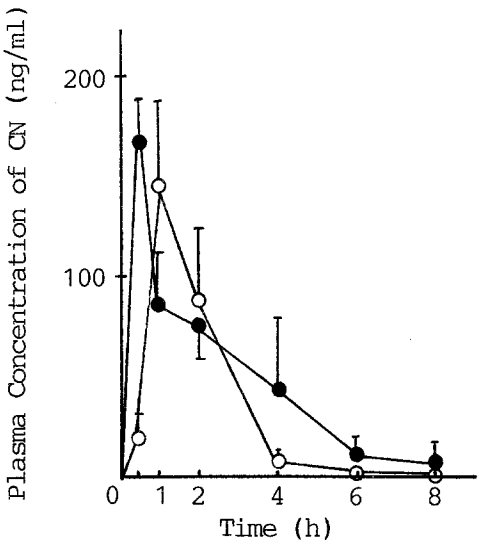


Figure 6. Time Course of Plasma CN Concentration in Dogs after Oral Administration of CN (50 mg) and Its β-CD Complex (containing 50 mg CN).
(o) CN. (●) CN/β-CD complex.
Each point represents the mean ± S.E. of 3 dogs.

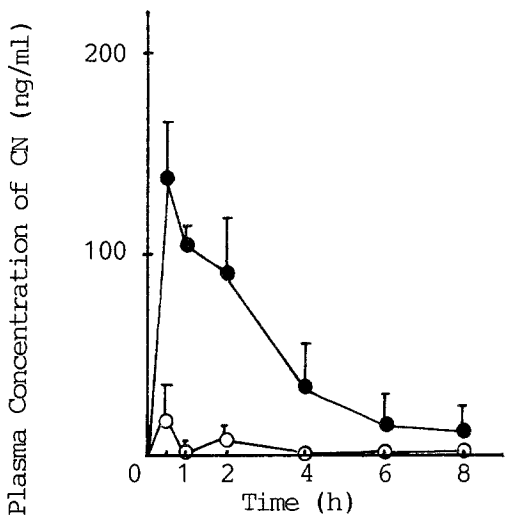


Figure 7. Time Course of Plasma CN Concentration in Dogs after Oral Administration of CN (50 mg) and Its β-CD Complex (containing 50 mg CN) with $NaHCO_3$ (500 mg).
(o) CN with $NaHCO_3$. (●) CN/β-CD complex with $NaHCO_3$.
Each point represents the mean ± S.E. of 3 dogs.

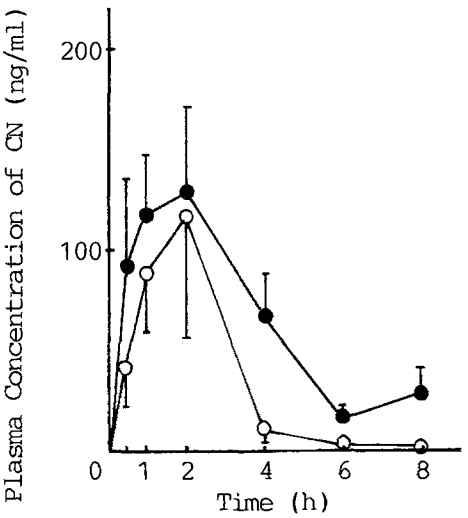


Figure 8. Time Course of Plasma CN Concentration in Dogs after Oral Administration of CN (50 mg) and Its β-CD Complex (containing 50 mg CN) with Citric Acid (400 mg).
(o) CN with citric acid. (●) CN/β-CD complex with citric acid.
Each point represents the mean ± S.E. of 3 dogs.

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