

Effects of Inorganic Salts on the Dissociation of a Complex of β -Cyclodextrin with an Azo Dye in an Aqueous Solution

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The apparent dissociation constant (K_{app}) of a complex between β -cyclodextrin and an azo dye, sodium *p*-*(4-hydroxy-1-naphthylazo)benzenesulfonate*, was determined by spectrophotometry in a phosphate buffer solution (pH 5.91) in the absence and in the presence of various inorganic salts at 25 °C. The cyclodextrin and the azo dye formed a 1 : 1 complex with the K_{app} value of 2.44×10^{-3} M in a 0.1 M phosphate buffer. K_{app} decreased with an increase in the concentration of the phosphate buffer and with the addition of some inorganic salts, such as Li_2SO_4 , Na_2SO_4 , K_2SO_4 , LiIO_3 , NaIO_3 , KIO_3 , and KF . These results were explained mainly in terms of the decrease in the activity of water, which takes part in the dissociation reaction of the inclusion complex, with an increase in the concentration of the inorganic salts. On the other hand, K_{app} increased with the addition of such salts as KCl , KBr , KI , KNO_3 , KSCN , and KClO_4 . These results were attributed mainly to the formation of the inclusion complexes of the cyclodextrin with the anions of these salts, competing with the azo dye for the cyclodextrin-binding site.

Cyclodextrins form inclusion compounds with various organic compounds. The equilibrium constants of the inclusion compounds have often been determined in aqueous solutions by spectrophotometric,^{1,2)} kinetic,³⁻⁶⁾ and solubility⁷⁻⁹⁾ measurements. In most of these measurements, inorganic salts have been added to the solution in order to control the pH and the ionic strength. However, the effect of the salts on the apparent equilibrium constants had generally been neglected until Schlenk and Sand⁷⁾ showed that some inorganic salts prevent the association of organic acids with α -cyclodextrin. Cramer *et al.*²⁾ clearly indicated that inorganic salts affect the equilibrium constant in two ways: (1) Inorganic salts change the structure of water, which has an influence on the hydrophobic interaction between cyclodextrins and guest organic molecules. (2) The anions of some inorganic salts compete with guest organic molecules for the binding sites of cyclodextrins. Cramer *et al.* showed that the perchlorate, nitrate, and iodide ions are trapped within

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2) F. Cramer, W. Saenger, and H. -Ch. Spatz, *J. Amer. Chem. Soc.*, **89**, 14 (1967).

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the void space of α -cyclodextrin molecules, preventing *p*-nitrophenol from forming inclusion complexes with the cyclodextrin, and that the sulfate and phosphate ions have no influence on the cyclodextrin-*p*-nitrophenol complex formation. However, the salts examined were somewhat limited.

In the present study, we attempted to examine in detail the effects of various inorganic salts on the apparent dissociation constant (K_{app}) of the inclusion compound of β -cyclodextrin (β -CD) with an azo dye. As the azo dye, sodium *p*-*(4-hydroxy-1-naphthylazo)benzenesulfonate* (Na^+HNB^-) was used because in it the difference in molar absorption coefficient between the free and the complexed HNB^- ions is largest among the dyes examined so far in our laboratory; therefore, it is possible to determine K_{app} by spectrophotometry with a high accuracy.

Results and Discussion

Determination of K_{app} of the β -CD-HNB⁻ Complex. Figure 1 shows the absorption spectra of Na^+HNB^- at varying β -CD concentrations in the 0.10 M phos-

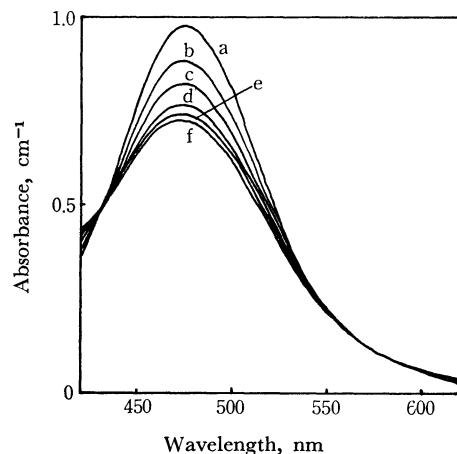


Fig. 1. Absorption spectra of 3.24×10^{-5} M HNB^- at varying β -CD concentrations in 0.1 M phosphate buffer (pH 5.91) at 25 °C.
 $[\beta\text{-CD}]$ (mM), a: 0.0, b: 1.0, c: 2.5, d: 5.0
e: 7.5, f: 10.0

phate buffer (pH 5.91) at 25 °C. Although the wavelength of the absorption maximum (475 nm) was virtually unchanged, the absorbance of HNB⁻ at the maximum decreased with an increase in the concentration of β -CD. There were two isosbestic points, at 431 and 576 nm, indicating the formation of a 1 : 1 complex between β -CD and HNB⁻. According to Cramer *et al.*,²⁾ the decrease in the absorbance (ΔE) of an HNB⁻ solution on the addition of β -CD is related to the total concentration (c_{β}°) of β -CD by the following equation:

$$c_a^{\circ} c_{\beta}^{\circ} / \Delta E = K_{app} / \Delta \epsilon + c_{\beta}^{\circ} / \Delta \epsilon \quad (1)$$

where c_a° is the total concentration of HNB⁻, and $\Delta \epsilon$, the difference in the molar absorption coefficient between the free and the complexed HNB⁻ ions. In fact, the plot of $c_a^{\circ} c_{\beta}^{\circ} / \Delta E$ vs. c_{β}° for the present case gave a straight line (Fig. 2), where each ΔE was measured at 475 nm. The values of $\Delta \epsilon$ and K_{app} were determined from the slope and the intercept of the line to be $9.59 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ and $2.44 \times 10^{-3} \text{ M}$ respectively.

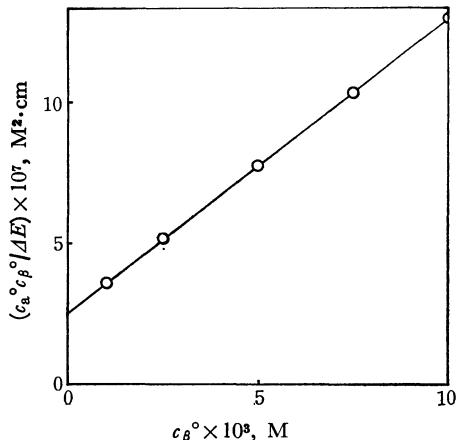


Fig. 2. Plot of $c_a^{\circ} c_{\beta}^{\circ} / \Delta E$ vs. c_{β}° .

TABLE 1. THE K_{app} VALUES AT VARYING CONCENTRATIONS OF A PHOSPHATE BUFFER (pH 5.91) AND AT 25°C

Concn., M	$K_{app} \times 10^3, \text{M}$
0.033	2.73
0.050	2.69
0.067	2.62
0.100	2.44
0.125	2.39
0.250	2.25

TABLE 2. EFFECT OF POTASSIUM SULFATE ON K_{app} IN 0.1 M PHOSPHATE BUFFER (pH 5.91) AT 25°C

$\text{K}_2\text{SO}_4, \text{M}$	$K_{app} \times 10^3, \text{M}$
0.030	2.41 ^{a)}
0.050	2.32
0.100	2.10
0.125	1.96
0.250	1.60
0.375	1.29

a) Determined in 0.050 M phosphate buffer.

TABLE 3. EFFECT OF POTASSIUM SALTS ON K_{app} IN 0.1 M PHOSPHATE BUFFER (pH 5.91) AT 25°C

Salt	$K_{app} = 10^3, \text{M}$	
	$c_s^{\text{a})} = 0.030 \text{ M}$	$c_s = 0.300 \text{ M}$
KIO_3	2.54	2.40 ^{b)}
KF	2.55	2.02
KCl	2.47	2.88
KBr	2.51	3.16
KI	2.83	6.52
KNO_3	2.61	3.74
KSCN	3.16	7.96
KClO_4	3.84	—

a) c_s : Concentration of a salt added to 0.1 M phosphate buffer. b) Determined at $c_s = 0.125 \text{ M}$.

spectively.

The K_{app} values were similarly determined at various concentrations of the phosphates (Table 1) or in the presence of potassium sulfate (Table 2) and the other potassium salts (Table 3), where the pH of each solution was adjusted to 5.91 by the use of a phosphate buffer. The $\Delta \epsilon$ value was virtually constant at $9.59 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ through all these measurements.

K_{app} decreased with increases in the concentrations of the phosphate, sulfate, iodate, and fluoride. On the other hand, K_{app} increased upon the addition of the chloride, bromide, iodide, nitrate, thiocyanate, and perchlorate. One can consider that the inorganic salts affect K_{app} in three different ways: (1) They change the activities of the free and the complexed HNB⁻ ions. Although the activity of β -CD may also somewhat vary upon the addition of the salts, the extent may be negligibly small because β -CD is a neutral molecule. (2) They change the activity of water, which probably takes part in the dissociation reaction of the inclusion complex. (3) Some of the salts are included in β -CD, competing with HNB⁻ for the binding site of β -CD. Among these effects, the first one was approximately estimated as follows.

Correction of K_{app} on the Change in the Activities of the Free and the Complexed HNB⁻ Ions. In order to estimate the activities of the free and the complexed HNB⁻ ions at various concentrations of inorganic salts, the activity coefficients (f 's) of them at an ionic strength, I , were calculated by the use of the Debye-Hückel equation:^{10a,11)}

$$\log f = -AZ^2 I^{1/2} / (1 + BaI^{1/2}) \quad (2)$$

where A and B are constants equal to $0.5115 \text{ M}^{-1/2}$

10) R. A. Robinson and R. H. Stokes, "Electrolyte Solutions," Butterworths, London (1970), a) p. 229; b) p. 468; c) p. 477; d) p. 465.

11) It is known that Eq. (2) holds up to ionic strength of about 0.1, but at higher ionic strengths the following equation is more adequate to estimate the f value:

$$\log f = -AZ^2 I^{1/2} / (1 + BaI^{1/2}) + bI$$

where b is a parameter relating to the dielectric constant of an electrolyte solution. However, it may be recognized that the f_f/f_c in Eq. (3) calculated by the use of this equation is virtually equal to that calculated by Eq. (2), provided that the b value for the free HNB⁻ is approximately equal to that for the complexed HNB⁻.

and $0.3291 \times 10^8 \text{ M}^{-1/2} \text{ cm}^{-1}$ respectively at 25°C ,^{10b} Z is the ionic valence, and a , the effective diameter of the hydrated ion. The a values for the free and the complexed HNB^- ions were conductometrically estimated to be about 6×10^{-8} and $12.5 \times 10^{-8} \text{ cm}$, as will be described in the Experimental section.

The corrected apparent dissociation constant (K_{corr}) was evaluated by the introduction of the activity coefficients thus calculated for the free and the complexed HNB^- ions (f_f and f_c , respectively) to Eq. (3):

$$K_{\text{corr}} = K_{\text{app}} \times (f_f/f_c) \quad (3)$$

Figure 3 shows the plot of K_{corr} vs. I , together with the plot of K_{app} vs. I , drawn on the basis of the data determined in the presence of the phosphate, sulfate, fluoride, and iodate. It is obvious that K_{corr} decreases more rapidly than K_{app} with an increase in I . This is caused by the fact that the size of the free HNB^- is smaller than that of the complexed HNB^- ; therefore, the activity of the former decreases more rapidly than that of the latter with an increase in I . On the other hand, K_{corr} increases with increasing concentrations of the chloride, bromide, iodide, nitrate, and thiocyanate (Table 4), similarly to the case of K_{app} . These results show that the change in K_{app} with increasing concentrations of inorganic salts can hardly be explained only in terms of the change in the activities of the free and complexed HNB^- ions. Therefore, we examined the second effect of inorganic salts des-

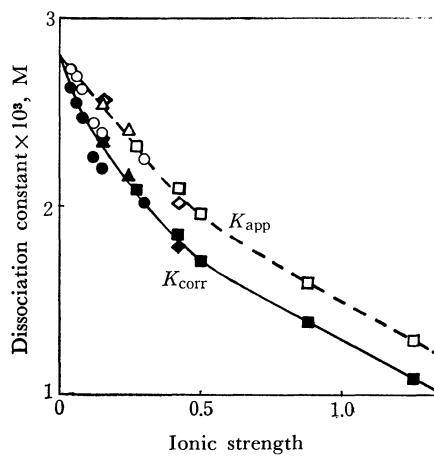


Fig. 3. Plots of K_{app} and K_{corr} vs. ionic strength.
 ○ and ●: the phosphate, □ and ■: the sulfate, △ and ▲: the iodate, ◇ and ◆: the fluoride.

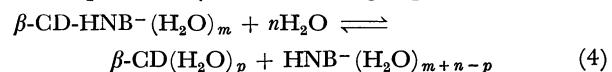
TABLE 4. THE K_{corr} VALUES IN THE PRESENCE OF POTASSIUM SALTS IN 0.10 M PHOSPHATE BUFFER AT 25°C

Salt	$K_{\text{corr}} \times 10^3, \text{ M}$	
	$c_s^{\text{a)}} = 0.030 \text{ M}$	$c_s = 0.300 \text{ M}$
KCl	2.28	2.54
KBr	2.31	2.78
KI	2.61	5.75
KNO_3	2.40	3.29
KSCN	2.91	7.01
KClO_4	3.54	—

a) c_s : Concentration of a salt added to 0.10 M phosphate buffer.

cribed above.

Effect of the Change in the Activity of Water on K_{corr} . In the preceding section we did not take into account the role of water in the dissociation of the β -CD-HNB⁻ complex. However, it is very probable that a large number of water molecules take part in the reaction, as was suggested by Cramer *et al.*,^{2b} for the water structure must be broken down around the complex during the dissociation reaction, and after that it must be reconstructed around β -CD and HNB^- . If the role of water is considered, the dissociation reaction can be expressed by the following equation:



The dissociation constant (K_d) for this reaction is given by:

$$K_d = K_{\text{corr}}/a_w^n \quad (5)$$

where a_w is the activity of water. The addition of inorganic salts may result in a decrease in a_w , causing a decrease in K_{corr} if K_d remains constant. Thus, we can qualitatively explain the effect on the K_{corr} of such inorganic salts as the phosphate, sulfate, iodate, and fluoride described above.

The a_w value changes not only with the salt concentration, but also with the kind of salt added. For example, the a_w of a potassium salt solution is generally larger than that of a sodium salt solution, which is in turn larger than that of a lithium salt solution, when these salts have the same anion and are in the same concentration. This is due to the fact that the extent of the hydration of these metallic cations increases in the order of K^+ , Na^+ , and Li^+ . Table 5 shows K_{corr} values in solutions containing different metallic cations at the same pH and the same ionic strength. It is obvious that K_{corr} decreases in the order of K^+ , Na^+ , and Li^+ salts in the cases of both sulfate and iodate, as had been expected. This also indicates that the role of the water activity is important in the dissociation equilibrium of the β -CD-HNB⁻ complex.

TABLE 5. THE K_{corr} VALUES IN PHOSPHATE BUFFER (pH 5.91) CONTAINING DIFFERENT METALLIC CATIONS AT 25°C

Cation	$K_{\text{corr}} \times 10^3, \text{ M}$	
	Sulfate ^{a)}	Iodate ^{b)}
Li^+	2.08	2.04
Na^+	2.20	2.16
K^+	2.22	2.33

a) Phosphate : 0.050 M, Sulfate : 0.030 M.

b) Phosphate : 0.100 M, Iodate : 0.030 M.

We have no data about the values of a_w at various concentrations of these salts, and the value of n is also unknown. Therefore, the K_d value can not be estimated directly from K_{corr} . In order to estimate the approximate n and K_d values, we tried, for convenience, to use the a_w values for H_2SO_4 solutions available in the literature^{10c)} as substitutes for those of the potassium sulfate solutions. The a_w values used

had been estimated by measurements of the depression in the vapor pressure. From Eq. (5), we obtain:

$$\log K_{\text{corr}} = \log K_d + n \log a_w \quad (6)$$

Figure 4 shows the plot of $\log K_{\text{corr}}$ in the sulfate solutions *vs.* $\log a_w$. For the sake of simplifying the calculation, we set a_w in the reference state as equal to its mole fraction. The plot was virtually linear. From the slope and the intercept at $\log a_w = 0$, the values of n and K_d were obtained as 50 and 2.66×10^{-3} M respectively. It seems that the value of n is somewhat larger than would be expected from the sizes of β -CD and HNB^- . This may mainly be due to the inadequacy of the a_w values used. However, the results suggest that a considerably large number of water molecules participate in the dissociation of the inclusion complex.

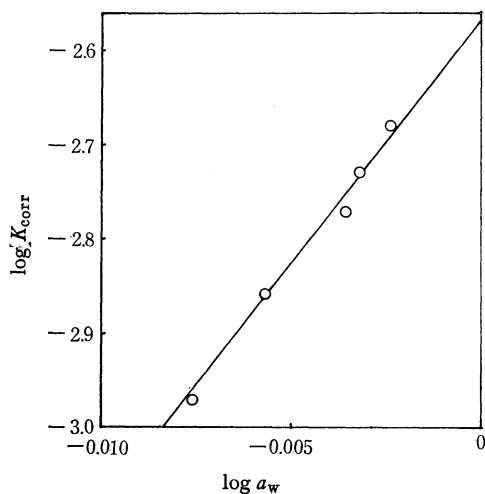


Fig. 4. Plot of $\log K_{\text{corr}}$ *vs.* $\log a_w$ for sulfate solutions.

In the above calculation, it was assumed that there is no appreciable interaction between β -CD and the inorganic salts. If these salts interact in some extent with β -CD, thus forming inclusion complexes, K_{corr} must increase with an increase in the concentrations of the salts, as will be described later in the case of such salts as the iodide, nitrate, and thiocyanate. In practice, however, the reverse was found here. Accordingly, it may be presumed that the interaction between them is negligibly small even if it does exist.

Inclusion of Inorganic Anions in β -CD. As has been shown in Table 4, K_{corr} 's in solutions containing KCl , KBr , KI , KNO_3 , KSCN , or KClO_4 increased with increases in the concentrations of these salts. These results can not be explained in terms of the decrease in water activity, because the correction for water activity results in a further increase in K_{corr} with an increase in the concentration of the salts. One can explain the results only in terms of the inclusion of the anions of these salts in β -CD. If the anions form adducts with β -CD in aqueous solutions, they compete with HNB^- for the binding site of β -CD, resulting in the increase in K_{app} or K_{corr} with the increase in the concentrations of the anions.

The dissociation constant (K_s) for an β -CD-anion complex can be calculated by the use of Eq. (7) below,

which one can easily derive on the assumption that the concentration of the inorganic salt is much higher than those of β -CD and HNB^- :

$$K_s = c_s K_{\text{corr}}^\circ / (K_{\text{corr}} - K_{\text{corr}}^\circ) \quad (7)$$

where K_{corr}° is the value of K_{corr} at the same ionic strength in a solution containing a salt which forms no adduct with β -CD, and where c_s is the total concentration of the salt. The values of K_s thus calculated at $I=0.15$ and 0.42 are given in Table 6. In these calculations, the K_{corr}° values at $I=0.15$ and 0.42 were taken as 2.20×10^{-3} and 1.82×10^{-3} M respectively; these are the mean values of K_{corr}° determined in solutions containing the phosphate, sulfate, iodate, and fluoride at the respective ionic strengths.

TABLE 6. THE K_s VALUES FOR SEVERAL ANIONS IN 0.10 M PHOSPHATE BUFFER (pH 5.91) AT 25 °C

Anion	K_s , M	
	c_s ^{a)} = 0.030 M ($I=0.15$)	c_s = 0.300 M ($I=0.42$)
Cl^-	0.8	0.8
Br^-	0.6	0.6
I^-	0.16	0.14
NO_3^-	0.3	0.4
SCN^-	0.09	0.11
ClO_4^-	0.05	—

a) c_s : Concentration of an anion added to 0.10 M phosphate buffer.

The K_s value for each salt was virtually invariable, regardless of the concentration. This indicates that the change in K_s with the decrease in water activity is small compared with the experimental error. The K_s values decreased in the order of Cl^- , Br^- , NO_3^- , I^- , SCN^- , ClO_4^- . This sequence is in agreement with that reported by Schlenk and Sand,⁷⁾ who showed that the degree of the association of halide ions with α -cyclodextrin increases in the order of Cl^- , Br^- , I^- . Cramer *et al.*²⁾ have also shown that ClO_4^- forms a fairly stable adduct with α -cyclodextrin.

One can consider that these anions are bound to β -CD by van der Waals force, hydrophobic interaction, and/or hydrogen bonding. In connection with this point, it was noticed that the hydrated anions forming no complex with β -CD have effective diameters of not less than 3.5 \AA ,¹⁶⁾ i.e., $4-4.5 \text{ \AA}$ for H_2PO_4^- and IO_3^- , 4 \AA for HPO_4^{2-} and SO_4^{2-} , and 3.5 \AA for F^- , whereas those forming complexes with β -CD have effective diameters of not more than 3.5 \AA , i.e., 3 \AA for Cl^- , Br^- , I^- , and NO_3^- , and 3.5 \AA for SCN^- and ClO_4^- . From this fact, it might be considered that the former anions are too large to be included within the void space of β -CD, whereas the latter anions are small enough to fit within it. However, the internal diameter of the β -CD ring is about 7.5 \AA , which is larger than the effective diameters of the hydrated ions. Furthermore, it has been reported that α -cyclodextrin, whose internal diameter is about 6.0 \AA , includes ClO_4^- to form a complex with K_{app} of 0.034 M ,²⁾ which is comparable with that of the complex between the ion and β -CD. These facts indicate that the role of the van der Waals

force is not very important in the formation of the inclusion complexes between inorganic anions and β -CD. The role of hydrophobic interaction and hydrogen bonding is not yet clear. It may be necessary to study further the physicochemical properties of β -CD and hydrated anions.

Experimental

Materials. The β -CD was isolated from a mixture of α , β , and γ -cyclodextrins given us by the Matsutani Kagaku Co. and was purified according to the directions of Cramer and Henglein.¹²⁾ $[\alpha]_D = +168^\circ$ (lit.,¹³⁾ $+162.5^\circ$). It was confirmed by paper chromatography with the two solvent systems (*s*-BuOH-pyridine-water (1:1:1)¹⁴⁾ and *n*-BuOH-DMF-water (2:1:1)¹⁵⁾ that the β -CD contained no appreciable amounts of α - or γ -cyclodextrins. The Na^+HNB^- , obtained commercially, was dissolved in hot distilled water and salted out by the addition of NaOAc . After this procedure had been repeated twice, the precipitate was recrystallized twice from water, washed with hot alcohol, and then recrystallized three times from alcohol. The KH_2PO_4 , Na_2HPO_4 , and KNO_3 were recrystallized from water. The Li_2SO_4 , Na_2SO_4 , K_2SO_4 , NaIO_3 , KIO_3 , KF , KCl , KBr , KI , and KSCN were obtained commercially and were used without further purification. The LiIO_3 was prepared from LiOH and HIO_3 and was recrystallized from water. The water used for the preparation of buffer solutions and for the conductivity measurements was obtained by the distillation of city water with a Kokura distillatory apparatus, Model 8000; its specific conductance was *ca.* $2 \times 10^{-6} \Omega^{-1} \text{ cm}^{-1}$ at 25°C . A 0.10 M phosphate buffer solution was made by mixing 10 ml of 0.10 M Na_2HPO_4 and 90 ml of 0.10 M KH_2PO_4 .¹⁶⁾

Apparatus. For the determination of the K_{app} of the β -CD-HNB⁻ complex, absorption spectra were recorded by the use of a Hitachi Model 124 spectrophotometer. The cell (1.0 cm) was maintained at $25 \pm 0.1^\circ\text{C}$ with a jacket through which water was circulated from a constant-temperature bath. The pH of each aqueous solution was measured by means of a Hitachi-Horiba pH meter, model M-4. For the conductivity measurements, a Yanagimoto conductivity outfit, model MY-7, was used. A conductivity cell was fitted with circular, blacked Pt electrodes separated by 10 mm. The cell constant was 0.4829. The viscosity of the aqueous solutions was determined by the use of an Ostwald viscometer. All the measurements were made at $25 \pm 0.1^\circ\text{C}$.

Determination of the *a* Values for the Hydrated Free and Complexed HNB⁻ Ions. The *a* values for the free and the com-

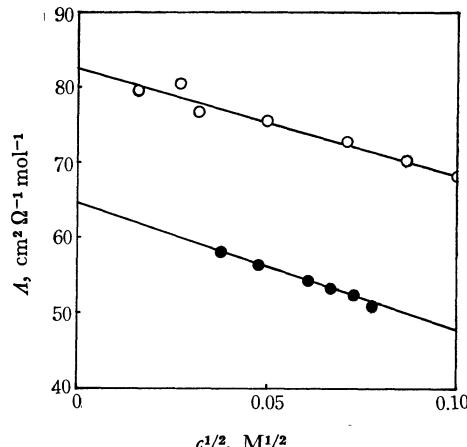


Fig. 5. Plots of the molar conductivity *vs.* the square root of concentration for the free (○) and the complexed (●) Na^+HNB^- salts in distilled water at 25°C .

plexed HNB⁻ ions were estimated by conductometry.¹⁷⁾ Figure 5 shows the relations between the molar conductivity (Λ) and the square root of the concentration for the free and the complexed Na^+HNB^- salts in distilled water at 25°C . Each Λ value for the complexed Na^+HNB^- (Λ_c) was determined as follows: (1) The conductivity (κ_t) of an aqueous solution containing 10.0 mM β -CD and a certain concentration of Na^+HNB^- was measured and multiplied by 1.020, a figure which corresponds to the relative viscosity of the solution against distilled water, in order to correct for the effect of the increase in the viscosity of the medium upon the addition of β -CD on the basis of Walden's rule. (2) The concentrations of the free and the complexed Na^+HNB^- salts (c_f and c_c , respectively) were calculated from the K_{app} value of 2.47×10^{-3} M, determined in distilled water at 25°C by the same spectrophotometric method as has been described above. (3) The conductivity (κ_f) of the free Na^+HNB^- salt at the calculated concentration was graphically estimated from the curve plotting the κ_f *vs.* the concentration of Na^+HNB^- measured in distilled water. (4) The conductivity (κ_c) of the complexed Na^+HNB^- salt was calculated by subtracting the κ_f value from $1.020 \times \kappa_t$. The Λ_c value was obtained by dividing κ_c by c_c .

By the extrapolation of the plots in Figure 5 to the intercept, the molar conductivities at an infinite dilution of the free and the complexed Na^+HNB^- salts (Λ_f° and Λ_c°) were evaluated to be 82.5 and $64.6 \text{ cm}^2 \Omega^{-1} \text{ mol}^{-1}$ respectively. Then, the molar conductivities of the free and the complexed HNB⁻ ions (λ_f° and λ_c° , respectively) were estimated to be 32.4 and $14.5 \text{ cm}^2 \Omega^{-1} \text{ mol}^{-1}$ respectively by subtracting $\lambda^\circ(\text{Na}^+) = 50.1 \text{ cm}^2 \Omega^{-1} \text{ mol}^{-1}$ ¹⁸⁾ from the Λ_f° (the Λ° value for the free Na^+HNB^- salt) and Λ_c° . By the use of this equation:¹⁷⁾ $a = k|Z|/\lambda^\circ$, where $k = 182 \times 10^{-8} \text{ cm}^3 \Omega^{-1} \text{ mol}^{-1}$ and where Z is the ionic valence, the *a* values for the free and the complexed HNB⁻ were calculated to be about 6×10^{-8} and $12.5 \times 10^{-8} \text{ cm}$ respectively.

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