chia coli penicillin amidohydrolase. In CEG synthesizing system, the replacement of water with other organic solvents was attempted for the enhancement of overall conversion (Table 2). Most solvents (10 v/v %) tested, however, did not result in any significant improvement on CEG production. Only 1-hexane showed a slight improvement of CEG yield. It was also observed that solvents having higher ability of proton donor than water was unfavorable to stimulating CEG production.

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Studies on the Cation Exchange Elution Behaviors of Metal Complexes

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The elution behaviors of a series of metal complexes, such as $Co(gly)_3$, $[Ni(en)_3]^{2+}$, $[Ni(phen)_3]^{2+}$, $[Fe(phen)_3]^{2+}$, $[Co(phen)_3]^{3+}$, $[Co(tn)_3]^{3+}$, $[Co(en)_3]^{3+}$ and $[Co(NH_3)_6]^{3+}$ (where gly; glycine, phen; phenanthroline, tn; trimethylenediamine, en; ethylenediamine), were studied in aqueous solution by measuring the retention volumes ($\bar{v}$ values) on SP-Sephadex C-25, cation exchange resin. It was found that the elution behaviors of metal complexes were apparently affected by salt concentrations, kinds of cations in eluent and kinds of anions in eluent, and according to the degrees of their effects coulombic forces, ion exchange capacities, the 'solvent effect' of resin backbone, hydrophobicity and hydrophilicity were applied to explain the elution mechanism.

Introduction

The variety of chromatographic techniques has been used to separate metal complexes.[1-5] Also, the widespread use of chromatographic methods has advanced considerably to the study of adsorption mechanism of metal complexes on ion exchange resin. In elution behaviors, in particular, the choice of elution methods and conditions seems to be largely empirical.[6] A brief review about the elutions of metal complexes by ion exchange resin is as follows: the adsorption behaviors of various Co(III) complexes were studied by paper chromatography and TLC[7-9]; the differences in $\bar{v}$ values of metal complexes due to the difference either in their electric charges or in their polarities[10,11]; ion association between complex ions and eluent anions was the main factor in the difference of $\bar{v}$ values[12,13]; electrostatic interaction between the complex ions and the functional groups in the stationary phase and/or between the complex ions and the anions in moving phase was used to interpret the adsorption mechanism.[13-15] Also, elution behaviors of metal complexes were explained by using water-structure maker and water-structure breaker.[16-19] These experiments were carried out by SP-Sephadex C-25 cation exchange resin which is the same as was used in the present work, and the mechanisms of adsorption were explained by electrostatic interaction, ion association, water-structure maker and water-structure breaker. This study was concerned with the elution behaviors of some complexes which have different central metal ions of +2 or +3 charge and with various kinds of anions in eluent. The aim of this work was to study the elution mechanism of the metal complexes and the effect of various kinds of factors which have influence on the elution behaviors of them.

Experimental

Reagents and Apparatus. All complexes used in this work were prepared according to the literature methods and identified by UV-VIS spectrophotometry. The complexes were confirmed to be stable against hydrolysis and isomerization during the chromatographic experiments. Also, the resin used in this work was SP-Sephadex C-25 (Pharamacia Fine Chemicals, Sweden), which is strongly acidic cation exchanger based on crosslinked dextran. All chromatographic experiments were carried out on a laboratory built borosilicate glass column (1.0 cm in diameter) furnished with a porous Teflon (Du pont) bottom and filled with two grams of SP-Sephadex C-25 (H^+-form) resin. The resin form was exchanged to required form by elution with 1.0 *M* MCl (where M:K^+, Na^+ and NH_4^+) aqueous solution.

Retention Volume ($\bar{v}$). To determine the $\bar{v}$ value, 1.0 mL of the stock solution of each metal complex ion was added to the column and then forced into the resin bed. A supply of appropriate eluent was then connected to the column. The flow rate during the elution was 0.3–0.5 m*l* per minute. The colored eluates were collected and analyzed by measuring the absorbance at λ_{max}(nm) of metal complex. All experiments were carried out at 20±1 °C.

Results and Discussion

The Effect of Charges in Metal Complexes. The elution behaviors of several metal complexes by various eluents of 0.25–0.6 *F* of salts are shown in Table 1. These results imply that several series of metal complexes are eluted by similar mechanism. It was confirmed that the more the formal concentration, the earlier the complex is eluted. This is accounted for by their differences of exchange capacities. The effect of salt concentrations on the $\bar{v}$ values of metal complexes is shown in Figure 1. On the other hand, in aqueous solution of metal halide the increasing order of $\bar{v}$ value is in accord with the increasing order of coulombic forces between the complexes and resin, which is generally related with the charges and hydrophilicities of complexes: $Co(gly)_3 < [Ni(en)_3]^{2+} < [Fe(phen)_3]^{2+} < [Co(phen)_3]^{3+} < [Ni(phen)_3]^{2+} < [Co(tn)_3]^{3+} < [Co(en)_3]^{3+} < [Co(NH_3)_6]^{3+}$. As seen in Table 1 and 2, $\bar{v}$ value of $[Ni(en)_3]^{2+}$ was smaller than that of $[Co(en)_3]^{3+}$ whereas $\bar{v}$ value of $[Co(phen)_3]^{3+}$ was smaller than that of $[Ni(phen)_3]^{2+}$, which is accounted for by the fact that +3 charged complexes seem to be less favorably forming the solvent effect' of resin backbone, being capable of forming strong hydrogen bonding to water molecules and strong attractive forces with functional group ($-SO_3^-$) of resin, whereas, +2 charged complexes seem to have strong influence on the 'solvent effect' of resin backbone, forming weak hydrogen bonding to water molecules and weak attractive forces with the functional group ($-SO_3^-$) of resin because of its small charge.

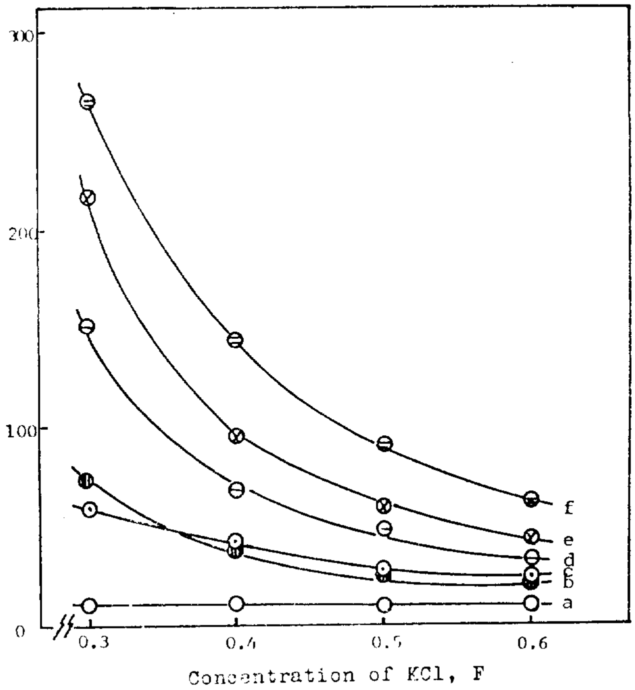

Figure 1. Effect of salt concentration on the retention volume Resin bed: 1.0×20 cm; flow rare: 0.5 m*l*/min.
a. $Co(gly)_3$; b. $[Co(phen)_3]^{3+}$; c. $[Fe(phen)_3]^{2+}$; d. $[Co(tn)_3]^{3+}$; e. $[Co(en)_3]^{3+}$; f. $[Co(NH_3)_6]^{3+}$.

TABLE 1: Effects of Salt Concentrations and Kinds of Cations on Retention Volume[a]

Eluent	Complex ion							
	$Co(gly)_3$	$[Fe(phen)_3]^{2+}$	$[Co(phen)_3]^{3+}$	$[Co(tn)_3]^{3+}$	$[Co(en)_3]^{3+}$	$[Co(NH_3)_6]^{3+}$	$[Ni(en)_3]^{2+}$	$[Ni(phen)_3]^{2+}$
0.3*F* KCl	11.0	59.2	72.0	150.5	216.8	264.8	.....	.....
0.4*F* KCl	10.7	40.4	36.5	67.0	94.0	144.2	25.8	39.8
0.5*F* KCl	10.0	27.0	28.6	48.3	59.4	90.4	17.5	34.5
0.6*F* KCl	9.5	25.0	21.7	33.5	41.8	62.1	16.0	28.8
0.4*F* NaCl	11.0	48.8	49.3	87.0	120.8	183.0	33.1	56.0
0.5*F* NaCl	10.5	39.0	36.0	53.5	71.1	113.5	27.6	40.5
0.6*F* NaCl	10.5	33.8	27.0	37.8	47.0	71.8	21.0	39.0
0.5*F* NH_4Cl	11.0	41.5	38.5	61.0	13.0	128.5	33.3	44.5

[a] Flow rate: 0.3 ml/min.

TABLE 2: Effect of the Kinds of Anions in Eluent on Retention Volumes of Metal Complexes*

Complex ion	Eluent					
	KF (0.5 *F*)	KCl (0.5 *F*)	KBr (0.5 *F*)	KI (0.5 *F*)	K_2SO_4 (0.25 *F*)	KNO_3 (0.5 *F*)
$[Ni(en)_3]^{2+}$	27.8	17.5	18.9	20.2	10.8	30.7
$[Ni(phen)_3]^{2+}$	59.0	34.5	24.9	....	82.4	34.1
$[Fe(phen)_3]^{2+}$	56.2	27.0	23.8	22.7	74.0	28.2
$[Co(phen)_3]^{3+}$	65.3	28.6	19.0	12.7	63.0	25.6
$[Co(tn)_3]^{3+}$	75.0	48.2	49.3	51.4	22.6	91.6
$[Co(en)_3]^{3+}$	90.2	57.1	59.4	66.0	21.2	109.0
$[Co(NH_3)_6]^{3+}$	102.0	90.4	93.9	127.2	21.0	144.8

*Resin bed: SP-Sephadex C-25, K^+-form, 1.0×20 cm; flow rate: 0.3 ml/min.

The Effect of the Kinds of Cations in Eluent. Table 1 also indicates that the decreasing order of $\bar{v}$ values of all the metal complexes for the kinds of cations in eluent is in accord with the increasing order of coulombic interaction between the cation in eluent and the resin; the $\bar{v}$ value of $Fe(phen)_3^{2+}$ in 0.5 *F* MCl (where $M = Na^+$, K^+ and NH^+_4) solutions decreased in the order of NH_4^+(41.5), Na^+(39.0) and K^+(27.0). So, it was considered that the predominent mechanism of adsorption of metal complexes by the resin is coulombic interaction.

The Effect of the Kinds of Anions in Eluent. The hydrophobicity and hydrophilicity of halogen atoms in KX ($X = F^-$, Cl^-, Br^- and I^-) as eluent studied here are expected to be related to their electronegativities and to the $\bar{v}$ values of metal complexes which are eluted by KX. This is confirmed by the results given in Figure 2 which shows that $\bar{v}$ values of metal complexes which have hydrophobic group, phen are increased in the order of $I^- < Br^- < Cl^- < F^-$, but those which have hydrophilic group, tn, en and NH_3, are decreased in the order of $I^- > Br^- > Cl^-$ and increased for only F^-. The reason why such phenomena take place is accounted for by which hydrophobic group associates more favorably with hydrophobic group and hydrophilic group with hydrophilic group, but fluoride ion as an eluent didn't give such a consistent $\bar{v}$ values because it reacts with water and forms hydrogen fluoride which brings about the decrease of effective fluoride ion concentration.

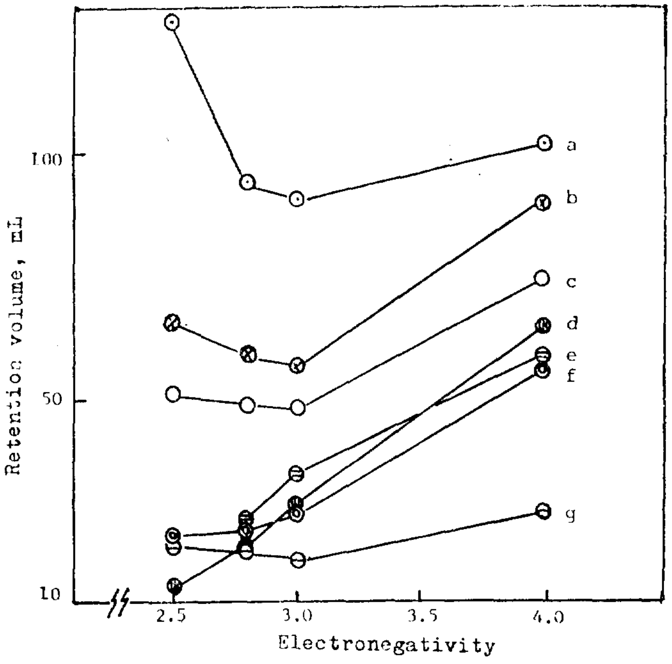

Figure 2. Plot of retention volume *vs.* electronegativity of anion in eluent
a. $[Co(NH_3)_6]^{3+}$; b. $[Co(en)_3]^{3+}$; c. $[Co(tn)^3]^{3+}$; d. $[Co(phen)_3]^{3+}$; e. $[Ni(phen)_3]^{2+}$; f. $[Fe(phen)_3]^{2+}$; g. $[Ni(en)_3]^{2+}$.

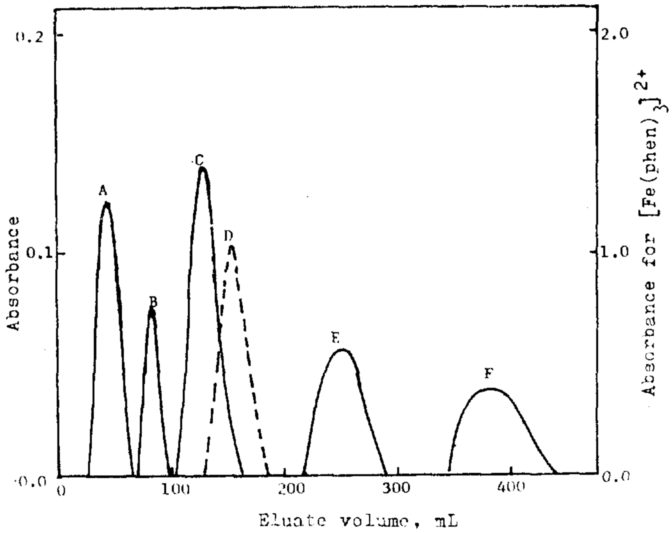

Figure 3. Separation of synthetic mixture of metal complexes by elution with 0.5 *F* KCl solution. Resin bed: 1.0×50 cm; flow rate: 0.3 ml/min.
A; $Co(gly)_3$, B; $[Ni(en)_3]^{2+}$, C; $[Fe(phen)_3]^{2+}$,
D; $[Co(phen)_3]^{3+}$, E; $[Co(en)_3]^{3+}$, F; $[Co(NH_3)_6]^{3+}$

In addition, the $\bar{v}$ values of $[Co(phen)_3]^{3+}$ were smaller than those of $[Ni(phen)_3]^{2+}$ and $[Fe(phen)_3]^{2+}$ by elution with 0.5 *F* KBr and KI, but this phenomenon is reversed by 0.5 *F* KCl and KF (Figure 2). This may be due to the reason that +3 charged complex which has hydrophobic group has more interaction with eluting hydrophobic anion and less interaction with eluting hydrophilic anion than +2 charged complexes. It was also observed that the increasing order of decreasing rate of $\bar{v}$ values of $[Co(tn)_3]^{3+}$, $[Co(en)_3]^{3+}$ and $[Co(NH_3)_6]^{3+}$ by 0.5 *F* KI, KBr and KCl was in accord with increasing order of hydrophilicity of coordinated groups:

Hydrophilicity: tn < en < NH_3

Decreasing rate of $\bar{v}$:

$$[Co(tn)_3]^{3+} < [Co(en)_3]^{3+} < [Co(NH_3)_6]^{3+}$$

This shows that the more hydrophilic group the Co (III) complex has, the larger the interaction between the complex and the hydrophilic anions in eluent. To confirm the above fact, the $\bar{\nu}$ values of metal complexes were measured by elution with potassium sulfate solution as eluent. The results show that the $\bar{\nu}$ values of Co(III) complexes which have hydrophilic groups have smaller values than those of metal-phen complexes by elution with $0.25F$ K_2SO_4 solution (Table 2). Still more, $0.5F$ KNO_3 solution was used as an eluent to compare with the effect of sulfate. As a consequence, $\bar{\nu}$ values of metal-tn, en and NH_3 complexes by elution with $0.5\ F$ KNO_3 solution were larger than those of metal-phen complexes. This phenomenon was the reverse compared with that with $0.25\ F$ K_2SO_4. This results also show that hydrophilic groups interact more strongly with hydrophilic ions in eluent and hydrophobic group with hydrophobic ions.[13]

Separation of Metal Complexes. From the results obtained in this study some complexes were separated from the synthetic mixtures. Figure 3 shows the separation of synthetic mixture of $Co(gly)_3$, $[Ni(en)_3]^{2+}$, $[Fe(phen)_3]^{2+}$ or $[Co(phen)_3]^{3+}$, $[Co(en)_3]^{3+}$ and $[Co(NH_3)_6]^{3+}$.

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The Crystal and Molecular Structure of Thiamphenicol

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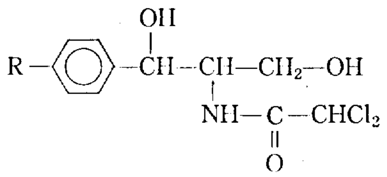
The structure of thiamphenicol, one of the congeners of chloramphenicol which is a well-known antibiotic, has been determined by single crystal x-ray diffraction techniques. The crystal structure was determined using diffractometer data obtained by the 2θ: ω scan technique with MoK_α radiation from a crystal having space group symmetry $P2_12_12_1$ and unit cell parameters $a=5.779$, $b=15.292$ and $c=17.322$ Å. The structure was solved by direct methods and refined by least squares to an $R=0.070$ for the 2116 reflections. The overall V-shaped conformation of thiamphenicol revealed in this study is consistent with those from the crystallographic studies and the proposed models from the theoretical and nmr studies of chloramphenicol. However there is no intramolecular hydrogen bond and the propanediol moiety is fully extended in the thiamphenicol molecule, while the crystal structures of chloramphenicol show the existence of the hydrogen bond between the two hydroxyl groups of the propanediol moiety forming an acyclic ring. All of the thiamphenicol molecules in the crystal are linked by a threedimensional hydrogen bonding network.

Introduction

Thiamphenicol(TPL), 2, 2-dichloro-N-[2-hydroxy-1-(hydroxymethyl)-2-[4-(methylsulfonyl) phenyl] ethyl] acetamide (I), is one of the hundreds of chloramphenicol congeners and shows much weaker antibiotic activity than chloramphenicol. Chloramphenicol (CPL) is one of the simplest antibiotics known and the first one synthesized by the organic-chemical method.[1]



(I)

Thiamphenicol: $R=-SO_2CH_3$. Chloramphenciol: $R=-NO_2$