# CHROMATOGRAPHIC STUDY OF OPTICAL RESOLUTION

## XI*. OPTIMUM CONDITIONS FOR OPTICAL RESOLUTION OF UNCHARGED COMPLEXES

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SUMMARY

Optical resolution of the uncharged metal complexes fac-[Co(D- or L-ser)$_3$], fac-[Co($\beta$-ala)$_3$] and mer-[Co($\beta$-ala)$_3$] (ser = serinate anion and $\beta$-ala = $\beta$-alaninate anion) was attempted by two types of chromatography using bis($\mu$-d-tartrato)diantimonate(2 −) as a chiral selector. The first type of chromatography (A) uses a chiral selector in the mobile phase, and the second type (B) uses it in the stationary phase. Trends in the retention volumes, their difference and ratio for enantiomers are discussed.

INTRODUCTION

It is difficult to separate uncharged metal complexes into enantiomeric forms. Chromatography may be practically the only means of achieving this purpose. In the first paper of this series[1] it was reported that fac-[Co($\beta$-ala)$_3$] ($\beta$-ala = $\beta$-alaninate anion) can be completely separated into its pure enantiomers on a column packed with CM-Sephadex using a solution of sodium d-tartrate (hereafter abbreviated as Na$_2$d-tart) in ethanol–water as an eluent. A similar chromatographic separation of enantiomers and diastereomers was reported[2] for a series of mixed amino acidato chelates of the type fac-[Co(D- or L-ser)$_{3-n}$($\beta$-ala)$_n$] (ser = serinate anion) using aqueous solutions of Na$_2$d-tart and sodium bis($\mu$-d-tartrato)diantimonate {hereafter abbreviated as Na$_2$[Sb$_2$(d-tart)$_2$]} as eluent. Uncharged complexes can be resolved into enantiomers also by a chiral selector immobilized on the stationary phase. For example, mer-[Co($\beta$-ala)$_3$] is completely separated into enantiomers on a column of the [Sb$_2$(d-tart)$_2$]$^{2-}$ form of the anion exchanger[3]. Using this so-called "ambush" chromatography, the separation mechanism with d-tart$^{2-}$ and [Sb(d-tart)$_2$]$^{2-}$ was

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\* Part X: S. Izumoto, U. Sakaguchi and H. Yoneda, *Bull. Chem. Soc. Jap.*, 56 (1983) 1646.
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extensively investigated for a series of mixed-ligand chelates of the type $fac$-[Co($\alpha$-ala)$_{3-n}$ ($\beta$-ala)$_n$]$^+$. In these studies, however, the attention was focused on elucidation of the separation mechanism from a stereochemical point of view. No experimental or theoretical studies of the chromatographic behaviour of uncharged complexes under various conditions have been made.

In the present study, the optimum conditions for optical resolution of un-charged complexes by two types of chromatography were investigated, and the results explained in terms of two equilibria, ion–dipole association and adsorption.

EXPERIMENTAL

*Materials*

The complexes $\varLambda$-$fac$-[Co(L-ser)$_3$] and $\varLambda$-$fac$-[Co(D-ser)$_3$], and racemic $fac$- and $mer$-[Co($\beta$-ala)$_3$], were prepared according to the method described previously[2]. The chiral selector, Na$_2$[Sb$_2$($d$-tart)$_2$], was prepared according to the method reported[5].

*Chromatographic experiments*

For type A chromatography, the sodium form of TSK 212 resin (cation ex-changer) was packed in a stainless-steel tube (200 × 4 mm I.D.). The column was equilibrated with the eluent. Aqueous solutions of Na$_2$[Sb$_2$($d$-tart)$_2$] at several con-centrations were used as eluents. A solution (20 $\mu$l) of a racemic mixture of $fac$-[Co(D- or L-ser)$_3$] and Blue Dextran 2000 which was used as a marker was injected by a syringe on top of the column. The retention volume was measured at flow-rate of 0.1 ml/min.

For type B chromatography, TSK 222 resin (anion exchanger) was packed in a column (500 × 4 mm I.D.) and saturated with [Sb$_2$($d$-tart)$_2$]$^{2-}$. The column was washed thoroughly with water and equilibrated with the eluent to be used. The eluents were ethanol–water mixtures having different ethanol contents.

RESULTS AND DISCUSSION

*Type A chromatography*

Fig. 1 shows typical elution curves for a racemic mixture of $\varLambda$-$fac$-[Co(D-ser)$_3$] and $\varLambda$-$fac$-[Co(L-ser)$_3$] eluted with aqueous solutions containing three different con-centrations of the chiral selector, Na$_2$[Sb$_2$($d$-tart)$_2$]. The retention volume for each enantiomer is largest at zero concentration of the chiral selector and decreases gradu-ally with increasing concentration of the chiral selector as shown in Fig. 2. A depen-dence on the concentration of the chiral selector is also shown by the difference between and the ratio of the retention volumes for two enantiomers, Fig. 3. Here, the separation of two elution bands, that is, the difference between the retention volumes for the two enantiomers, has a maximum at a certain concentration of the chiral selector. In other words, there is an optimum concentration of the chiral se-lector for optical resolution. It is interesting to recall the case of complex cations which were separated into enantiomers on a cation-exchange column[5]. There the separation of the two elution bands decreased monotonously with increasing con-centration of the chiral selector. The origin of this difference will be discussed later.

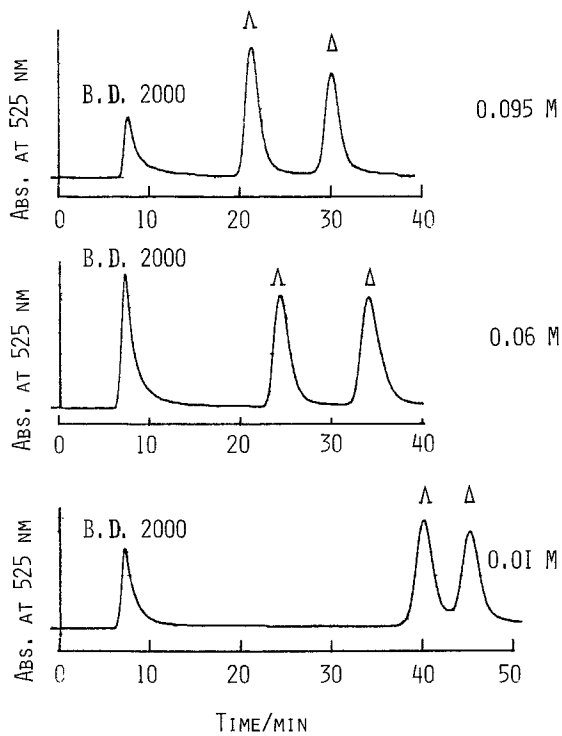The separation factor, $\alpha$, shows no maximum but a gradual increase with in-

Fig. 1. Typical elution curves for a racemic mixture of $\Lambda$-$fac$-[Co(D-ser)$_3$] and $\Delta$-$fac$-[Co(L-ser)$_3$] with three concentrations of Na$_2$[Sb$_2$($d$-tart)$_2$] in the eluent. B.D. 2000 = Blue Dextran 2000.
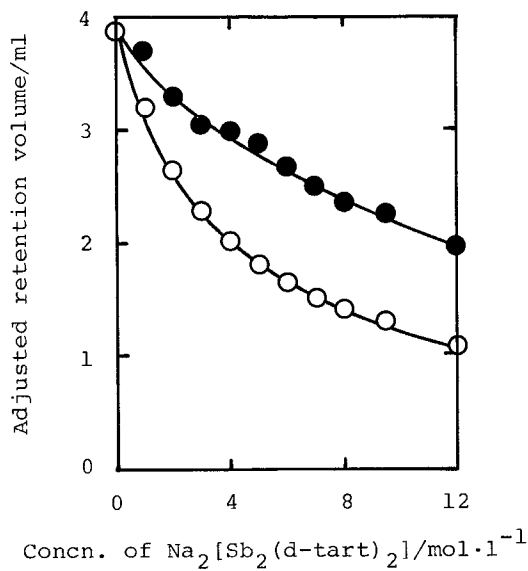


Fig. 2. Dependences of the adjusted retention volumes of $\Lambda$-$fac$-[Co(D-ser)$_3$] (O) and $\Delta$-$fac$-[Co(L-ser)$_3$] (●) on the concentration of Na$_2$[Sb($d$-tart)$_2$] in the eluent.
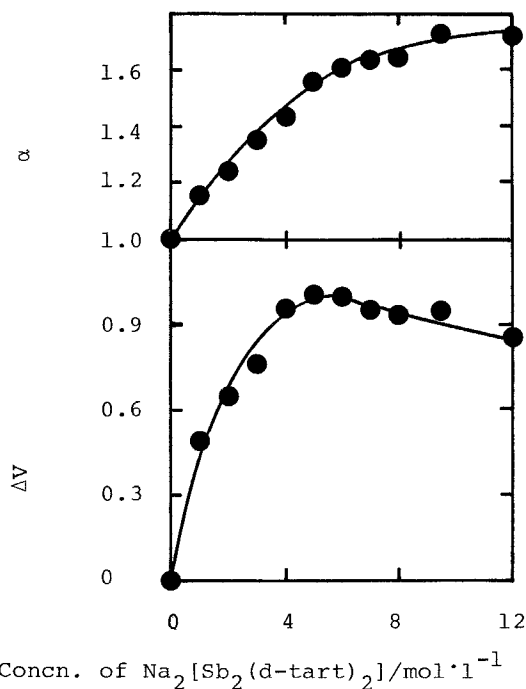
Fig. 3. Dependence of the separation factor, $\alpha$, and the difference in retention volume, $\Delta V$, on the concentration of $Na_2[Sb(d\text{-tart})_2]$ in the eluent for a racemic mixture of $\Lambda\text{-}fac\text{-}[Co(D\text{-ser})_3]$ and $\Delta\text{-}fac\text{-}[Co(L\text{-ser})_3]$.

creasing concentration of the chiral selector and seems to approach a definite value at infinite concentration. The trends in these parameters, the retention volumes, their difference and their ratio can be derived from expressions for two equilibria, ion association and adsorption.

    Let us consider the case in which an uncharged complex M is eluted on a cation-exchange column with an aqueous solution containing a chiral selector anion X which can associate with a complex M. The association constant, $\beta$, for M and X in aqueous solution is expressed by:

$$\beta = \frac{[MX]}{[M][X]} \tag{1}$$

    Although the complex is electrically neutral, it has some affinity for the ion-exchange resin, and some fraction is adsorbed on the latter. Thus, an adsorption equilibrium is formed between the cation exchanger and the eluent. For simplicity, the concentration of M in the stationary phase, (M), is assumed to be proportional to that in the mobile phase, [M]. Thus, we have

$$(M) = A[M] \tag{2}$$

where $A$ is a constant. This assumption is reasonable since the amount of sample is very small compared with the amount of adsorbent.

The distribution ratio, $D_M$, is described, to a good approximation, by:

$$D_M = \frac{(M)}{[M] + [MX]} \tag{3}$$

Replacing (M) and [MX] by eqns. 1 and 2, we obtain:

$$D_M = \frac{A[M]}{[M] + \beta[M][X]} = \frac{A}{1 + \beta[X]} \tag{4}$$

The adjusted retention volume, $V_{adj}$, for M can be related to $D_M$ and is expressed by

$$V_{adj} = D_M V_{resin} \tag{5}$$

where $V_{resin}$ is the volume of the resin in the column. Hereafter, the adjusted retention volume is called simply the retention volume, $V$, and described by

$$V = \frac{K}{1 + \beta[X]} \tag{6}$$

where $K$ is a constant equal to $A V_{resin}$.

Since the complex exists in two enantiomeric forms, $\Delta$-M and $\Lambda$-M, we have to discriminate their parameters by use of the subscripts $\Delta$ and $\Lambda$. As shown in Fig. 2, $V_\Delta$ is greater than $V_\Lambda$, so that $\beta_\Delta$ is greater than $\beta_\Lambda$. The separation factor, $\alpha$, which is the ratio of $V_\Delta$ and $V_\Lambda$ is expressed by:

$$\alpha = \frac{V_\Delta}{V_\Lambda} = \frac{1 + \beta_\Lambda[X]}{1 + \beta_\Delta[X]} \tag{7}$$

Inspection of eqn. 7 reveals that the value of $\alpha$ increases with increasing concentration of X and approaches a definite value, $\beta_\Lambda/\beta_\Delta$, at infinite concentration of X. The difference between the two retention volumes, $\Delta V = V_\Delta - V_\Lambda$, is expressed by:

$$\Delta V = V_\Delta - V_\Lambda = K \left( \frac{1}{1 + \beta_\Delta[X]} - \frac{1}{1 + \beta_\Lambda[X]} \right) \tag{8}$$

Differentiation of $\Delta V$ with respect to [X] leads to:

$$\frac{d(\Delta V)}{d[X]} = \frac{K(\beta_\Lambda - \beta_\Delta)(1 - \beta_\Delta\beta_\Lambda[X])}{(1 + \beta_\Delta[X])^2 (1 + \beta_\Lambda[X])^2} \tag{9}$$

It is easily seen that the value of $d(\Delta V)/d[X]$ is zero, when $(1 - \beta_\Delta\beta_\Lambda[X])$ is zero, that is, when $[X] = 1/\beta_\Delta\beta_\Lambda$. Thus, we have shown that the difference between the retention volumes of the enantiomers, $\Delta V$, has a maximum at $[X] = 1/\beta_\Delta\beta_\Lambda$.

However, the situation is different in the elution of cationic complexes. The elution of adsorbed cations is affected not only by ion association with chiral selector anions but also by ion exchange with counter ions of the chiral selector anions. Thus,

the right-hand side of eqn. 8 should be multiplied by a factor $1/[Na]$ which is equal to $1/2[X]$. Thus, we have:

$$V = K\left(\frac{1}{1 + \beta_A[X]} - \frac{1}{1 + \beta_A[X]}\right)\frac{1}{2[X]} \tag{10}$$

This function does not have a maximum in the region of $[X] > 0$, which means that a variation in $[X]$ is inevitably accompanied by a variation in $[Na]$ which obscures the chiral discrimination by $X^{2-}$.

## Type B chromatography

Now let us turn to the case of the so-called "ambush" type chromatography where the chiral selector anion is immobilized on an anion-exchange resin. In this case, the easy way to change the elution conditions is to use mixed solvents of various compositions. Ethanol–water mixtures are used in the present case. Figs. 4 and 5 show plots of the retention volumes, their differences and ratio for two kinds of neutral tris($\beta$-alaninato)cobalt(III) chelates against the ethanol content of the eluent. It is seen that the facial isomer shows a larger retention volume than the corresponding meridional isomer. This is because the facial isomer has a larger dipole moment and is more strongly adsorbed by the chiral selector anion on an anion-exchange resin than is the meridional isomer. As to the effect of ethanol, the following seems reasonable. The chiral selector anion immobilized on the ion-exchange resin is considered to be more or less hydrated, as is the complex in the eluent. Chiral discrimination takes place when the complex approaches close to the selector anion. This requires dehydration to some extent. The addition of ethanol may cause dehyration
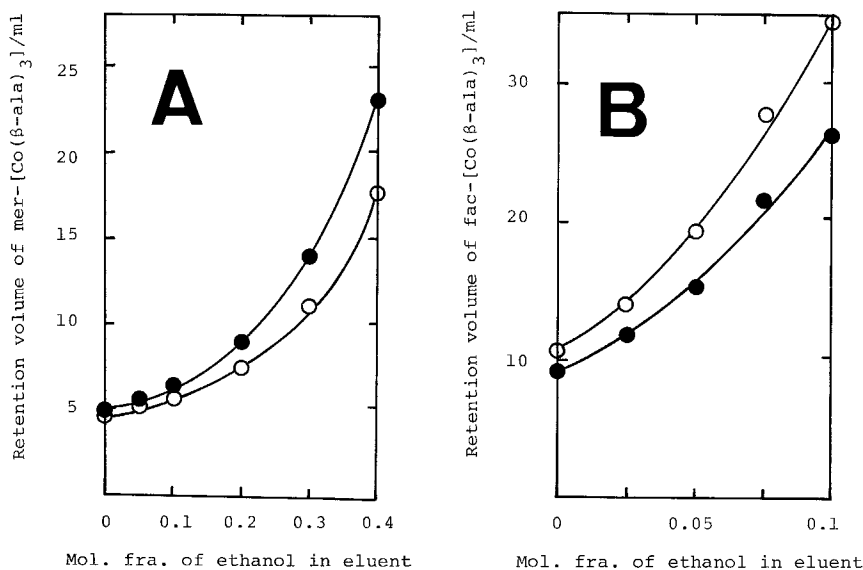


Fig. 4. Dependence of the adjusted retention volume on the concentration of ethanol in the eluent for mer-[Co($\beta$-ala)$_3$] (A) and fac-[Co($\beta$-ala)$_3$] (B) enantiomers. O—O, ($-$) enantiomer; ●—●, ($+$) enantiomer. ($+$) and ($-$) indicate the sign of the circular dichroism band of the eluate at 500 nm.
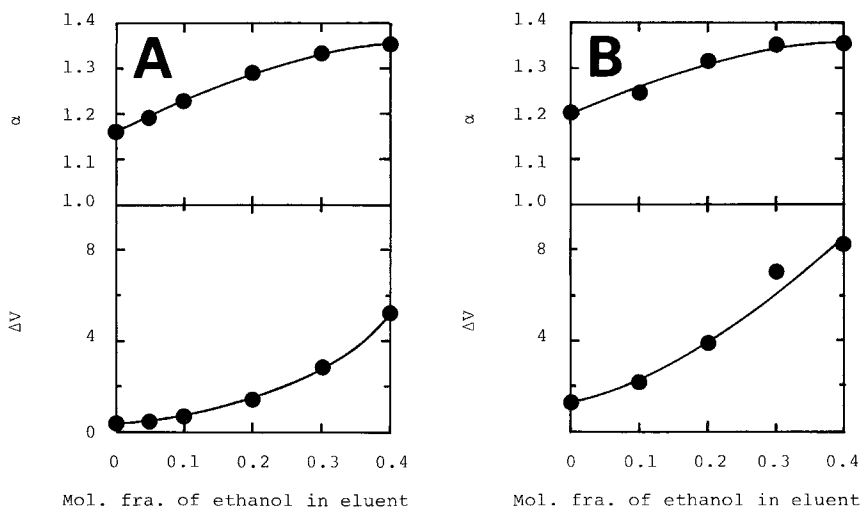
Fig. 5. Dependence of the separation factor, $\alpha$, and the difference in the retention volume, $\Delta V$, on the concentration of ethanol in the eluent for *mer*-[Co($\beta$-ala)$_3$] (A) and *fac*-[Co($\beta$-ala)$_3$] (B) enantiomers.

of the hydrated complex as well as of the hydrated chiral selector anion, so that it enhances chiral discrimination. Such an enhancement is expected to increase with increasing mole fraction of ethanol in the eluent. The trends shown in Figs. 4 and 5 can thus be understood. In conclusion, for good separation of enantiomers by chromatography of type B, we have to use an eluent containing as high an ethanol mole fraction as possible.

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