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CHROMATOGRAPHIC STUDY OF OPTICAL RESOLUTION

VIII. THEORETICAL STUDY OF THE CHROMATOGRAPHIC BEHAVIOUR OF THE ENANTIOMERS OF RACEMIC COMPLEX CATIONS ON A CATION-EXCHANGE COLUMN

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SUMMARY

Optical resolution of several mono- and divalent complex cations was achieved with a cation-exchange column using a solution containing various concentrations of a resolving agent as the eluent. The retention volumes of enantiomers, the differences in their retention volumes and the separation factors were determined for various concentrations of the eluent. The trends in these parameters could be expressed by functions derived from the equilibrium expressions of ion association and ion exchange.

INTRODUCTION

In previous papers of this series[1,2] the separation mechanism of enantiomers of metal complexes was discussed mainly from the stereochemical point of view. No consideration was given to the optimal conditions for optical resolution based on the general theory of chromatographic separation. This also applies to most other papers on the chromatographic resolution of racemic mixtures[3]. There have been few papers in which attempts have been made to improve the efficiency of optical resolution by systematically varying the separation conditions. Mikeš *et al.*[4] reported that the separation factor for the enantiomers of helicenes depends on temperature and has an optimum at a certain temperature. Davankov *et al.*[5] reported that in ligand-exchange chromatography using Cu^{2+} complex formation a decrease in temperature or in the concentration of a displacing ligand in an eluent solution leads to an increase in the separation factor of D- and L-prolines. In Part V of this series[6] we reported that *cis*-$[Co(N_3)_2(en)_2]^+$ was resolved into enantiomers on a cation-exchange column and that the separation factor of the enantiomers increases with increasing concentration of the chiral selector anion, antimony *d*-tartrate, $[Sb_2(d\text{-tart})_2]^{2-}$, in the eluent. How-

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ever, no papers appear to have considered the optical resolution from the point of view of chromatographic separation theory. In this paper, we discuss the separation mechanism of enantiomers and the dependence of the separation factor on the concentration of a chiral selector anion in an eluent.

THEORETICAL

Let us consider the case in which a monovalent complex cation M^+ is eluted on a column packed with a cation exchanger. An aqueous solution containing a divalent anion X^{2-}, which potentially forms ion pairs with the complex cation M^+, is used as the eluent. On increasing the concentration of X^{2-}, the concentration of the negatively charged ion pair MX^- increases. This effect overlaps with the effect of the cation exchange and leads to a decrease in the adsorption of the complex cation M^+. Thus, we have to consider two equilibria, ion exchange and ion pair formation.

The ion-exchange equilibrium is

$$RNa + M^+ \rightleftharpoons RM + Na^+$$

where RNa and RM are sodium and complex cations retained by the resin, respectively, and the equilibrium constant K_1 is defined by

$$K_1 = \frac{[RM][Na^+]}{[RNa][M^+]} \tag{1}$$

The ion-pair equilibrium is

$$M^+ + X^{2-} \rightleftharpoons MX^-$$

where the association constant β is defined by

$$\beta = \frac{[MX^-]}{[M^+][X^{2-}]} \tag{2}$$

As the total concentration of the complex M^+ is low in both the resin and eluent phases, *i.e.*, $[Na^+] \gg [M^+]$ and $[RNa] \gg [RM]$, the distribution ratio D_M is described, to a good approximation, by

$$D_M = \frac{[RM]}{[M^+] + [MX^-]} = \frac{K_1}{1 + \beta [X^{2-}]} \cdot \frac{Q}{[Na^+]} \tag{3}$$

where Q is the ion-exchange capacity of the resin (mequiv./ml).

The adjusted retention volume, V_{adj}, for the elution of M^+ can be related to D_M by

$$V_{adj} = D_M V_{resin} \tag{4}$$

where V_{resin} is the volume of the resin in the column.

Hereafter the adjusted retention volume is called simply the retention volume and described by eqn. 5 below. By replacing D_M in eqn. 4 with eqn. 3, we obtain

$$V = \frac{V_{resin} K_1 Q}{1 + \beta [X^{2-}]} \cdot \frac{1}{[Na^+]}$$

$$= \frac{0.5 V_{resin} K_1 Q}{[X^{2-}] + \beta [X^{2-}]^2} = \frac{C_1}{[X^{2-}](1 + \beta [X^{2-}])^2} \quad (5)$$

where $[Na^+] = 2[X^{2-}]$ and $0.5 V_{resin} K_1 Q$ can be regarded as a constant C_1. As the monovalent complex cation M^+ exists in two enantiomeric forms, Δ-M^+ and Λ-M^+, we have to discriminate the quantities concerning them by using the subscripts Δ and Λ. The retention volumes V_Δ and V_Λ are plotted against the concentration of X^{2-} according to eqn. 5 in Fig. 1a, where β_Λ is assumed to be greater than β_Δ. The difference in their retention volumes, $\Delta V = V_\Delta - V_\Lambda$, can be expressed by

$$\Delta V = \frac{C_1 (\beta_\Lambda - \beta_\Delta)}{(1 + \beta_\Delta [X^{2-}])(1 + \beta_\Lambda [X^{2-}])}$$

$$= \frac{C_2}{(1 + \beta_\Delta [X^{2-}])(1 + \beta_\Lambda [X^{2-}])} \quad (6)$$

where $C_1 (\beta_\Lambda - \beta_\Delta)$ is a constant C_2. Fig. 1b shows the plot of eqn. 6.

The separation factor α is given by

$$\alpha = \frac{V_\Delta}{V_\Lambda} = \frac{1 + \beta_\Lambda [X^{2-}]}{1 + \beta_\Delta [X^{2-}]} \quad (7)$$

The separation factor varies with $[X^{2-}]$ and reaches a constant value $\beta_\Lambda/\beta_\Delta$ at a very large value of $[X^{2-}]$ (Fig. 1c). For a particular enantiomeric pair β_Δ and β_Λ have

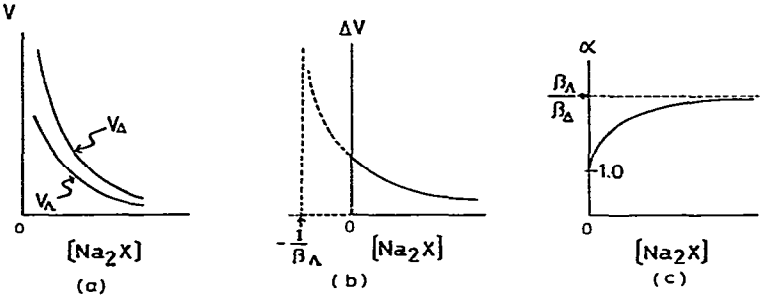

Fig. 1. (a) Dependence of adjusted retention volumes (V) on the concentration of Na_2X. V_Δ and V_Λ are V values for the Δ- and Λ-enantiomers, respectively. (b) Dependence of difference in retention volume (ΔV) on the concentration of Na_2X. (c) Dependence of separation factor (α) for the enantiomers on the concentration of Na_2X.

definite values, so that the separation of two elution peaks (ΔV) and the separation factor α have definite values for a definite value of $[X^{2-}]$. When the separation factor α is fairly large, a good separation (ΔV) is obtained with an appropriate concentration of $[X^{2-}]$. However, when the separation factor is small, we have to use a very low concentration of X^{2-} for a good separation (ΔV) (see Fig. 1b). A very long time would be required for elution. To shorten the elution time, it is necessary to use a concentrated eluent which gives a small ΔV. To resolve this dilemma, we have to use a longer column. If we double the column length, we double the separation (ΔV). Thus, we can rewrite eqns. 5 and 6 as a function of the length of the column:

$$V = \frac{C_1'}{(1 + \beta [X^{2-}])([X^{2-}])} \cdot L \tag{5'}$$

$$\Delta V = \frac{C_2'}{(1 + \beta_A [X^{2-}])(1 + \beta_A [X^{2-}])} \cdot L \tag{6'}$$

From eqn. 6′ we obtain

$$L = \frac{\Delta V (1 + \beta_A [X^{2-}])(1 + \beta_A [X^{2-}])}{C_2'} \tag{8}$$

Eqn. 8 is plotted in Fig. 2a.

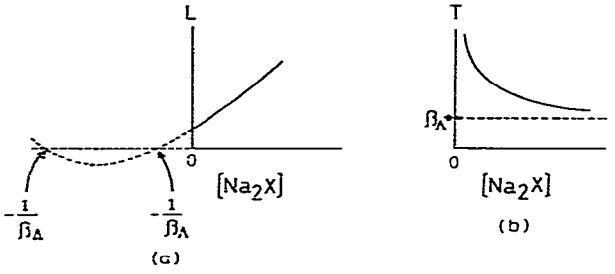

Fig. 2. (a) Column length (L) at various concentrations of Na_2X when the difference in retention volumes for enantiomers is constant. (b) Time (T) required for the second enantiomer (Δ-form) to be eluted at various concentrations of Na_2X, when the difference in the retention volumes of the enantiomers is constant.

We introduce the time (T) which is required for the second enantiomer to be eluted. Let F be a constant flow-rate, then $T = V/F$. From eqns. 5′ and 8

$$T = \frac{C' L}{[X^{2-}] + \beta_\Delta [X^{2-}]^2} \cdot \frac{1}{F}$$

$$= \frac{C_1'}{C_2'} \cdot \frac{\Delta V (1 + \beta_\Delta [X^{2-}])(1 + \beta_A [X^{2-}])}{[X^{2-}] + \beta_\Delta [X^{2-}]^2} \cdot \frac{1}{F}$$

$$= C_3 \left(\frac{1}{[X^{2-}]} + \beta_A \right) \tag{9}$$

where $C_3 = C_1'/(C_2' F)$. Eqn. 9 is plotted in Fig. 2b. Thus, when the separation factor is small, higher concentrations of the eluent and longer column lengths are recommended in order to obtain a good separation.

EXPERIMENTAL

Materials

The complexes used were *cis*(O)–*cis*(N)-$[CO(gly)_2en]Cl \cdot H_2O$ (ref. 7), *cis*(O)-*trans*(N)-*cis*(NH_3)-$[Co(gly)_2(NH_3)_2]Cl \cdot H_2O$ (ref. 8), $[Cogly(en)_2]Cl_2 \cdot H_2O$ (ref. 9), $[Cogly(tn)_2]Cl_2 \cdot H_2O$ (ref. 10) and *cis*-α-$[Co(N_3)_2trien]I$. The first four complexes were prepared according to the methods described in the literature, and the last complex was prepared as follows.

A solution containing 3.2 g of *cis*-α-$[CoCl_2trien]Cl$ in 50 ml of water was mixed with a solution of 2 g of sodium azide in 15 ml of water and heated at 60°C for 30 min. After the solution had been allowed to stand at room temperature, dark violet crystals were deposited, which were presumed to be the azide salt of the desired complex and were separated by filtration. The crystals were converted into the iodide by dissolving them in a small amount of water and adding an excess of sodium iodide. The dark violet crystalline powder was filtered, washed with ethanol and dried in air. The β-isomer of this complex was prepared from *cis*-β-$[CoCl_2trien]Cl$ by the same method in order to identify the isomers.

All complexes used were identified by elemental analyses and PMR and UV absorption spectroscopy.

The eluents used were aqueous solutions of various concentrations of sodium antimony *d*-tartrate dihydrate, $Na_2[Sb_2(d\text{-tart})_2] \cdot 2H_2O$, which was prepared from $K_2[Sb_2(d\text{-tart})_2] \cdot 3H_2O$ and sodium perchlorate.

Apparatus

The chromatographic unit was a JASCO LCP-150 pump, PM-150 pressure gauge, PC-150 pump controller, septum injection kit and Shimazu UV-140 double-beam spectrophotometer.

The column was a 20-cm × 3 mm I.D. stainless-steel tube packed with IEX-510 strong-acid cation-exchange resin (Toyo Soda, Japan). A dual-pen strip-chart recorder (Rigakudenki Electronic Recorder Model B-161) was used. The detector, equipped with a 20-μl flow cell, was operated at the first absorption band of each complex. Blue Dextran 2000 (Pharmacia, Sweden) was used as a marker to measure the void volume of the column.

RESULTS AND DISCUSSION

To establish whether the conclusions in the theoretical section are valid in actual chromatographic runs, chromatographic separations of five racemic complexes were performed. The complexes *cis*-α-$[Co(N_3)_2trien]^+$ and $[Co(gly)_2(NH_3)_2]^+$ have already been reported to be separable into enantiomers, but the other three complexes were found to be separable for the first time in this study. Typical elution curves of mono- and divalent complex cations eluted with 0.06 and 0.1 *M* $Na_2[Sb_2(d\text{-tart})_2]$ solution are shown in Figs. 3–5. Fig. 6 shows the dependence of retention volumes,

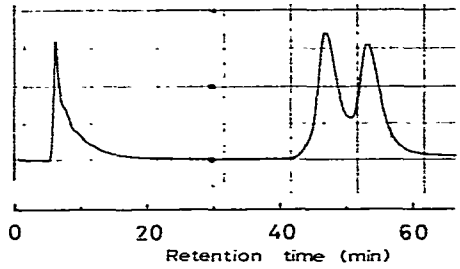

Fig. 3. Elution curve for *cis*-α-$[Co(N_3)_2trien]^+$. Eluent: 0.06 *M* $Na_2[Sb_2(d\text{-tart})_2]$.

differences in retention volumes and separation factors on the eluent concentration for the enantiomers of the complexes.

There is a close resemblance between the curves in Fig. 1 and the corresponding curves in Fig. 6, which indicates the validity of the above theory. As shown in Fig. 6 for the monovalent complexes, the separation factor increases with increasing eluent concentration and reaches a certain limiting value at a high concentration of the eluent. This is just the trend that is expected from eqn. 7. On the other hand, for the divalent complexes the separation factor was found to be almost constant in the experimental concentration range, as shown in Fig. 6. In general, the association constant for the divalent complex cation is presumed to be fairly large so that the term $1 + \beta[X^{2-}]$ in eqn. 7 will come close to $\beta[X^{2-}]$. Thus, the separation factor reaches a definite value even at low concentrations of X^{2-}.

Next, let us compare the trends of the separation factors of two monovalent complexes, *cis*-α-$[Co(N_3)_2trien]^+$ and *cis*(O)-*cis*(N)-*cis*(NH_3)-$[Co(gly)_2(NH_3)_2]^+$. At low concentration of X^{2-} (0.02 *M*), the separation factor is smaller for the diazido complex than for the diammine complex. However, when the concentration of X^{2-} exceeds 0.1 *M*, the separation factor is larger for the diazido complex than for the

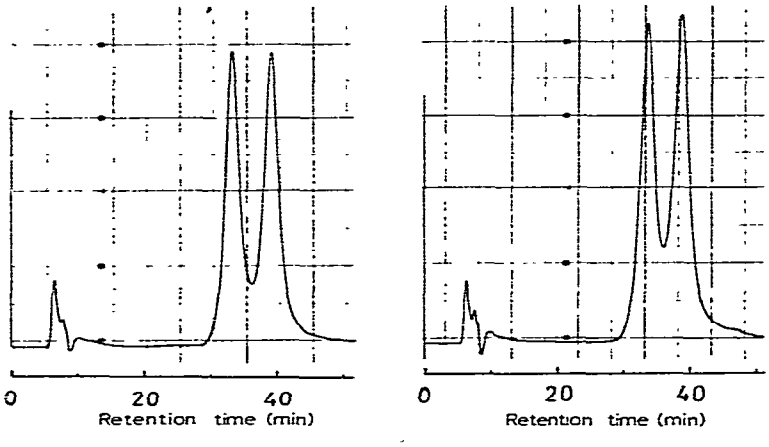

Fig. 4. (a) Elution curve for *cis*(O)-*cis*(N)-$[Co(gly)_2en]^+$. (b) Elution curve for *cis*(O)-*trans*(N)-*cis*(NH_3)-$[Co(gly)_2(NH_3)_2]^+$. Eluent: 0.06 *M* $Na_2[Sb_2(d\text{-tart})_2]$.

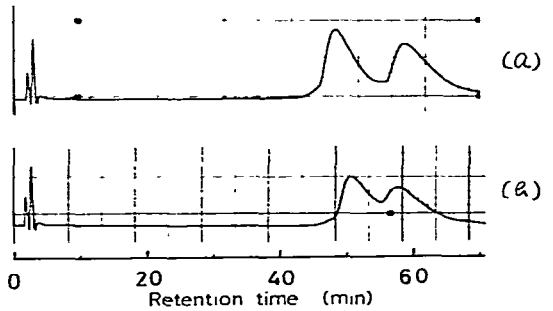

Fig. 5. (a) Elution curve for $[Cogly(en)_2]^{2+}$. (b) Elution curve for $[Cogly(tn)_2]^{2+}$. Eluent: 0.1 *M* $Na_2[Sb_2(d\text{-tart})_2]$.

diammine complex, as shown in Fig. 6. This can be explained by assuming that the association constant with antimony *d*-tartrate is smaller for the azido complex than for the diammine complex but the ratio β_A/β_Δ is larger for the former complex than for the latter.

Concerning the elution time, let us consider the case of the diazido complex (Fig. 6a). In this study we used a column length of 20 cm. The time (adjusted) required for the elution of the second peak was 21 min with 0.12 *M* eluent and the peak separation (ΔV) was 0.43 ml. With 0.02 *M* eluent, the elution time was 160 min and the peak separation (ΔV) was 1.17 ml. If we want a 1-ml peak separation with 0.02 *M* eluent, we have to use a 17-cm column length, which will require 137 min for elution. With 0.12 *M* eluent we have to use a 46.5-cm column and the elution time (adjusted will be 48.8 min. These relations are illustrated in Fig. 7. Thus, in order to obtain the same separation (ΔV) it is desirable, in order to save time, to use the longer column and more concentrated eluent than to use the shorter column and more dilute eluent.

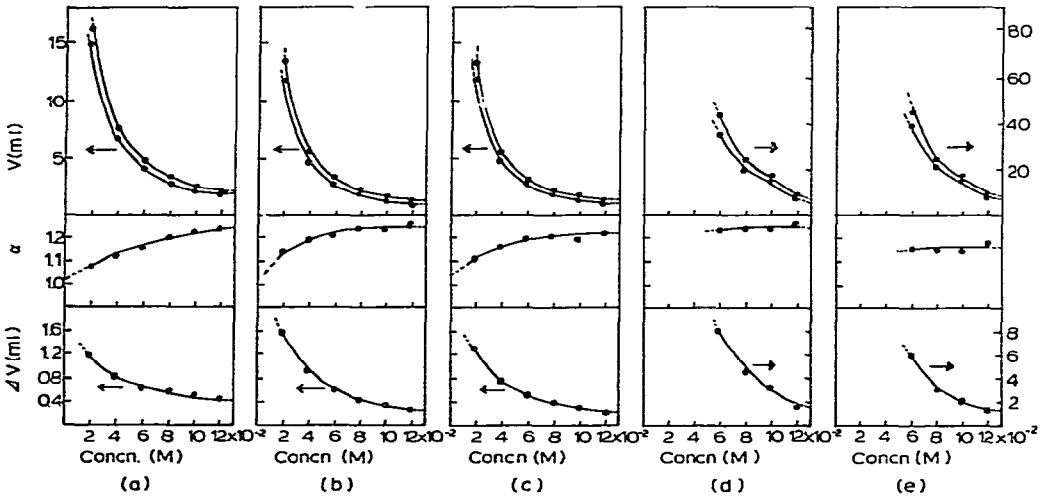

Fig. 6. Dependence of adjusted retention volumes (V), separation factor (α) and difference in retention volume (ΔV) for the enantiomers on the concentration of $Na_2[Sb_2(d\text{-tart})]$. (a) *cis*-α-$[Co(N_3)_2trien]^+$; (b) *cis*(O)-*cis*(N)-$[Co(gly)_2en]^+$; (c) *cis*(O)-*trans*(N)-*cis*(NH_3)-$[Co(gly)_2(NH_3)_2]^+$; (d) $[Cogly(en)_2]^{2+}$; (e) $[Cogly(tn)_2]^{2+}$.

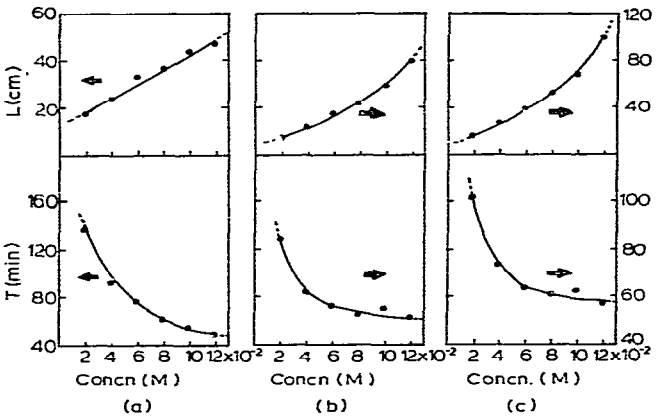

Fig. 7. Column length (L) and elution time (T) for the second enantiomer to be eluted at various concentrations of $Na_2[Sb_2(d\text{-tart})_2]$ when ΔV = 1 ml. (a) *cis*-α-$[Co(N_3)_2trien]^+$; (b) *cis*(O)-*cis*(N)-$[Co(gly)_2en]^-$; (c) *cis*(O)-*trans*(N)-*cis*(NH_3)-$[Co(gly)_2(NH_3)_2]^-$; (d) $[Cogly(en)_2]^{2-}$; (e) $[Cogly(tn)_2]^{2-}$.

CONCLUSIONS

(1) When the complex cation and the chiral selector anion associate only slightly with each other, the separation factor for the enantiomers increases with increasing concentration of the anionic chiral selector, and approaches a certain limiting value.

(2) When strong association takes place between the sample complex cation and the chiral selector anion, the separation factor is expressed as the ratio of the association constants of the enantiomers.

(3) At a constant column length, the lower the concentration of anionic chiral selector in the eluent, the larger is the difference in the retention volumes of the enantiomers.

(4) When the length of the column and the concentration of the chiral selector are both variable, the longer the column and the higher the concentration, the larger is the difference in the retention volume per unit time.

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REFERENCES

1 S. Yamazaki and H. Yoneda, *Inorg. Nucl. Chem. Lett.*, 15 (1979) 195.
2 S. Yamazaki, T. Yukimoto and H. Yoneda, *J. Chromatogr.*, 175 (1979) 317.
3 I. S. Krull, *Advan. Chromatogr.*, 16 (1978) 175–210.
4 F. Mikeš, G. Boshart and E. Gil-Av, *J. Chromatogr.*, 122 (1976) 205.
5 V. A. Davankov, S. V. Rogozhin, A. V. Semechkin, V. A. Baranov and G. S. Sannikova, *J. Chromatogr.*, 93 (1974) 363.
6 S. Yamazaki and H. Yoneda, *J. Chromatogr.*, 177 (1979) 227.
7 N. Matsuoka, J. Hidaka, Y. Shimura, *Bull. Chem. Soc. Jap.*, 45 (1972) 2491.
8 K. Kobayashi and M. Shibata, *Bull. Chem. Soc. Jap.*, 48 (1975) 2561.
9 C. T. Liu and B. E. Douglas, *Inorg. Chem.*, 3 (1964) 1356.
10 G. R. Brubaker and D. P. Schaefer, *Inorg. Chem.*, 10 (1971) 2170.