BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 52 (6), 1859—1860 (1979)

Studies of the Separation Mechanism in Ion-exchange Chromatography. I. Inversion of the Elution Order of cis and trans Isomers of Metal Complexes

Hayami YONEDA* and Shigeo YAMAZAKI

Department of Chemistry, Faculty of Science, Hiroshima University, Hiroshima 730

(Received December 4, 1978)

Synopsis. The inversion of the elution order in ion-exchange chromatography was found for cis and trans isomers of $[Co(X)_2(en)_2]^+$ ($X=N_3^-$, NO_2^-, and NCS^-) by the addition of dioxane to NH_4Cl or $(NH_4)_2SO_4$ aqueous solution as eluent, and the separation mechanism was discussed.

Since King and Walters[1)] reported the chromatographic separation of the cis and trans isomers of $[Co(NO_2)_2(NH_3)_4]^+$ by ion-exchange technique, it has been accepted as a general rule that the trans isomer is eluted earlier than the cis isomer.[2,3)] This is quite understandable when we consider that the cis isomer has a dipole moment and is adsorbed more strongly than the trans isomer having no dipole moment. This explanation is based on the assumption that separation is carried out mainly by adsorption, although chromatography consists of two processes, adsorption and elution. Here it must be pointed out that the dielectric constant is fairly smaller in the stationary phase (resin phase) than in the moving phase (aqueous solution), so that ion association in the stationary phase should be more effective than in the moving phase, that is, adsorption should be more decisive than elution. Thus, it is natural to consider that the elution order is determined by the strength of adsorption. Therefore, if we set up conditions under which ion association in the moving phase is more effective than that in the stationary phase, the elution order of the cis and trans isomers is expected to be reversed.

Based on this consideration, we tried to chromatograph the cis and trans pairs of three cobalt(III) complexes through the column packed with SP-Sephadex C-25 using dioxane–water mixed solution of an electrolyte as an eluent. As such Sephadex contains many hydrophilic functional groups, the dielectric constant in the stationary phase should be fairly large and it can be easily overtaken by the dielectric constant in the moving phase (aqeuous solution) with the addition of some amount of dioxane to the eluent, which should result in the inversion of the elution order. This expectation was fulfilled in the present study.

Experimental

Samples. All the complexes were prepared according to the literature, and identified by UV absorption spectra and elemental analyses. Complexes used were the cis and trans pairs of $[Co(NO_2)_2(en)_2]ClO_4$, $[Co(NCS)_2(en)_2]Cl\cdot H_2O$, and $[Co(N_3)_2(en)_2]ClO_4$.

Sample solutions were prepared by dissolving 10 mg of the complex in a few ml of an eluent.

Eluent. The eluents were aqueous solutions of 0.4 mol dm^{-3} NH_4Cl, 0.4 mol dm^{-3} NH_4NCS and 0.05 mol dm^{-3} $(NH_4)_2SO_4$ containing various amounts of dioxane. Dioxane was distilled before use.

Apparatus. All the works were carried out on a laboratory-built chromatographic unit at room temperature. The column size was 25 cm long and 6.5 mm in diameter. SP-Sephadex C-25 (Pharmacia, Sweden) was used as a packing material. The flow rates were ranged from 0.8 ml/min for zero dioxane to 0.25 ml/min for 60% dioxane content. The detector was operated at the first absorption band of each complex. The adjusted retention volume was measured from the elution curve. $(NH_4)_3[Cr(NCS)_6]$ was used as a marker.[4)] The sample solution 20 μl being typical was injected by a syringe into the column.

Results and Discussion

Fig. 1(a) shows the trend of the retention volumes of the cis and trans pairs of three cobalt(III) complexes with increasing dioxane content of 0.4 mol dm^{-3} NH_4Cl solution. As expected, in the low dioxane content region, the retention volume of the cis isomer having a dipole moment is larger in all three cases than that of the trans isomer having no dipole moment. This suggests that the electrostatic interaction between the complex cation and the functional group (SO_3^-) of Sephadex ion-exchanger plays a decisive role in chromatographic separation. The situation is reversed in the high dioxane content region where the retention volume of the cis isomer becomes smaller than that of the trans isomer. This means that the electrostatic interaction between the complex cation and the counter anion in the moving phase plays a dominant role in chromatographic separation.

The most important factors which govern the electrostatic interaction are the charge density of the counter ion (ionic charge times concentration) and the dielectric constant of the medium. In the case of SP-Sephadex C-25, the exchange capacity and the bed volume of the gel at ionic strength 0.4 are known to be 2.3 m equivalent/g(dry gel) and 6 ml/g(dry gel) respectively. Since the real volume of the swelling gel is estimated to be 2/3 of the bed volume, the concentration of the SO_3^- group in the swelling gel is calculated to be 0.57 mol dm^{-3} which is larger than 0.4 mol dm^{-3}, the Cl^- concentration of the eluent. In addition, the water caught in the Sephadex matrix is presumed to be less structured than the water in the moving phase. Since the electrostatic interaction is dependent upon the product of the charge density of the counter ion and the inverse of the dielectric constant, it should be larger in the stationary phase than in the moving phase in the absence or at the low content of dioxane.

Although the addition of dioxane decreases the magni-

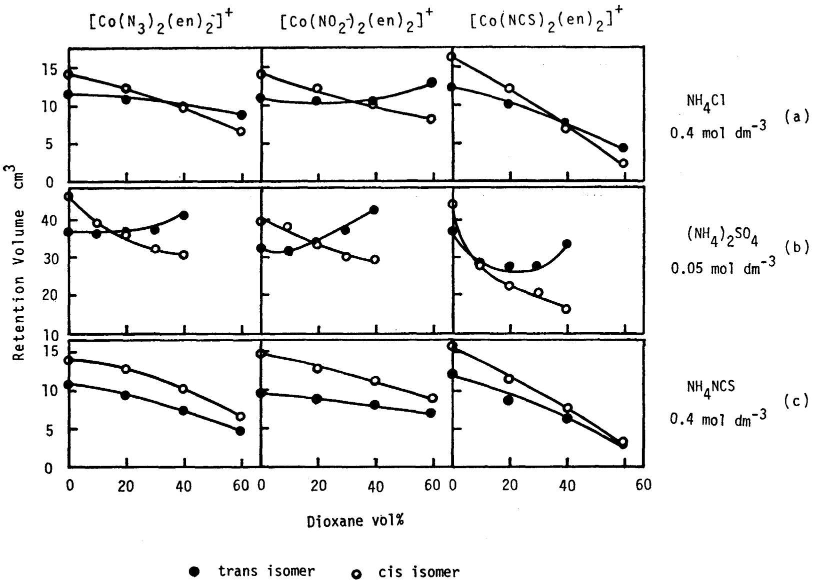

Fig. 1. Retention volume of trans(●) and cis(○) pairs of $[Co(X)_2(en)_2]^+$ ($X=N_3^-$, NO_2^-, and NCS^-) *vs.* dioxane content in 0.4 mol dm^{-3} NH_4Cl, 0.05 mol dm^{-3} $(NH_4)SO_4$ and 0.4 mol dm^{-3} NH_4NCS solution as eluent.

tude of the dielectric constant both in the stationary and moving phases, the rate of decrease should be larger in the moving phase. Since Sephadex contains many hydrophilic groups, it can hold much water strongly. Thus, the Sephadex matrix contains more water than the moving phase when the dioxane–water mixed solution is kept in equilibrium with Sephadex.[5] Therefore, the increase of dioxane content in the eluent results in a more gradual decrease of the dielectric constant in the stationary phase than in the moving phase. Thus, the magnitude of the dielectric constant in both phases becomes closer with the increasing content of dioxane, and at a certain content of dioxane the inversion of the magnitude of the dielectric constant takes place. The inversion of the elution order can be thus understood. In the case of 0.4 mol dm^{-3} NH_4Cl, the inversion of the elution order takes place at about 35—40% (v/v) dioxane content above which the cis isomer is eluted earlier than the trans isomer. The inversion should take place at much lower dioxane content for the bivalent anion SO_4^{2-} than for the univalent anion Cl^-. Thus, for 0.05 mol dm^{-3} $(NH_4)_2SO_4$ solution, the inversion takes place at about 10—20% dioxane content as shown in Fig. 1(b).

However, it must be noted that for $(NH_4)_2SO_4$ solution, the retention volume of the trans isomer does not decrease but rather increase with increasing content of dioxane. This means that the effective concentration of SO_4^{2-} to catch the complex cation decreases with increasing content of dioxane. The addition of dioxane decreases the dielectric constant of the moving phase which results in strengthening the association of SO_4^{2-} not only with the complex cation but also with NH_4^+. Since the cis isomer has a dipole moment and a great tendency toward association with SO_4^{2-}, it is not so much influenced by the presence of NH_4^+. However, for the trans isomer having no dipole moment, the existence of a large amount of NH_4^+ results in the decrease of the effective concentration of SO_4^{2-} in the eluent of high dioxane content, so that the retention volume increases.

If this interpretation is valid, the retention volume should decrease regularly with increasing content of dioxane, when an eluent contains a salt which has little tendency toward ion association even in the solution of high dioxane content. Ammonium thiocyanate solution as eluent is considered to be the case as shown in Fig. 1(c). Here, NCS^- solvates dioxane strongly and is presumed to be dissociated from NH_4^+ even in the high dioxane content. Such strong solvation of NCS^- anion causes the decrease in its association with the complex (cis and trans) too, so that the association-promoting tendency due to the decrease of the dielectric constant is almost cancelled. Thus the inversion of the elution does not take place.

Reference

1) E. L. King, and R. R. Walter, *J. Am. Chem. Soc.*, **74**, 4471 (1952).

2) M. Mori, M. Shibata, and M. Nanasawa, *Bull. Chem. Soc. Jpn.*, **29**, 947 (1956); M. Mori, M. Shibata, and K. Hori, *ibid.*, **34**, 1809 (1961).

3) Y. Yoshikawa, M. Kojima, M. Fujita, M. Iida, and H. Yamatera, *Chem. Lett.*, **1974**, 163.

4) S. Yamazaki and H. Yoneda, *J. Chromatogr.*, in press.

5) N. Yoza, *Kagaku No Ryoiki*, **25**, 1041 (1971).