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Note

Gel filtration of a series of cobalt(III) complexes

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During the last 10 years, we have examined the paper electrophoretic and chromatographic behaviour of a series of cobalt(III) complexes, notably $Co(NH_3)_6^{3+}$, $Co(en)_3^{3+}$, $Co(dip)_3^{3+}$ and $Co(o\text{-phen})_3^{3+}$, because we had observed that these complexes lend themselves to the study of outer-sphere complexing. The complexes are extremely stable in even high concentrations of electrolytes and they permit the study of outer-sphere complexing and adsorption with cations over a range of sizes from an ionic weight of 160.9 to 599.

As we have at our disposal such a range of sizes, we felt that we should also examine the gel filtration properties of these cations, as numerous mechanisms for the movement of ions on Sephadex gels have been proposed. Our findings are reported in this paper.

EXPERIMENTAL AND RESULTS

Thin-layer chromatography

The following compounds at concentrations of *ca.* 10^{-2} *M* in water were studied: (1) a mixture of $Co(o\text{-phen})_3Cl_3$, $Co(dip)_3Cl_3$, $Co(tn)_3Cl_3$, $Co(en)_3Cl_3$ and $Co(NH_3)_6Cl_3$, (2) $Co(o\text{-phen})_3Cl_3$, (3) $Co(dip)_3Cl_3$, (4) $Co(tn)_3Cl_3$, (5) $Co(en)_3Cl_3$, (6) $Co(NH_3)_6Cl_3$, (7) $Co(pn_2dip)Cl_3$, (8) a mixture of $Co(en_2dip)Cl_3$ and $Co(endip_2)Cl_3$ and (9) $Co(pn)_3Cl_3$, where the following abbreviations are used: en = ethylenediamine; pn = propylenediamine; tn = 1,3-diaminopropane; dip = dipyridyl; *o*-phen = *o*-phenanthroline.

We examined the complexes on two gels that should separate substances with ionic weights in the range 100–600, namely Bio-Gel P-2 and Sephadex G-25, and also on a gel with much larger pores (Sephadex G-75). The technique was that described previously[1] employing the Pharmacia TLC chamber using Blue Dextran 2000 (BD) as reference for the exclusion limit.

The chromatograms obtained (*i.e.*, "prints" on Whatman No. 1 paper detected by spraying with aqueous ammonium sulphide) are shown in Figs. 1–6.

Figs. 1 and 2 show that on Sephadex G-25 there is a pronounced separation effect in both sodium chloride and sodium trichloroacetate but that the actual concentration of the electrolytes has relatively little effect in the range 0.1–1.0 *N*. Further, the sequence follows that of increasing ionic size with the exception of $Co(o\text{-phen})_3^{3+}$, which seems to be adsorbed on the Sephadex and thus moves more slowly than

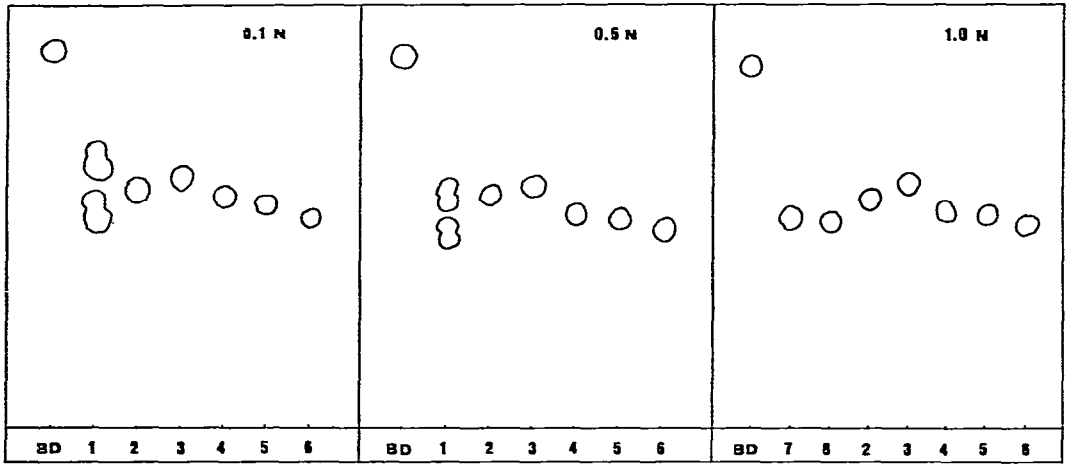

Fig. 1. Thin-layer chromatograms on Sephadex G-25 using as eluents aqueous NaCl solutions. The numbers refer to the solutions listed under *Thin-layer chromatography*. BD = blue dextran.

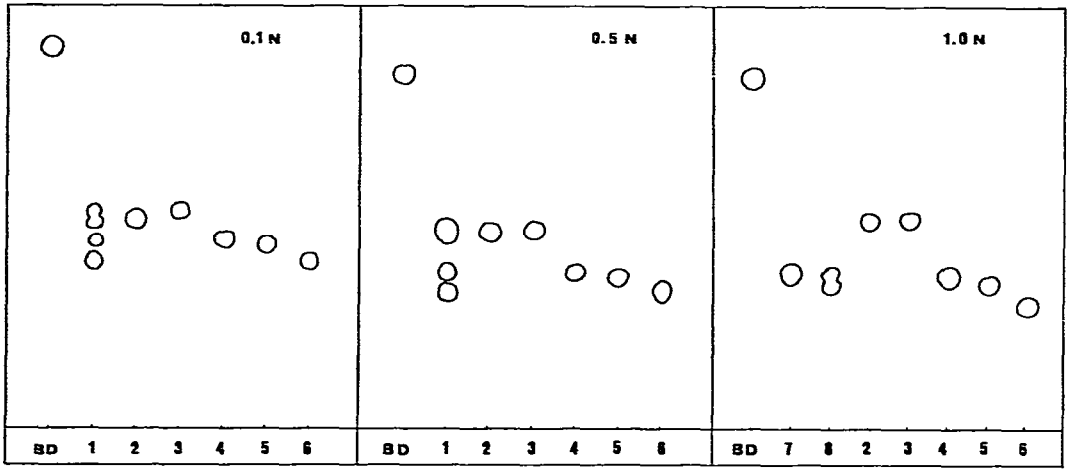

Fig. 2. Thin-layer chromatograms on Sephadex G-25 with CCl_3COONa solutions as eluents.

$Co(dip)_3^{3+}$. These two electrolytes were employed because in electrophoretic studies a strong ion-pairing tendency was observed between the trichloroacetate ions and the heavier cobalt(III) complexes of the type $Co(dip)_3^{3+}$ or $Co(o\text{-phen})_3^{3+}$. In chloride, on the other hand, there is a general tendency for ion pairing with no preference towards the larger complexes. Hence the better separation in trichloroacetate than in chloride is probably due to the enhanced size differences caused by this ion pairing.

The chromatograms on Sephadex G-75 in Fig. 3 show that on this gel with pores that are too large for an exclusion effect there are no differences in movement in trichloroacetate and chloride, which suggests that on Sephadex neither adsorption nor ion exchange play a predominant role.

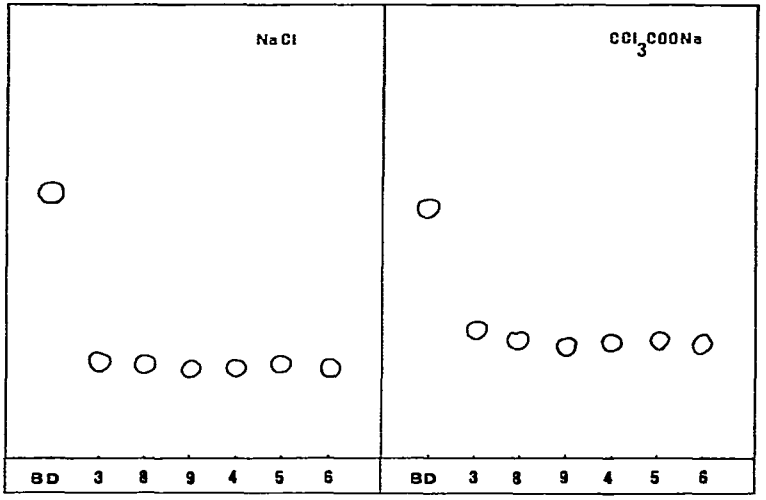

Fig. 3. Thin-layer chromatograms obtained on Sephadex G-75. Eluents: 0.5 *N* NaCl (left) and 0.5 *N* CCl_3COONa (right).

Figs. 4 and 5 show the chromatograms obtained on Bio-Gel P-2 with sodium chloride and sodium trichloroacetate as eluents. As we found later in column experiments, there is considerable retardation (mainly by ion exchange) on Bio-Gel P-2, which accounts for the good separations.

The inversion of the pair $Co(dip)_3^{3+}$ and $Co(o\text{-phen})_3^{3+}$, which is very pronounced in sodium chloride and less in sodium trichloroacetate, is probably again due to an adsorption effect which is reduced when $Co(o\text{-phen})_3^{3+}$ is in the form of an ion pair with trichloroacetate.

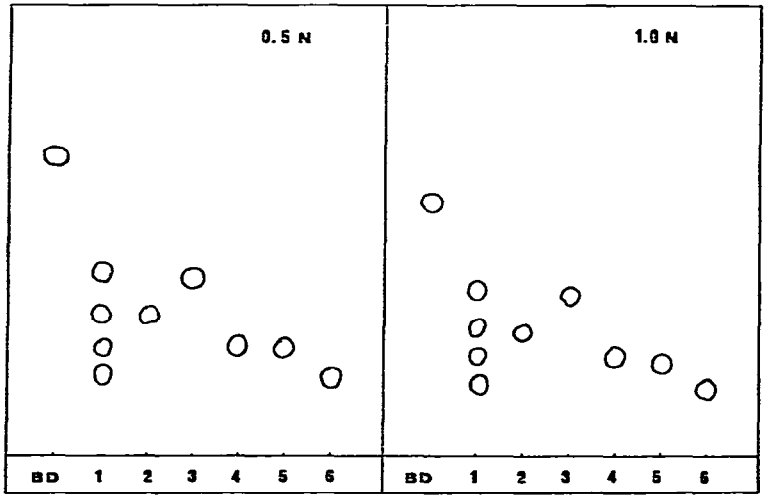

Fig. 4. Thin-layer chromatograms obtained on Bio-Gel P-2. Eluents: 0.5 *N* and 1.0 *N* NaCl.

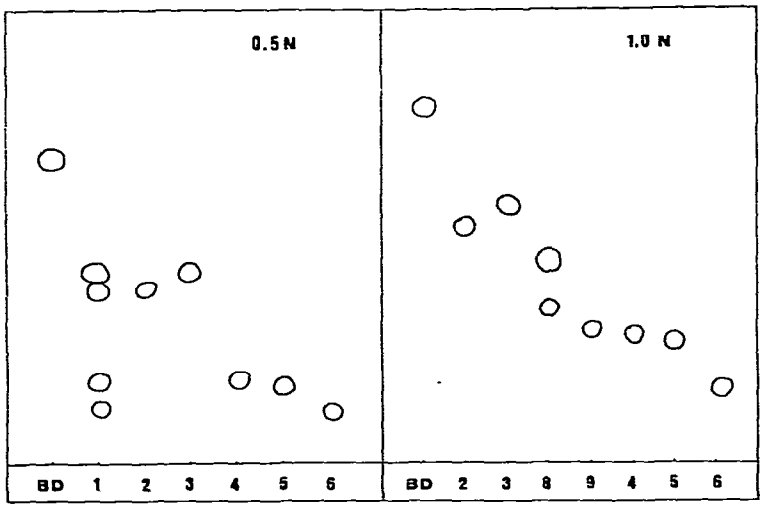

Fig. 5. Thin-layer chromatograms obtained on Bio-Gel P-2. Eluents: 0.5 *N* and 1.0 *N* CCl_3COONa.

Fig. 6 shows the chromatogram obtained in 1 *M* sodium sulphate solution. Here the complexes $Co(NH_3)_6^{3+}$, $Co(en)_3^{3+}$, $Co(tn)_3^{3+}$ and $Co(pn)_3^{3+}$ form strong ion pairs by hydrogen bonding and hence the smaller ions move as fast as the larger ions because the ion pairs formed will have sizes comparable to those of the heavier free ions.

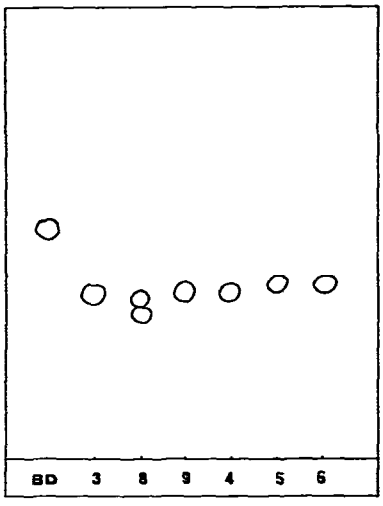

Fig. 6. Thin-layer chromatograms obtained on Bio-Gel P-2. Eluent: 1.0 *M* Na_2SO_4.

Column experiments

Sephadex G-10. The elution curves of some single complexes and some mixtures are shown in Fig. 7. All complexes are eluted well ahead of sodium chloride and thus there seems to be a separation due essentially to differences in size. A plot of

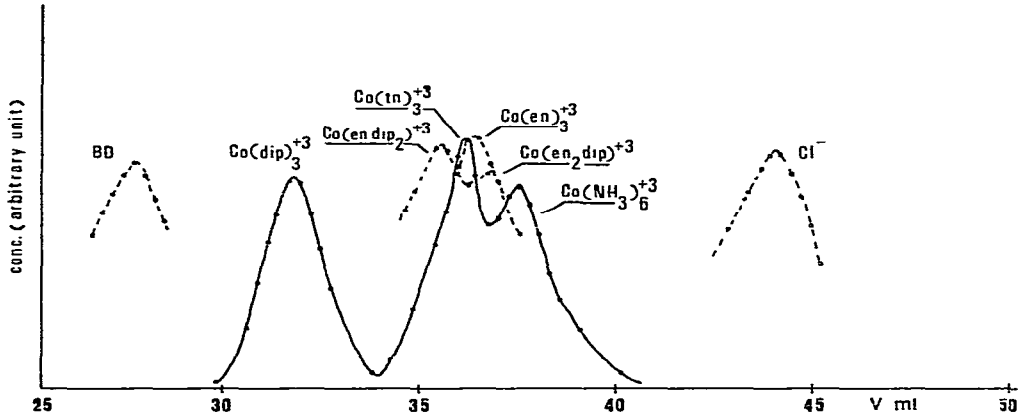

Fig. 7. Elution curve on Sephadex G-10 column. Column bed, 91 × 1.0 cm; eluent, 0.1 *N* NaCl; flow-rate, 27 ml/h; sample volume, 0.5 ml. Dotted lines indicate compounds chromatographed separately.

K_{av} against ionic weight gives an approximately straight line (Fig. 8); in view of the well known ion pairing with chloride ions, which is also influenced by the symmetry of the complexes, we really have no knowledge of the actual "ionic weight" of the hydrated and ion-paired complexes in solution and thus such a correlation has little significance.

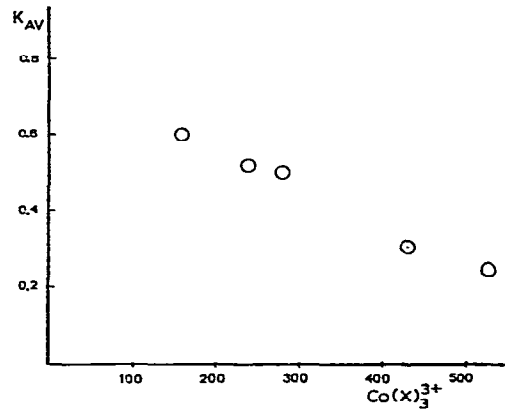

Fig. 8. Plot of K_{av} values against ionic weight for complexes eluted on Sephadex G-10 column.

Sephadex G-15. There is a good differentiation between complexes and all move ahead of sodium chloride, as shown in Fig. 9. The plot of K_{av} against ionic weight is approximately a straight line (Fig. 10).

Bio-Gel P-2. We have separated on a column of Biogel P-2 a more complex mixture than on the Sephadex gels (Fig. 11); however, not all complexes are eluted ahead of NaCl indicating that a certain retention presumably due to ion exchange occurs.

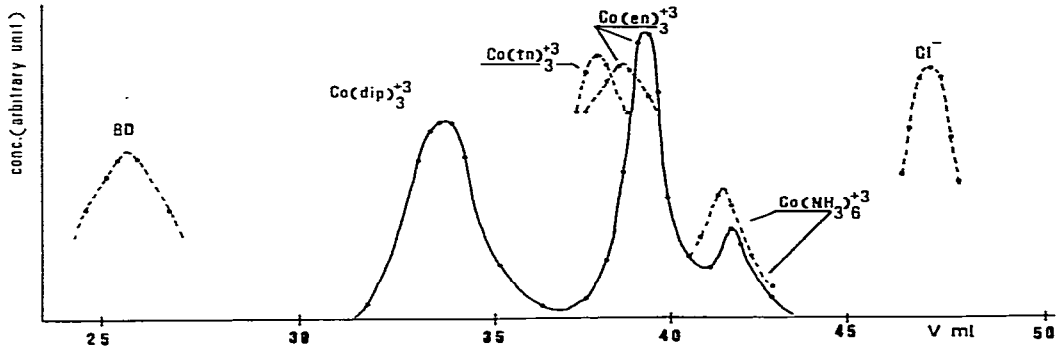

Fig. 9. Elution curve on Sephadex G-15 column. Column bed, 85 × 1.0 cm; eluent, 0.1 *N* NaCl; flow-rate, 26 ml/h; sample volume, 0.5 ml. Dotted lines indicate compounds eluted separately.

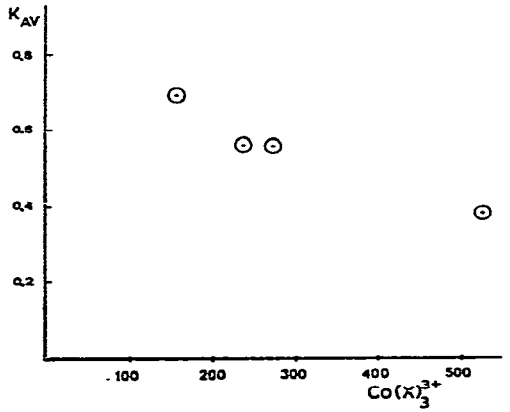

Fig. 10. Plot of K_{av} values obtained on Sephadex G-15 column against ionic weight.

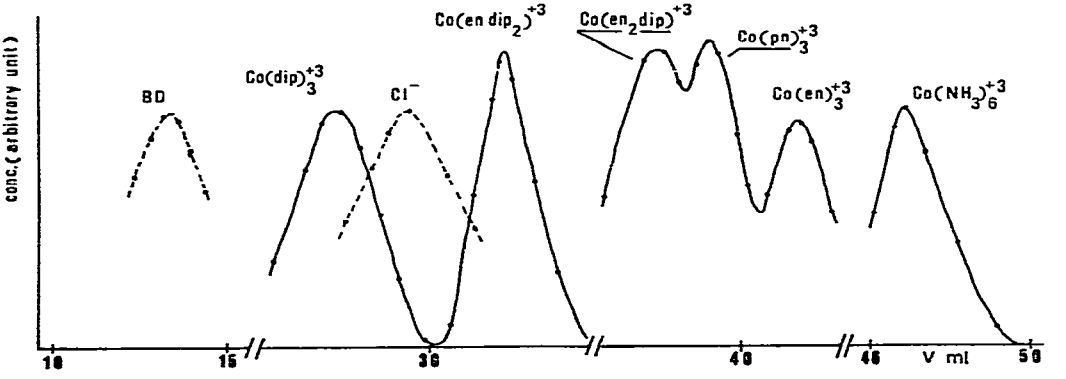

Fig. 11. Elution curve on Bio-Gel P-2 column. Column bed, 46 × 1.0 cm; eluent, 0.1 *N* NaCl; flow-rate, 22 ml/h; sample volume, 0.5 ml.

DISCUSSION

From our work on the electrophoresis of the cobalt(III) complexes examined here, we know that all complexes with three positive charges attract anions strongly and with several different distinct types of attraction (hydrophobic, electrostatic, hydrogen bonding). Hence in any case the species present in solution in electrolytes in the range 0.1–1.0 *N* are an agglomerate that is much larger than the actual complex.

The exclusion limits for "molecular weights" given by Pharmacia for various Sephadex gels are as follows: G-10, 100–400; G-15, 100–1500; G-25, 300–2000; and G-75, 5500–40,000. It is evident from our results that good separations are obtained on Sephadex G-10, G-15 and G-25. On Sephadex G-25 it is surprising that measurable differences exist, as it is just on the limit of the actual "ionic weights".

On the other hand, the results in sulphate medium (Fig. 6) show how ion pairing influences the gel chromatographic behaviour of these complexes. The complex $Co(o\text{-phen})_3^{3+}$ is anomalous in gel filtration as it moves more slowly than the smaller $Co(dip)_3^{3+}$. It is tempting to ascribe this to an adsorption effect on Sephadex, because in electrophoretic experiments $Co(o\text{-phen})_3^{3+}$ is very strongly ion paired with trichloroacetate and indeed in the presence of trichloroacetate it moves faster than in chloride [relative to $Co(dip)_3^{3+}$].

On Bio-Gel P-2 much better separations are obtained because not only the gel filtration effect but also ion exchange takes place. Bio-Gel P-2, being more hydrophobic than the Sephadex gels, adsorbs $Co(o\text{-phen})_3^{3+}$ more strongly than Sephadex.

In conclusion, gel filtration separations of cobalt(III) complexes are possible and, with some exceptions, follow the order of increasing size of the complexes. Ion pairing is as important in gel filtration as in other separations methods such as electrophoresis or ion exchange.

REFERENCE

1 M. Sinibaldi and M. Lederer, *J. Chromatogr.*, 107 (1975) 210.