

High Performance Liquid Chromatographic Separations of Some Bis(ethylenediamine)cobalt (III) Complexes

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The high performance liquid chromatographic separations of a variety of bis(ethylenediamine)cobalt (III) complexes $[\text{Co}(\text{en})_2\text{XY}]^n$ ($n=1-, 0, 1+$) have been carried out. Good separations are achieved on $\text{C}_{18}-\mu$ -Bondapak reversed phase octadecyldimethylsilane (ODS) column using tributylmethylammonium cation and octyl sulphonate anion as ion-pairing reagents. The efficiency and speed of the separation are superior to those obtained on a partisol SCX ion exchanged column.

High performance liquid chromatography (HPLC) has been largely employed for the separation of uncharged coordination complexes¹. The separation of some mono-^{2,3}, di-^{4,5} and tri-cationic⁴ complexes has also been reported. HPLC and reversed phase high performance liquid chromatography (RP-HPLC) have been extensively used for the separation of amino acids⁶ and low molecular weight peptides^{6,7}. We have presently employed RP-HPLC and HPIEC (high performance ion exchange chromatography) techniques for the separation of complexes of the type $[\text{Co}(\text{en})_2(\text{X})(\text{Y})]^n$ ($n=1-, 0$, or $1+$; en = ethylenediamine).

Perchloric acid (AR) was used to adjust the pH of the eluent to 5. Tributylmethylammonium bromide, lithium and sodium perchlorates and octyl sulphonate, all of GP grade were used as received. Separations were carried out on μ -Bondapak C_{18} reversed phase column ($25 \times 0.5 \text{ cm}^2$) of octadecyldimethylsilane (ODS) bonded on inert silica support which was originally end-capped with trimethylsilane. Some experiments were carried out using a partisol μ SCX ion exchange column.

The columns were initially washed for 15 min with the required eluent and allowed to equilibrate with the mobile phase. A sample of a standard complex solution was usually injected to check the column, detector and retention time. On completion of the run the column was equilibrated with the initial mobile phase.

The chromatograms were recorded using a Perkin-Elmer series 3B instrument at a flow rate of

$\text{cm}^3 \text{ min}^{-1}$. The sample (20μ litre; 10^{-3} M) were introduced through a Rheodyne valve and the peaks measured by UV spectroscopy at 254 nm using a Hewlett-Packard integrator. The eluents were made up in water or in methanol-water mixtures and filtered using $1.2 \mu\text{m}$ porosity filters (Millipore) before use.

The following bis(ethylenediamine)cobalt (III) complexes were prepared by standard procedures.

cis- $\text{Na} - [\text{Co}(\text{en})_2(\text{S}_2\text{O}_3)_2]$, *trans*- $\text{Na} - [\text{Co}(\text{en})_2(\text{S}_2\text{O}_3)_2]$, *cis*- $\text{Na}_2 - [\text{Co}(\text{en})_2(\text{SO}_3)_2]\text{ClO}_4 \cdot 3\text{H}_2\text{O}$, *trans*- $\text{Na}[\text{Co}(\text{en})_2(\text{SO}_3)_2]3\text{H}_2\text{O}$, *cis*- $[\text{Co}(\text{en})_2(\text{N}_3)]\text{NO}_3$, *cis*- $[\text{Co}(\text{en})_2\text{Cl}_2]\text{Cl} \cdot \text{H}_2\text{O}$ and *trans*- $\{\text{Co}(\text{en})_2\text{Cl}_2 = \text{Cl}\}$.

Solution of the complex $[\text{Co}(\text{en})_2(\text{S}_2\text{O}_3)(\text{OH}_2)]$ was prepared using Deutsch's method⁹, while solutions of $[\text{Co}(\text{en})_2(\text{S}_2\text{O}_3)(\text{N}_3)]$ and $[\text{Co}(\text{en})_2(\text{SO}_3)(\text{N}_3)]$ were prepared by allowing $[\text{Co}(\text{en})_2(\text{S}_2\text{O}_3)(\text{OH}_2)]$ or $[\text{Co}(\text{en})_2(\text{SO}_3)_2]$ to react with 0.1 M NaN_3 at 25°C for 24 hr. The purity of these solutions were established by HPLC, while satisfactory microanalyses and UV-vis spectra confirmed the purity of the solid compounds.

The octahedral complexes reported here exist as cations, anions and uncharged species in solution and are quite stable^{8,9}.

The complete separation using an ion exchange or ion-pair column would require one sample for the anion and a second for the cation. The positively charged complexes are paired with octyl sulphonate anion ($\text{C}_8\text{H}_{17}\text{SO}_3^-$), while the negatively charged complexes are paired with tributylmethylammonium cation (NMeBu_3^+) to provide suitable retention for separation on a C_{18} bonded (ODS) column. Some complexes have also been eluted with ClO_4^- anions on a partisol SCX ion exchange column. The data presented in Table 1 are representative of the performance of the columns in use. The results of separations of the complexes (a-g) of the type

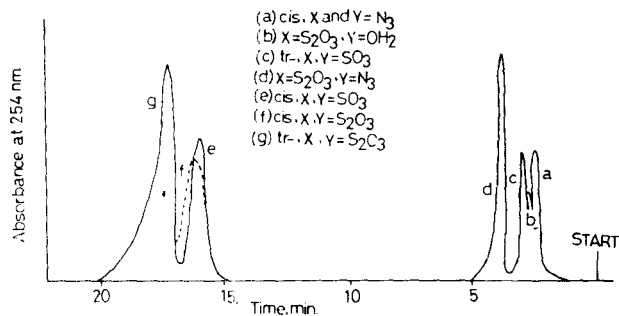


Fig. 1—Chromatogram of a mixture of $[\text{Co}(\text{en})_2\text{XY}]^n$ complexes on ODS column using $0.3\% \text{ NMeBu}_3^+$ in water as an eluent at a flow rate $1 \text{ cm}^3 \text{ min}^{-1}$ [chart speed 0.5 cm min^{-1}]

Table 1—Retention Volumes (cm^3) of Some Bis(ethylenediamine)cobalt (III) Complexes on ODS and SCX Columns

	using eluent ^(a)		SCX column using eluent ^b	
	0.3% NMeBu ⁺	0.2% C ₈ H ₁₇ SO ₃ ⁻ in 10% MeOH	0.1M ClO ₄ ⁻	0.2M ClO ₄ ⁻
<i>trans</i> -[Co(en) ₂ (S ₂ O ₃) ₂] ⁻ (g)	17.4	2.8	3.6	3.7
<i>cis</i> -[Co(en) ₂ (S ₂ O ₃) ₂] ⁻ (f)	16.4	—	3.4	3.5
<i>cis</i> -[Co(en) ₂ (SO ₃) ₂] ⁻ (e)	16.2	—	—	—
[Co(en) ₂ (S ₂ O ₃)(N ₃) ⁰ (d)	4	4.4	4.7	4.5
<i>trans</i> -[Co(en) ₂ (SO ₃) ₂] ⁻ (c)	3.0	—	—	—
[Co(en) ₂ (S ₂ O ₃)(OH ₂) ⁺ (b)	2.8	18	10.7	7.4
<i>cis</i> -[Co(en) ₂ (N ₃) ₂] ⁺ (a)	2.6	48	9.9	7.3
<i>trans</i> -[Co(en) ₂ Cl ₂] ⁺	—	60.4	—	—
<i>cis</i> -[Co(en) ₂ Cl ₂] ⁺	—	42.2	—	—
<i>cis</i> -[Co(en) ₂ (SO ₃)(N ₃) ⁰	—	5.6	—	—
		4.6		

(a) Chart speed 0.5 cm min⁻¹.(b) Chart speed 1 cm min⁻¹

[Co(en)₂XY]ⁿ using 0.3% tributylmethylammonium cation and reverse phase ODS column show a clear separation (Fig. 1). The retention times follow the order **a** < **b** < **c** < **d** < **e** < **f** < **g** (Fig. 1). This suggests that polarizability factors including interactions of the negatively charged complexes with the ion-pairing reagent and the stationary phase are important. The extent of separation of complex (e) from (f) seems small. Both are *cis*-complexes with a single negative charge. The separation of (f) from (g) is quite reasonable. The cation complexes (a, b and d) appear at a retention volume of about 3 cm³, a volume slightly exceeding the void volume (2 cm³). The unexpected elution order of complex (c) may be due to the rapid hydrolysis into [Co(en)₂(SO₃)(OH₂)⁺. The bonded hydrocarbon surface layer and the eluent electrolyte have the effect of reducing the column's capacity for uncharged hydrophilic complexes. This leads to the earlier separation of uncharged hydrophilic complexes as shown in Fig. 1.

The results of separation of the complexes using ODS column and octyl sulphonate in 10% MeOH as eluent are also given in Table 1. Each complex is separated from the other, the order being that expected for increasing interaction with the stationary phase.

The positively charged molecules are thought to form neutral ion-pairs with one octyl sulphonate counterion present in the mobile phase. The hydrophobic interaction of the hydrocarbon chain of this non-pair with the bonded octadecyl hydrocarbon surface of the stationary phase provides the basis for separation. Alternatively, the octyl sulphonate

additives might initially adsorb onto hydrophobic surface of the substrate to change it into a sulphonate-based ion exchange column¹⁰.

In an attempt to reduce the retention time of the cationic complexes, the percentage of MeOH in octyl sulphonate was raised to 30% instead of 10%, but this tended to reduce the differences between the complexes rendering the separation difficult.

Using a partisol high performance ion exchange (HPIEC) column and aqueous ClO₄⁻ anion gave, in general, poor results. Elution times were shorter than those obtained by RP-HPLC, peaks were broad and the separations were poor. The elution order as expected from cation exchanger column (Table 1) is the same as that observed using ODS column. On increasing the anion concentration, i.e. ClO₄⁻ concentration from 0.1 to 0.2 M (Table 1) the negatively charged complexes *cis*-[Co(en)₂(S₂O₃)₂]⁻ and *trans*-[Co(en)₂(S₂O₃)₂]⁻ eluted with a little bit longer retention times. The uncharged complex [Co(en)₂(S₂O₃)(N₃)⁰ eluted with shorter retention time. The discrimination amongst the complexes *cis*- and *trans*-[Co(en)₂(S₂O₃)₂]⁻ and [Co(en)₂(S₂O₃)(N₃)⁰ is not as good. The positively charged complexes *cis*-[Co(en)₂(N₃)₂]⁺ and [Co(en)₂(S₂O₃)(OH₂)⁺ have lesser retention times in 0.2 M ClO₄⁻ than those observed with 0.1 M ClO₄⁻ and the peaks overlapped. This phenomenon has been noted before¹¹, and has been attributed to interactions between the different complexes. The reagent and the stationary phase. Finally, the result indicated that RP-HPLC has considerable potential for the analysis of

investigated complexes. The method is characterized by reasonable separations with excellent peak shapes and high column efficiencies.

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