# Analysis of Cobalt Complexes by High-performance Liquid Chromatography using Ion-pair Reversed-phase, Silica, or Alumina Columns

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High-performance liquid chromatography on unmodified silica or alumina columns either separately or in series has been compared with the ion-pair reversed-phase method for the analysis of pentacyanocobaltate(III) and bis(ethylenediamine)thiosulphatocobalt(III) complexes.

Analysis during the reaction of metal complexes is usually performed by spectroscopic techniques but, if the compounds have half-lives of the order of $10^2$ min, high-performance liquid chromatography (h.p.l.c.) is a far superior method. Nucleophilic competition in substitution reactions of cobalt(III) complexes is a particularly sensitive test for reaction mechanism [1] but it depends on the availability of rapid and quantitative analyses of mixtures of compounds differing by only one ligand. Our present studies involve two groups of complexes, $[Co(CN)_5X]^{x-}$ ($x = 3$ or 2) and $[Co(en)_2(S_2O_3)X]^y$ ($y = 1-$, 0 or $1+$; en = ethylenediamine), both of which may be analysed by h.p.l.c. The analyses exemplify the usefulness of liquid–solid h.p.l.c. compared to the reversed-phase method and the value of combined alumina–silica columns for the simultaneous analysis of cationic, anionic and neutral species.

## Experimental

*Reagents.*—G.P. grade tributylmethylammonium bromide, sodium perchlorate, azide, thiocyanate, propanesulphonate, and acetate were used as received. The remaining sodium salts and perchloric acid were Analar grade.

The salt *trans*-$Na[Co(en)_2(S_2O_3)_2]$ was prepared by Deutsch's method.[2] The complexes *trans*-$[Co(en)_2(S_2O_3)X]$, X = $NCS^-$, $N_3^-$, $CH_3CO_2^-$, or $NO_2^-$, were prepared without isolation from 0.01 mol dm$^{-3}$ $Na[Co(en)_2(S_2O_3)_2]$ by reaction in 0.1—1 mol dm$^{-3}$ solutions of the appropriate anion in water at 25 °C for 24 h; $K_3[Co(CN)_5X]$, X = $N_3^-$ or $Cl^-$, were prepared by the cyanide substitution of corresponding penta-ammines.[3]

Satisfactory microanalyses and u.v.–visible spectra confirmed the purity of the solid compounds, while h.p.l.c. analysis was used to establish the purity of the compounds prepared in solution, $[Co(en)_2(S_2O_3)X]$.

*Apparatus.*—H.p.l.c. columns, 25 × 0.45 cm, were packed with Spherisorb 10-µm alumina, Partisil 10-µm silica, or Partisil 5-µm ODS (a bonded 5% $C_{18}$-type reversed phase). The chromatograms were recorded using both a Perkin-Elmer Series 3B and also a Pye-Unicam LC3 instrument with flow rates of *ca.* 1 cm$^3$ min$^{-1}$ (reversed phase), 2 cm$^3$ min$^{-1}$ (alumina–silica combination column) and 4 cm$^3$ min$^{-1}$ (alumina column). The samples, of volume 20 µl and concentration $10^{-3}$ mol dm$^{-3}$, were introduced by a Rheodyne valve and the peaks measured by u.v. spectroscopy at 254 or 289 nm {for $[Co(en)_2(S_2O_3)X]$}, using either a Hewlett-Packard 3380 or a Pye-Unicam CDPl integrator. The eluants were made up in methanol–water mixtures, adjusted to pH 5 with perchloric acid, and filtered with Millipore 1.2-µm porosity filters before use.

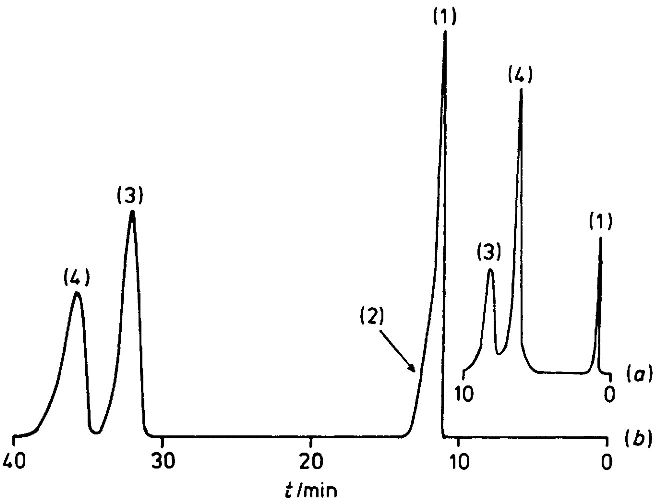

**Figure 1.** Chromatograms of $[CO(CN)_5X]^{x-}$ obtained on (*a*) alumina (flow rate 4 cm$^3$ min$^{-1}$, eluant 0.25 mol dm$^{-3}$ aqueous $Na[ClO_4]$, detection at 254 nm), and (*b*) reversed phase (flow rate 0.70 cm$^3$ min$^{-1}$, eluant 45% methanol–water + 0.017 mol dm$^{-3}$ $[NMeBu_3]Br$, detection at 254 nm). Peaks: (1) $[Co(CN)_5(OH_2)]^{2-}$ [with side-band (2) due to polymeric cyano-complex in chromatogram (*b*)]; (3) $[Co(CN)_5Cl]^{3-}$; (4) $[Co(CN)_5(N_3)]^{3-}$

## Results

$[Co(CN)_5X]^{x-}$.—Although silica-bonded anion-exchange columns did not apparently resolve this family of complexes, separations could be achieved using tributylmethylammonium bromide as ion-pairing agent [Figure 1(*a*)]. The same analysis could also be performed using an alumina column [Figure 1(*b*)]. Although the retention volumes are slightly greater in the latter case the very low pressure drop of the alumina column allowed a flow rate of 4 cm$^3$ min$^{-1}$ so that the analysis was complete in 10 min compared to 40 min using the reversed-phase column.

$[Co(en)_2(S_2O_3)X]^y$.—In this case the reactant under examination, $[Co(en)_2(S_2O_3)_2]^-$, produced cationic and uncharged products so that a complete analysis using an ion-exchange or ion-pair column would require one sample for the anion and a second for the cation separations. However, although the alumina column preferentially retains anions and the silica column cations, they have the great advantage that they may be coupled in series and used to give a complete analysis for

**Table.** Retention volumes [a] of *trans*-[$Co(en)_2(S_2O_3)X$] and $NO_2^-$ on alumina–silica combined and reversed-phase anion-pairing columns [b]

| Analysis | Solvent (% MeOH) | Electrolyte | Retention volume/cm$^3$ X = NCS | $N_3$ | $CH_3CO_2$ | $NO_2$ | $NO_2^-$ | Column |
|---|---|---|---|---|---|---|---|---|
| 1 | 99 | 0.01 mol dm$^{-3}$ Na[$C_3H_7SO_3$] | 11.2 | — | — | 15.0 | 24.9 | *c* |
| 2 | 90 | 0.01 mol dm$^{-3}$ Na[$C_3H_7SO_3$] | 8.5 | — | — | 10.3 | 17.1 | *c* |
| 3 | 75 | 0.01 mol dm$^{-3}$ Na[$C_3H_7SO_3$] | 7.7 | — | — | 8.6 | 13.7 | *c* |
| 4 | 0 | 0.04 mol dm$^{-3}$ Na[$C_3H_7SO_3$] | 6.9 | — | — | 7.4 | 10.5 | *c* |
| 5 | 90 | 0.04 mol dm$^{-3}$ Na[$ClO_4$] | 9.1 | — | — | 11.4 | 21.1 | *c* |
| 6 | 90 | 0.01 mol dm$^{-3}$ Na[$ClO_4$] | 10.4 | — | — | 14.4 | 30.1 | *c* |
| 7 | 90 | 0.04 mol dm$^{-3}$ Na[$ClO_4$] | 13.4 | 18.2 | 19.0 | 23.3 | >30 | *d* |
| 8 | 0 | 0.01 mol dm$^{-3}$ [$NMeBu_3$]Br | 4.7 | 4.2 | 5.5 | 4.3 | 9.0 | *e* |
| 9 | 30 | 0.01 mol dm$^{-3}$ [$NMeBu_3$]Br | 3.3 | 3.6 | 4.2 | 3.8 | 5.4 | *e* |

[a] Relative and absolute retention volumes vary during use and are unreliable for qualitative analysis. [b] Alumina–silica column: efficiency *ca.* 500 plates (before activation), *ca.* 340 plates (after activation). Reversed-phase column: efficiency *ca.* 1 000 plates. Void volumes: 4.5 cm$^3$ (alumina–silica), 2.0 cm$^3$ (reversed phase). Efficiencies fall as the columns age. [c] Alumina–silica. [d] Activated alumina–silica. [e] Reversed phase.

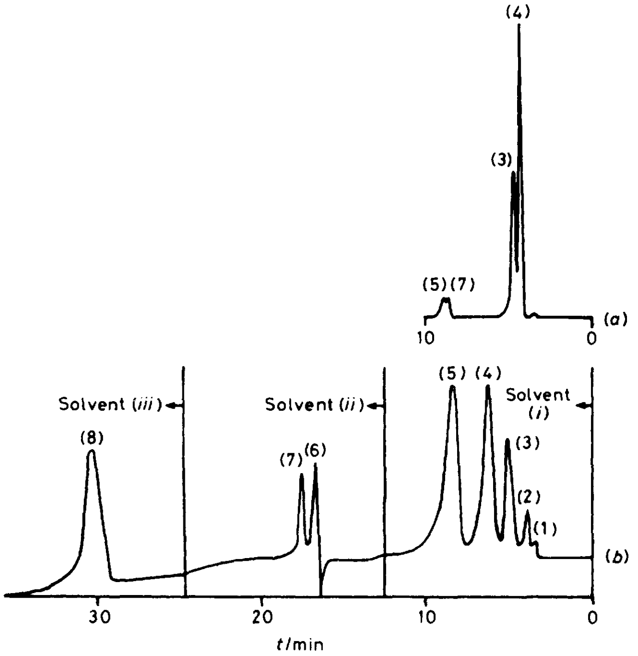

Figure 2. Chromatograms of [$Co(en)_2(S_2O_3)X$]$^y$ obtained on (*a*) reversed phase {flow rate 1 cm$^3$ min$^{-1}$, eluant 0.01 mol dm$^{-3}$ [$NMeBu_3$]Br, detection at 289 nm; peak (8) is not detectable at this wavelength} and (*b*) alumina–silica {eluant 0.04 mol dm$^{-3}$ Na[$ClO_4$] in (*i*) 90% methanol–water, flow rate 1.87 cm$^3$ min$^{-1}$, (*ii*) 50% methanol–water, flow rate 1.37 cm$^3$ min$^{-1}$, and (*iii*) water, flow rate 1.46 cm$^3$ min$^{-1}$, detection at 254 nm]. Peaks: (1) and (2), unidentified; (3) [$Co(en)_2(S_2O_3)(NCS)$]; (4) [$Co(en)_2(S_2O_3)(NO_2)$]; (5) $NO_2^-$; (6) solvent front; (7) [$Co(en)_2(S_2O_3)_2$]$^-$; (8) $NCS^-$

a single sample. Furthermore, their combined resolution * can, as in this case, prove superior to the reversed-phase system.

In Figure 2 the best result for the reversed-phase column, chromatogram (*a*), is compared to that for the alumina–silica combination, chromatogram (*b*), for a reaction mixture (0.01 mol dm$^{-3}$ complex) containing 0.5 mol dm$^{-3}$ nitrite and 0.5 mol dm$^{-3}$ thiocyanate anions diluted ten-fold before injection. Cation pairing by alkanesulphonates was abandoned in this application since the anion [$Co(en)_2(S_2O_3)_2$]$^-$ obscured the uncharged complexes and the latter were insufficiently resolved. The alumina–silica column requires a solvent program to cover the whole range and the analysis takes longer to complete, but it does separate the positive, negative, and uncharged species. In practice in this instance the ion-pair system was preferable since it yielded accurate relative concentrations for the reactant and uncharged products in less than 10 min. The cation products appear at about 3.5 cm$^3$, a volume slightly exceeding the void volume, 2 cm$^3$, and the nitrite peak, eluted close to [$Co(en)_2(S_2O_3)_2$]$^-$, can be allowed for by a small blank correction. The cation products were eluted in much larger volumes from the alumina–silica column, appearing in the second and third solvent stages [Figure 2(*b*)].

The most striking difference between the two systems is that the reversed-phase retention volumes decrease roughly in proportion to the percentage of methanol in the eluant whereas the liquid–solid retentions increase non-linearly (Table, analyses 1—3). Changes in solvent and electrolyte produced similar effects in charged and uncharged species {Table, $NO_2^-$ compared to [$Co(en)_2(S_2O_3)X$]}.

The alumina–silica column combination could be activated by washing with about 0.5 l of carbonate buffer (pH = 10) followed by the same volume of an aqueous acetate buffer (Table, analysis 7 after activation compared to 5 before). After activation the improved capacity and separation allowed the resolution of all except [$Co(en)_2(S_2O_3)(N_3)$] and [$Co(en)_2(S_2O_3)(CH_3CO_2)$]. Fortunately, the reversed-phase system could resolve this combination although it failed for [$Co(en)_2(S_2O_3)(NCS)$] and [$Co(en)_2(S_2O_3)(N_3)$].

## Discussion

The principal methods applied to the analysis of ionic mixtures by h.p.l.c. include liquid–solid, reversed phase, and ion exchange, but recently a group of papers [5—7] in this area have highlighted the efficiency of the reversed-phase method using alkane- or arene-sulphonates as ion-pairing agents for the resolution of cations. Although such methods can be very useful, the columns and reagents are relatively expensive and are not always as efficient as the cheaper liquid–solid technique. The two analytical problems described in this work show that there is often a considerable advantage to be derived from a general comparison of the performance of the two methods. The selectivity of an alumina or silica column can be extremely large so that the frequently poor column efficiency is unimportant. In addition the resistance to flow is low [28 bar (2.8 × 10$^6$ Pa) per cm$^3$ min$^{-1}$ flow for the alumina column used here], so that columns of about 1 m in length are practicable even with relatively cheap equipment. The re-

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* Resolution allows for column efficiency, *N*, separation factor, α, and the capacity factor, *k*, in assessing a column's performance.[4]

versed-phase system gives satisfactory results by virtue of its superior column efficiency which in fact often exceeds that obtained in the present examples. Its advantage is usually the speed of the analysis.

The bonded hydrocarbon surface layer and the eluant electrolyte has the effect of reducing the columns' capacity for uncharged hydrophilic complexes. This leads to the relatively poor separation shown in Figure 2(*a*). In order to improve the retention of the anionic complexes a relatively long-chain tertiary ammonium cation is necessary but this tends to dwarf the differences between the complexes so that the separation declines. The description of such systems as 'ion pairing' can hardly be strictly accurate since the uncharged, although doubtless polar, complexes are also retained to a small extent and their retention volume increases as the water content of the eluant rises (Table, analyses 8 and 9). This means that an uncharged hydrophilic species is now behaving as if it were hydrophobic, presumably because of association with the tertiary ammonium ion.

In summary, this work has illustrated that in any project requiring rapid quantitative analyses of multi-component ionic and uncharged complexes it is always worth comparing the performance of the liquid–solid and the liquid–reversed-phase systems both alone and in combination with one another.

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## References

1 M. H. M. Abou-El-Wafa and M. G. Burnett, *J. Chem. Soc., Chem. Commun.*, 1983, 833.
2 J. N. Cooper, J. D. McCoy, M. G. Katz, and E. Deutsch, *Inorg. Chem.*, 1980, **19**, 2265.
3 P. Barca, J. Ellis, M-S. Tsao, and W. K. Wilmarth, *Inorg. Chem.*, 1967, **6**, 243.
4 C. F. Simpson, 'Practical High Performance Liquid Chromatography,' Heyden and Son Ltd., London, 1979, p. 12.
5 D. A. Buckingham, R. A. Clark, M. M. Deva, and R. F. Tasker, *Inorg. Chem.*, 1983, **22**, 2754.
6 D. A. Buckingham, C. R. Clark, and R. F. Tasker, *Inorg. Chem.*, 1983, **22**, 2722.
7 D. A. Buckingham, C. R. Clark, M. M. Deva, and R. F. Tasker, *J. Chromatogr.*, 1983, **262**, 219.

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