

Note

High-performance liquid chromatographic separation of mixtures of cobalt(III) complexes

G. GRASSINI STRAZZA and C. M. POLCARO

CNR, Laboratorio di Cromatografia, Via Romagnosi 18A, Rome (Italy)

(Received June 17th, 1977)

High-performance liquid chromatography (HPLC) has been applied to the separation of inorganic compounds, mainly complexes, by a number of workers¹. In most of this work there does not seem to be an obvious advantage in a fast separation except in the separation of radioisotopes.

This paper reports the use of HPLC in the identification of relatively labile cobalt(III) complexes formed in solid-state reactions, in particular for two examples where the labile species were separated and identified by their spectra measured during the elution.

EXPERIMENTAL

Preparation of complexes

The complexes were prepared by methods which have been reported in the literature: the *trans* and *cis* forms of $[\text{Co}(\text{en})_2\text{Cl}_2]^+$ as the chloride and nitrate salts according to Bailar³ and Yamada and Tsuchida⁴, respectively; the two isomeric forms of $[\text{Co}(\text{en})_2(\text{OCOC}_6\text{H}_5)_2]^+$ as the nitrate salts, according to Aprile *et al.*⁵; *trans*- $[\text{Co}(\text{en})_2(\text{OCOC}_6\text{H}_5)\text{Cl}]^+$, as the perchlorate salt, after Dasgupta *et al.*⁶; $[\text{Co}(\text{en})_2\text{CO}_3]^+$ as the chloride salt, by the method outlined for the corresponding perchlorate⁷, substituting *trans*-dichlorobis(ethylenediamine) chloride in the appropriate step of the synthesis.

Eluents

All solvents were ERBA RP, except 2-propanol which was from Merck (Darmstadt, G.F.R.). The two eluents were (A) 95% ethanol-2-propanol-25% aqueous ammonium acetate-1 N acetic acid in methanol (140:60:2.5:0.15) for the separation of the benzoato complexes and (B) 95% ethanol-methanol-25% aqueous ammonium acetate-1 N acetic acid in methanol (60:140:2.5:0.15) for the separation of the carbonato complexes. The eluents were degassed by stirring with a stream of nitrogen for 15 min and heating to 30-35°.

Solutions of samples

Isothermal reactions were carried out as previously described⁸, using a mixture of the complex (between 0.04 and 0.1 g, accurately weighed) and ammonium chloride (1:80 molar ratio in the case of the benzoato complex and 1:5 in the case of the

carbonato complex). The first mixture was heated at 130° for 10 min and, after cooling, was dissolved in acetone. After filtration, the solution was evaporated to dryness at room temperature by passing a stream of nitrogen and then redissolved in methanol-water (9:1). The mixture of carbonato complexes was heated at 165° for 90 min and, after cooling, dissolved in water.

All preliminary experiments were carried out with two standard mixtures (*cis*-[Co(en)₂(OCOC₆H₅)₂]NO₃, *trans*-[Co(en)₂(OCOC₆H₅)₂]NO₃ and *trans*-[Co(en)₂(OCOC₆H₅)Cl]ClO₄; [Co(en)₂CO₃]NO₃, *cis*-[Co(en)₂Cl₂]NO₃ and *trans*-[Co(en)₂Cl₂]NO₃, with a concentration of *ca.* 1 mg/μl for each benzoato complex and 1 mg/10 μl for the carbonato complexes.

HPLC procedure

A stop-flow injection of 50 μl of the above solutions of standard benzoato complexes or of the heated mixture (15 μl for the carbonato complexes), was performed with a Supelco (Bellefonte, Pa., U.S.A.) pressure-lock liquid syringe (Series B-110; 50 μl). Chromatography was performed at ambient temperature on a Micropak-Si 10 μm column (50 cm × 2 mm I.D.) (Varian Aerograph, Walnut Creek, Calif., U.S.A.).

The eluent flow-rate was 1 ml/min, at pressures of 5500 p.s.i. for eluent A and 2500 p.s.i. for eluent B, generated by a Model 6000 solvent delivery system (Waters Assoc., Milford, Mass., U.S.A.). The column was regenerated by washing with methanol. A continuously variable wavelength Aerograph Variscan LC detector (Varian), monitoring at 580 nm for the benzoato complexes and at 340 nm for carbonato complexes, was used.

RESULTS AND DISCUSSION

In earlier work^{8,9} the isothermal reactions between [Co(en)₂(OCOC₆H₅)₂]NO₃ or [Co(en)₂CO₃]Cl and ammonium chloride were studied by reflectance spectroscopy and thin-layer chromatography (TLC). The final product of both reactions was *trans*-[Co(en)₂Cl₂]Y (Y = NO₃⁻ or Cl⁻) but the most interesting result was the chromatographic evidence for a stepwise reaction, with the formation of intermediate species. TLC was performed at -25° in the case of benzoato complexes to minimize reactions during development⁸.

During the reaction of *cis*-[Co(en)₂(OCOC₆H₅)₂]NO₃ with ammonium chloride, it was found that excess ammonium chloride interferes both in HPLC and in TLC. This could be avoided by dissolving the mixture in acetone, which dissolves ammonium chloride only. Also, using acetone, the final product, *i.e.* *trans*-[Co(en)₂Cl₂]⁺ is insoluble, but the intermediate products and the starting compound go readily into solution. The eluent employed in TLC, 95% ethanol-methanol-25% aqueous ammonium acetate-1 *N* acetic acid in methanol (60:140:5:0.3) gave only partial separation in HPLC. Complete separation (Fig. 1) was achieved by eluent A, which has lower ionic strength and polarity than the first eluent. The elution sequence is the same as in TLC: *trans*-dibenzoato, intermediate species, *cis*-dibenzoato. No hydrolysis and/or isomerization of the products seems to occur during chromatography. The spectrum of the intermediate peak (Fig. 2) shows the characteristic shoulder¹⁰ at 450 nm of the *trans*-isomers of the series

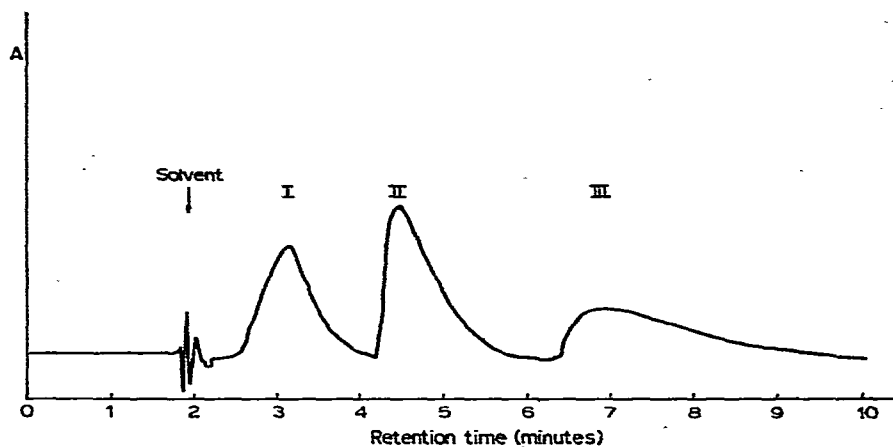


Fig. 1. Chromatogram of a mixture of *cis*-[Co(en)₂(OCOC₆H₅)₂]NO₃ and NH₄Cl (1:80 molar ratio) after heating at 130° for 10 min. Injection: 50 μl of a 1.3 M solution. Column: (50 × 0.2 cm I.D.) Micropak-Si; particle size: 10 μm, Mobile phase (A) 95% ethanol-2-propanol-25% aqueous ammonium acetate-1 N acetic acid in methanol (140:60:2.5:0.15). Flow-rate: 1 ml/min. Pressure: 5500 p.s.i. λ = 580 nm. I = *trans*-[Co(en)₂(OCOC₆H₅)₂]⁺; II = intermediate species; III = *cis*-[Co(en)₂(OCOC₆H₅)₂]⁺.

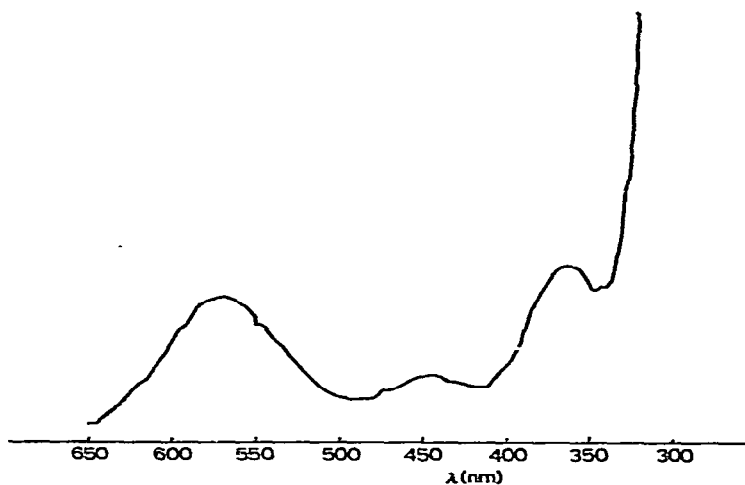


Fig. 2. Directly scanned spectrum of the second peak of the chromatogram in Fig. 1.

[M(AA)₂B₂]⁺ or [M(AA)₂BC]⁺ and a maximum around 570 nm. This spectrum could be that of *trans*-[Co(en)₂(OCOC₆H₅)Cl]⁺ (ref. 6). Further evidence for this assignment was obtained from the *R_F* value in TLC compared with that of a sample of *trans*-chlorobenzoate prepared as detailed in ref. 6.

Following the reaction of [Co(en)₂CO₃]Cl with ammonium chloride, three TLC spots were obtained with formamide-methanol-water-70% HClO₄ (40:60:2:0.3), with another faint spot of cobalt(II) at the point of application. This solvent was

unsatisfactory for HPLC as it eluted the various complexes too fast. After a few preliminary experiments, eluent B was found to give a good elution, as shown in Fig. 3. The elution sequence was again the same as in TLC: *trans*-[Co(en)₂Cl₂]⁺, intermediate species, [Co(en)₂CO₃]⁺.

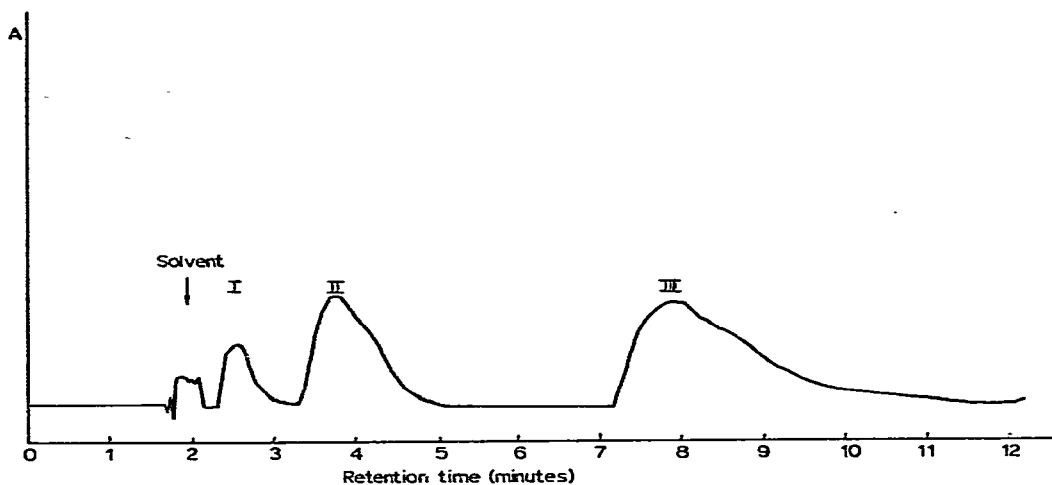


Fig. 3. Chromatogram of a mixture of [Co(en)₂CO₃]Cl and NH₄Cl (1:5 molar ratio) after heating at 165° for 90 min. Injection: 15 μl of a 0.4 M solution. Column: as in Fig. 1. Mobile phase (B) 95% ethanol-methanol-25% aqueous ammonium acetate-1 N acetic acid in methanol (60:140:2.5:0.15). Flow-rate: 1 ml/min. Pressure: 2500 p.s.i. λ = 340 nm. I = *trans*-[Co(en)₂Cl₂]⁺; II = intermediate species; III = [Co(en)₂CO₃]⁺.

The spectrum of the second peak scanned during the elution is shown in Fig. 4, and it corresponds¹¹ to that of the *cis*-[Co(en)₂Cl₂]⁺. From these results it seems that the reaction is:

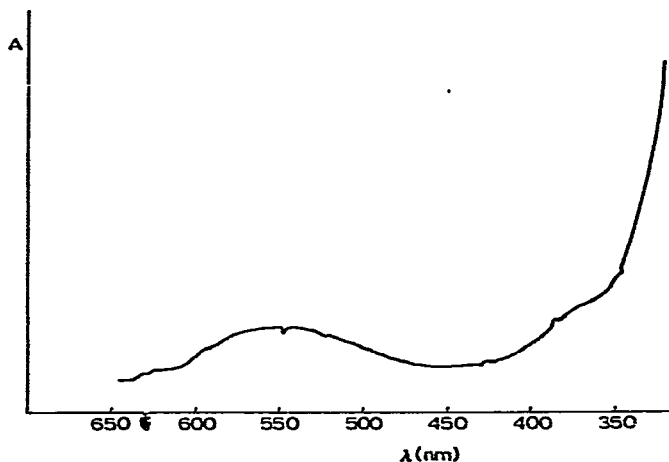


Fig. 4. Directly scanned spectrum of the second peak of the chromatogram in Fig. 3.

HPLC has the advantage in the study of reactions which yield a labile intermediate that separation can be carried out in a few minutes instead of *ca.* 30 min for thin layers. What is more important, however, is that the spectra can be scanned directly without any transfer from the layer to a new solvent.

REFERENCES

- 1 J. F. K. Huber, J. C. Kraak and H. Veenig, *Anal. Chem.*, 44 (1972) 1554; J. S. Fritz and J. N. Story, *Anal. Chem.*, 46 (1974) 25; P. C. Uden and F. H. Walters, *Anal. Chim. Acta*, 79 (1975) 175; E. Gaetani, C. F. Laureri, A. Mangia and G. Parolari, *Anal. Chem.*, 48 (1976) 1725.
- 2 E. P. Horwitz and C. A. A. Bloomquist, *J. Inorg. Nucl. Chem.*, 35 (1973) 271; *J. Chromatogr. Sci.*, 12 (1974) 200; D. O. Campbell, *J. Inorg. Nucl. Chem.*, 35 (1973) 3911.
- 3 J. C. Bailar, *Inorg. Synthesis*, 2 (1946) 222.
- 4 S. Yamada and R. Tsuchida, *Bull. Chem. Soc. Jap.*, 25 (1952) 127.
- 5 F. Aprile, V. Caglioti and G. Illuminati, *J. Inorg. Nucl. Chem.*, 21 (1961) 300.
- 6 T. P. Dasgupta, W. Fitzgerald and M. L. Tobe, *Inorg. Chem.*, 11 (1972) 2046.
- 7 M. Linhard and G. Stirn, *Z. Anorg. Allg. Chem.*, 268 (1952) 105.
- 8 V. Carunchio, G. Grassini Strazza, A. Messina and C. M. Polcaro, *Thermochim. Acta*, 19 (1977) 85.
- 9 V. Carunchio, G. Grassini Strazza, A. Messina and C. M. Polcaro, in press.
- 10 L. E. Orgel, *J. Chem. Phys.*, 23 (1955) 1004.
- 11 F. Basolo, C. J. Ballhausen and J. Bjerrum, *Acta Chem. Scand.*, 9 (1955) 810.