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Note

Separation and analysis of 2-(5-cyanotetrazolato)pentaamminecobalt(III) and related cobalt(III) complexes by capillary tube isotachopheresis

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The perchlorate salt of 2-(5-cyanotetrazolato)pentaamminecobalt(III), designated CP, is a relatively new explosive which is much less sensitive to accidental initiation than other primary explosives such as lead azide, yet it can rapidly grow to detonation when properly confined¹. The main impurities in CP explosive after it is synthesized are (5-carbamoyltetrazolato)pentaamminecobalt(III) perchlorate and (5-amidinotetrazolato)tetraamminecobalt(III) perchlorate (Fig. 1). Many common separation and analysis approaches, *i.e.*, gas chromatography (GC), electron ionization and chemical ionization mass spectrometry (EI-MS, CI-MS) and GC-MS, are not feasible due to the very nature of these species being ions of unprecedented polarity and negligible vapor pressure. Analysis approaches based on thin-layer chromatography (TLC) have met with some success, but complete resolution of the complexes was not achieved². Quantitative estimation of the amide by IR measurements at 1675 cm^{-1} are complicated/hindered by the presence of a broad band in the same region arising from a deformation vibration of the NH_3 ligands^{2,3}. Modern liquid chromatographic approaches which are currently being explored are hindered by the limited solubility, and great differences in concentration are a source of problems due to limitations imposed by sample capacity of ion-exchange resins. Furthermore, a reproducible high-performance liquid chromatographic (HPLC) method suitable for routine analysis has yet to be found⁴.

Since most of the above procedures have not proven to date to be as straightforward or fruitful as desired, we investigated the possibility of using isotachopheresis (ITP) for the analysis of CP. The ITP technique has been described previously^{5,6} and recently in extensive reviews^{7,8}.

EXPERIMENTAL

The LKB 2127 Tachophor® isotachoelectrophoresis equipment was used to separate cations in a PTFE capillary tube, length 61 cm, 0.5 mm I.D., thermostated at 20°C. The leading electrolyte was 10 mM K^+ with $\approx 10\text{ mM}$ acetate as counter ion at pH 5.5. The terminating electrolyte was 10 mM Tris. Separations required about 20 min at a current of 150 μA . Separated zones were detected by their UV absorption at 254 nm. A recorder-paper speed of at least 10 cm/min was required for accurate measurement of the records.

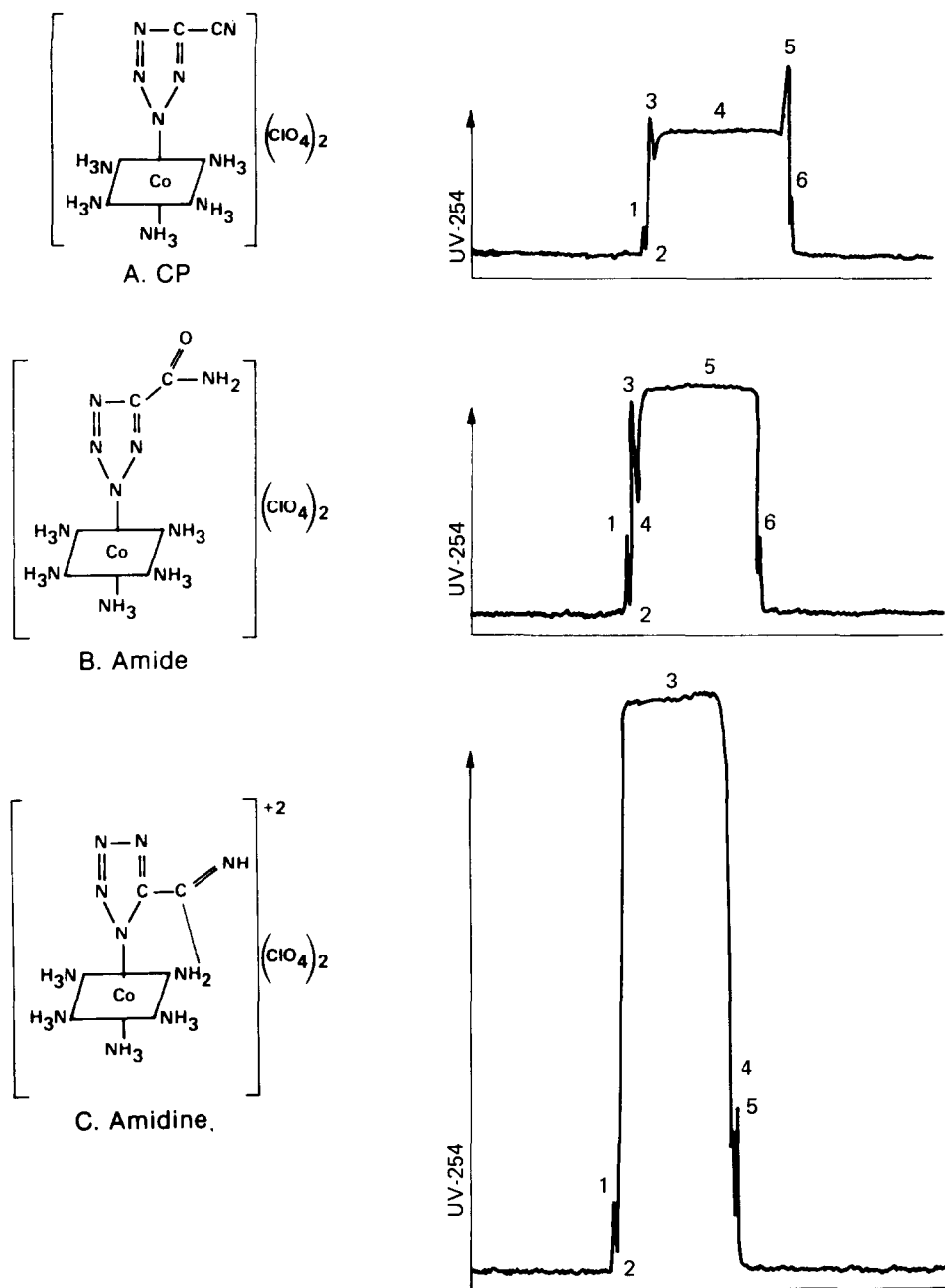


Fig. 1. Isotachopherograms resulting from injections ($3 \mu\text{l}$) of 7.5 nanomoles of CP/EL58633 (A), amide complex/EL-43182 (B) and amidine complex/EL52685 (C). Zones identified: 1 = electrolyte contaminant; 2 = non-UV-absorbing unknown; 3 = amidine; 4 = CP; 5 = amide; 6 = UV-absorbing unknown.

RESULTS AND DISCUSSION

Isotachopherograms resulting from separate injections with samples (3 μ l injection) of CP, amide and amidine containing about 7.5 nmole each are shown in Fig. 1. The assignment of contaminants will be discussed below. The absorbance observed at 254 nm for each major zone was consistent with extinction coefficients obtained from the UV spectra of authentic standards. In the instance of UV absorbing species, the area under the peak records, after transform from a transmittance scale to an absorbance scale, is linearly related to the amount of material. A typical calibration curve over the 0.1–2.0 nmole concentration range of CP is depicted in Fig. 2. These data clearly demonstrate the direct proportionality of zone areas to CP concentration which is essentially linear over the concentration range studied.

In Fig. 3 the separation of an equal molar mixture of CP and its two major contaminants is shown. The resolution of the cations appears satisfactory. Three methods were employed to investigate the purity and identity of the indicated zones. (1) Molar extinction coefficients obtained from the UV-visible spectra of authentic standards and absorbance ratios of respective ITP zones were compared at both 254 and 280 nm in Table I. It should be noted that zone concentration cannot be determined directly, therefore, only observed absorbance ratios under identical conditions are compared to extinction data. (2) Thermal step heights, a relative index of

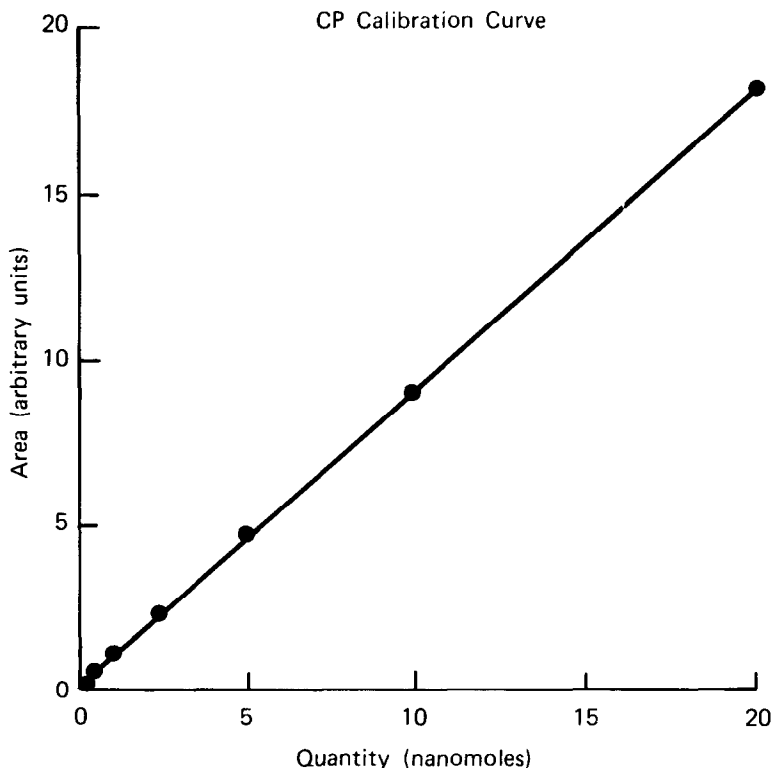


Fig. 2. The direct proportionality of zone areas to concentration, which was essentially linear over the concentration range studied, is shown by a typical calibration curve.

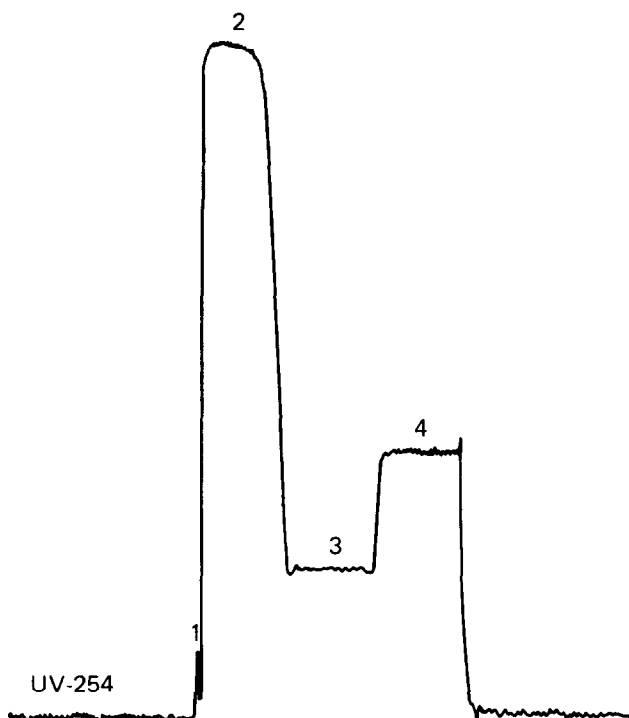


Fig. 3. Isotachopherogram of an equal molar mixture of amidine (2), CP (3) and amide (4). Zone 1 is due to an electrolyte contaminant.

the mobility for each species producing a zone, were compared to step heights obtained from analysis of purified standards under identical conditions. These relative step heights are somewhat analogous to relative retention indexes employed for similar purposes in chromatographic analytical procedures. Table II lists the values observed under the condition of these experiments. Values have been normalized relative to potassium where the step height of sodium is 100 arbitrary units. Under our experimental conditions a minimum of 10 units difference in step height was required for complete separation. It should be pointed out that step heights could not be determined precisely for very small UV zones observed because of the lower sensi-

TABLE I

COMPARISON OF ZONE ABSORBANCES AT 254 AND 280 nm TO EXTINCTION DATA FROM UV SPECTRA OF AUTHENTIC STANDARDS

Compound	UV molar extinction		ITP zone absorbance	
	E_{254}	E_{280}	A_{254}	A_{280}
CP	510	30	0.080	0.0056
Amide	920	60	0.16	0.011
Amidine	3800	1100	0.71	0.22

TABLE II

THERMAL STEP HEIGHTS AT 150 μ A

Values normalized relative to potassium so that the step height of sodium is 100 arbitrary units. A minimum of 10 units difference in step height is required for complete separation under the experimental conditions employed.

<i>Ion</i>	<i>Thermal step height</i>
K ⁺	0
Na ⁺	100
Amidine ²⁺	105
Co ²⁺	114
CP ²⁺	117
Amide ²⁺	131
Tris ²⁺	358

tivity of the thermoionic detector. (3) A third confirmation test was the chemical modification of samples by spiking with authentic standards followed by reanalysis. This approach demonstrated in Fig. 4 was particularly useful for very small UV zones where precise step height determination was not possible. The assignments of small UV zones in Fig. 1 were made in a similar fashion.

Taking advantage of the high sensitivity and resolving power of the Tachophor combined with the wide dynamic range of the method, the purity of CP Lot EL58633 was calculated from the relative areas of the resulting zones shown in Fig. 5. The precision of the ITP method has also been calculated from a standard calibration curve, Fig. 2, where eight different concentrations were measured three times each. The relative standard deviation was 0.0004. Samples giving areas below 0.1 units ought to be rerun at double volume to give a more reliable result. ITP analysis of CP

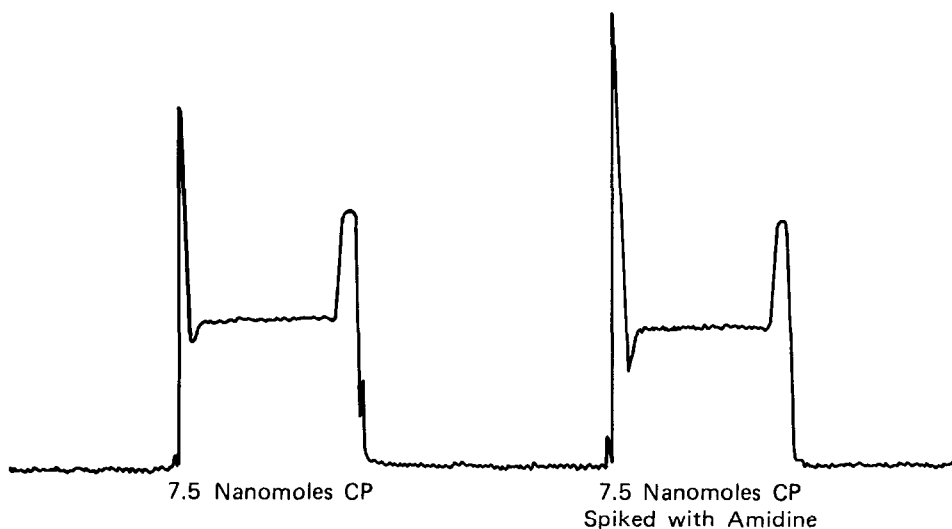


Fig. 4. Effect of spiking with amidine on isotachopherogram.



Fig. 5. Isotachopherogram resulting from a 7.5 nanomole injection of CP Lot EL58633. Zones: 1 = electrolyte contaminant; 2 = non-UV-absorbing unknown; 3 = amidine ($0.5 \pm 0.02\%$); 4 = CP ($95.6 \pm 0.3\%$); 5 = amide ($3.9 \pm 0.04\%$); 6 = UV-absorbing unknown. Percentages were calculated from relative areas, with uncertainties at the 95% confidence level based on three replicates.

should contain as a minimum about 10 pmole. Below this level the coefficient of variation is more than 1.0%. Using of an internal standard should improve the reproducibility because a significant source of error can be traced to the reproducibility of making microliter injections.

CONCLUSIONS

These results demonstrate that ITP can provide useful qualitative and quantitative data on charged cobalt(III) complexes and their salts. Its advantages are that it resolves several ions in one experiment, that it is sensitive to picomole amounts, that both large and small amounts of different compounds can be measured simultaneously, that it is relatively quick (3 runs per hour), and little or no sample pre-treatment is required.

It is concluded from the present experiments with ITP that qualitative and quantitative analysis of charged complexes and their salts by electrophoretic methods offers a viable tool for studies of such materials with advantages over more commonly employed analytical methodology for many applications. Areas of direct applicability include: quality control/quality assurance (QC/QA), stability/compatibility studies, purification both prior to further analysis work, *i.e.*, spectroscopic study and as a preparation step as well as utility in certain physical chemical studies. Work in this laboratory has already begun in all of the above areas. The recent development of a high resolution capillary adaptation of conventional zone electrophoretic techniques⁹ should also offer new expanded possibilities in these and other areas as refinements and interest develop.

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