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SEPARATION, IDENTIFICATION AND ESTIMATION OF Cr(III)
COMPLEX IONSTHIN-LAYER CHROMATOGRAPHY AND DIFFUSE REFLECTANCE
SPECTROSCOPY

A. D. KIRK, K. C. MOSS AND J. G. VALENTIN

Chemistry Department, University of Victoria, Victoria, B.C. (Canada)

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SUMMARY

A number of complex ions of Cr(III) have been separated by thin-layer chromatography in silica gel, alumina and cellulose. Silica gel yielded the most acceptable results. Diffuse reflectance spectroscopy has been used for identification of the eluted spots and good spectra may be obtained from spots containing only 10^{-7} mole of complex ion. An application of these techniques to the analysis of the photolysis products of $[\text{Cr}(\text{en})_2\text{ox}]^+$ is described.

We wish to report some results on the analyses of mixtures of Cr(III) complex ions, results which we feel will be of interest to other workers faced with similar problems. The technique we have developed makes use of thin-layer chromatography for separation of the complex ions, diffuse reflectance spectroscopy for identification of the individual eluted spots and colorimetric analysis for quantitative determination of the Cr(III) content of the spots.

As far as we are aware, previous applications of thin-layer chromatography to separations of complex ions have been restricted to cobalt(III)¹ and platinum(II) species^{1a}; DRUDING AND HAGEL¹ have studied the separation of *cis/trans* isomers by silica gel TLC following on from an earlier study by ERLLENMEYER *et al.*². The coupling of TLC with reflectance spectroscopy has received little attention to date. Previous work has been limited to organic systems³⁻⁶.

Frodyma *et al.*⁷ separated dye mixtures by silica gel and alumina TLC, and identified and estimated the components both by direct reflectance spectroscopy on the plates and also by reflectance spectroscopy on the thin-layer absorbent after its removal from the plate.

Now that TLC sheets of silica gel, alumina, cellulose, etc., with reproducible characteristics are readily available from commercial sources, we thought it of value to develop these techniques for the analysis of mixtures of complex ions.

In studies of the kinetics and photochemistry of complex ions, the types of

investigations that can be carried out are often restricted by the limitations of presently available analytical methods. This is particularly so in studies of the photochemistry of octahedral Cr(III) complexes. Because of our interest in this area, and in view of the previous demonstration of the utility of TLC in the analysis of Co(III) complexes, we chose to begin our work with chromium complexes.

In the main, all thin-layer work has been done on silica gel (Eastman 6061 without fluorescent indicator) or alumina (Eastman 6062 without fluorescent indicator), although a few runs have been carried out on cellulose (Eastman 6064 without fluorescent indicator). Running times for the chromatograms have been found to be in the order of 15 to 30 min, depending on the composition of the eluting phase. This time is sufficiently short that decomposition does not occur for most of the complexes. In one or two cases, trouble was experienced with components of the eluting phase substituting into the coordination sphere of the complex, *e.g.* dimethyl sulphoxide replaced water in $[\text{Cr}(\text{NH}_3)_5 \cdot \text{H}_2\text{O}]^{3+}$, but this could be avoided by appropriate alteration of the eluent. All chromatograms were run in the Eastman TLC Developing Apparatus. The solvent systems used varied, as did the procedures for activating the plates, and are discussed individually below.

One major advantage of the commercially prepared sheets, over and above their reproducibility, is that they are on a plastic backing. This means that individual spots may be cut from the developed chromatogram, masked by a black cut-out (so that the white silica gel or alumina surrounding the spot is covered), and the diffuse reflectance spectrum of the spot measured. This masking procedure enabled good spectra to be obtained from spots containing only 10^{-7} mole of complex; this for Cr(III) complexes in which the molar extinction coefficient at the maximum for the d-d ligand field bands is only of the order of 6×10^1 . For more intensely coloured complexes, correspondingly higher sensitivity would be obtained. All spectra were measured using the reflectance accessory for the Unicam SP700 spectrophotometer. Fig. 1 shows the arrangement and dimensions of the sample/reference holder, the masks, the chromatogram spot and the reference (the reference was cut from the

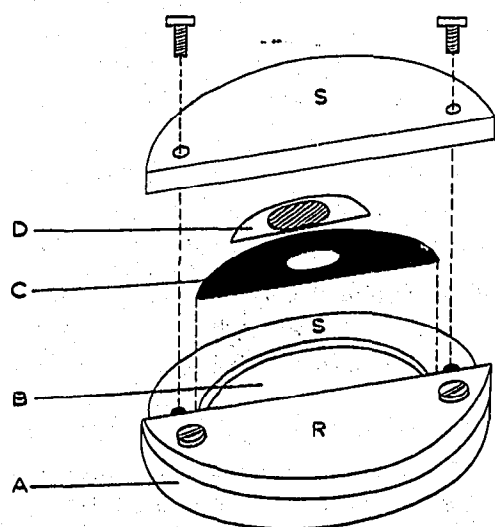


Fig. 1. Unicam SP 700 reflectance cell. A = Cell body; B = silica window; C = black mask (hole diameter 8 mm); D = sample TLC sheet containing spot. The reference side of the cell contains a black mask and a piece of unused TLC sheet.

corresponding unused chromatogram sheet). This technique avoids the danger of contamination or loss of material present when the adsorbent has to be scraped from the backing. No attempt was made to use reflectance spectroscopy as a quantitative technique for estimation of the Cr content of the eluted spots, as too many obvious difficulties and sources of inaccuracies present themselves. Quantitative analysis was therefore carried out on the eluted spots by the colorimetric technique described later.

The first system studied was irradiated $[\text{Cr}^{\text{III}}(\text{en})_2\text{ox}]^+$; on irradiation of the charge transfer or the d-d bands of this ion, marked spectral changes occur, suggesting aquation. Very careful analysis for oxalate shows it to be absent in the irradiated solutions⁸; and therefore ethylenediamine aquation was suspected to be the photochemical process, leading to the production of $[\text{Cr}^{\text{III}}\text{en ox}(\text{H}_2\text{O})_2]^+$. Since both reactant and product ions are unipositive ions, the analysis of this system was expected to provide a reasonably good test of the value of TLC for such analyses.

The separation was found to be remarkably simple. Irradiated and non-irradiated $[\text{Cr}^{\text{III}}(\text{en})_2\text{ox}]\text{NO}_3$ solutions were spotted side by side on silica gel sheets (activated at 100° for 15 min), and eluted with the solvent system methanol-dimethyl sulphoxide-70% perchloric acid in the volume ratio 100:2.5:2.5. The non-irradiated solution gave a single orange spot (R_F 0.0) and the irradiated solution two spots, one orange (R_F 0.0) and the other red (R_F 0.85). The R_F value of the fast running component was reproducible within $\pm 0.01 R_F$. A genuine sample of $[\text{Cr}^{\text{III}}\text{en ox}(\text{H}_2\text{O})_2]\text{Br} \cdot 2\text{H}_2\text{O}$ (ref. 9) was prepared, and when run under identical conditions gave a red spot (R_F 0.85). That the product of the photolysis is, in fact, $[\text{Cr en ox}(\text{H}_2\text{O})_2]^+$ is further suggested by examination of the reflectance spectra. Table I gives the reflectance spectra data for the eluted chromatographic spots discussed above. The table shows that the product of photolysis has a spectrum identical (within experimental error) with that of the authentic $[\text{Cr}^{\text{III}}\text{en ox}(\text{H}_2\text{O})_2]^+$. A comparison of the solution spectrum of $[\text{Cr}^{\text{III}}\text{en ox}(\text{H}_2\text{O})_2]^+$ with its reflectance spectrum on silica gel shows that a spectral shift of about 2% towards lower energy occurs on adsorption. Fig. 2 shows these spectra and is presented to illustrate the quality of reflectance spectra possible.

Since our expectation was that it would be difficult to obtain good quantitative

TABLE I

REFLECTANCE SPECTRA OF ELUTED CHROMATOGRAPHIC SPOTS

Complex ion	R_F value (silica gel)	Spectral data			
		ν_1 Peak max. ($\text{cm}^{-1} \times 10^{-3}$)	Band width at half ht. ($\text{cm}^{-1} \times 10^{-3}$)	ν_2 Peak max. ($\text{cm}^{-1} \times 10^{-3}$)	Band width at half ht. ($\text{cm}^{-1} \times 10^{-3}$)
Pure $[\text{Cr}(\text{en})_2\text{ox}]^+$	0.0	26.75 ± 0.15	4.3 ± 0.4	20.30 ± 0.15	3.5 ± 0.4
Pure $[\text{Cr en ox}(\text{H}_2\text{O})_2]^+$	0.85	24.85 ± 0.15	4.7 ± 0.3	18.65 ± 0.15	3.8 ± 0.3
Irradiated $[\text{Cr}(\text{en})_2\text{ox}]^+$	0.0	26.75 ± 0.15	4.3 ± 0.5	20.30 ± 0.15	3.5 ± 0.4
	0.85	24.75 ± 0.15	4.3 ± 0.5	18.45 ± 0.15	3.5 ± 0.5
Solution spectrum of pure $[\text{Cr en ox}(\text{H}_2\text{O})_2]^+$		25.30 ± 0.15	4.9 ± 0.3	19.00 ± 0.15	4.0 ± 0.3

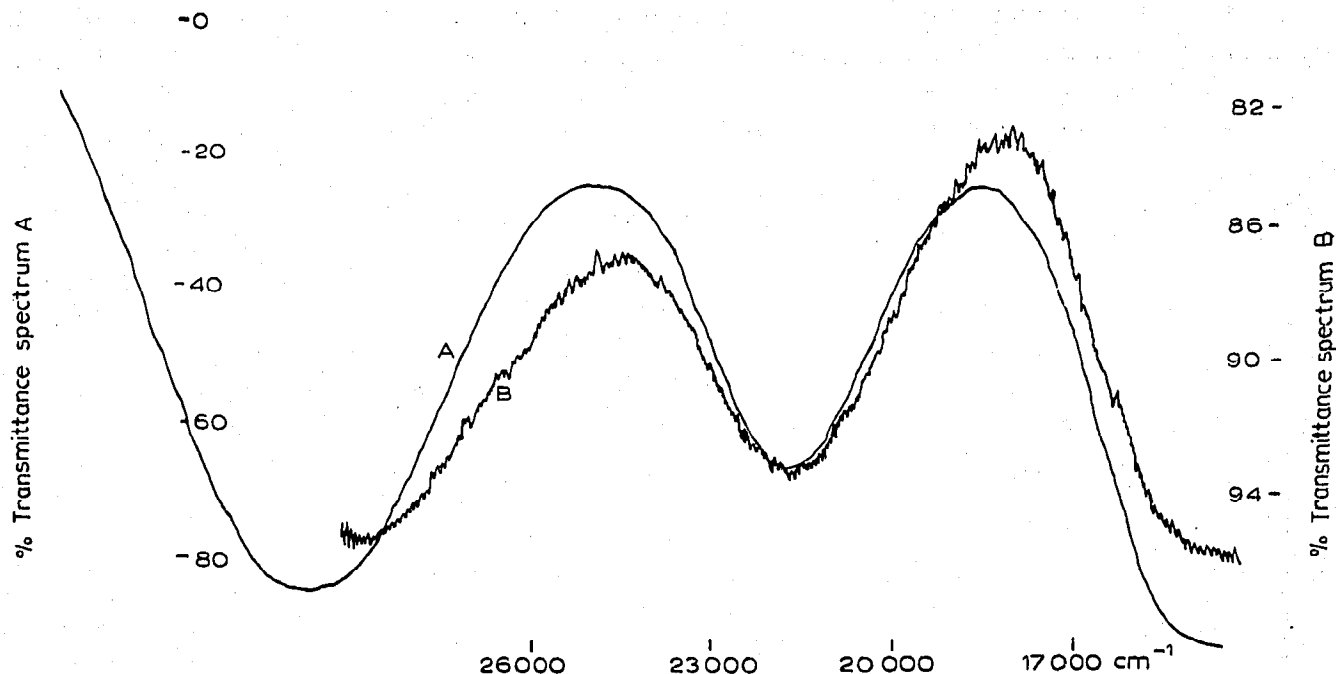


Fig. 2. Solution and reflectance spectra of $[\text{Cr}^{\text{III}}\text{en ox}(\text{H}_2\text{O})_2]^+$. (A) Solution spectrum ($10^{-2} M$); (B) reflectance spectrum.

analytical data for mixtures from the reflectance spectra of spots containing such small amounts of material (but see ref. 6), we adopted extraction and colorimetric analysis for this purpose. The total Cr(III) content of eluted spots was determined as follows. The adsorbent was scraped from the backing sheet into an 0000 porcelain crucible, and was then evaporated to dryness three times with concentrated nitric acid (five drops). Four drops of 0.25 M sulphuric acid were then added and the crucible contents transferred to a centrifuge tube, with careful washing with 0.25 M H_2SO_4 . The mixture was then heated at 100° for 10 min and the supernatant solution transferred to a second centrifuge tube containing *ca.* 50 mg of potassium persulphate. The crucible residue was treated twice more with 0.25 M H_2SO_4 and the washings combined with the original solution. This solution was then heated, with the addition of one drop of 0.05 M silver sulphate, for half an hour at 50° and then at 100° for another half hour to destroy excess persulphate. The Cr(VI) solution was then transferred to a 1 ml volumetric flask, four drops of a 0.25% diphenylcarbazide solution in ethanol were added, and the solution made up to the mark with distilled water. The optical densities were then measured at $540 m\mu$, using a 10 mm path length microcell in a Unicam SP700 spectrophotometer. This rather elaborate procedure was found to be necessary because of the need to destroy the polyvinyl alcohol binder in the TLC sheets before oxidising the chromium. If commercially available sheets on a plastic backing with an inorganic binder were available, then the procedure could be simplified considerably.

Fig. 3 shows the calibration curve obtained using standard $\text{K}_2\text{Cr}_2\text{O}_7$ solutions and the experimental points obtained by applying the above procedure to known samples of $[\text{Cr}^{\text{III}}(\text{H}_2\text{O})_6]^{3+}$ spotted on silica gel plates. The agreement between the two is excellent; the experimental points derived from the Cr(III) spots deviate from

the calibration curve by an average of 2.6% with a slight bias, as to be expected, towards low values. The least squares lines for the two sets of data agree very well.

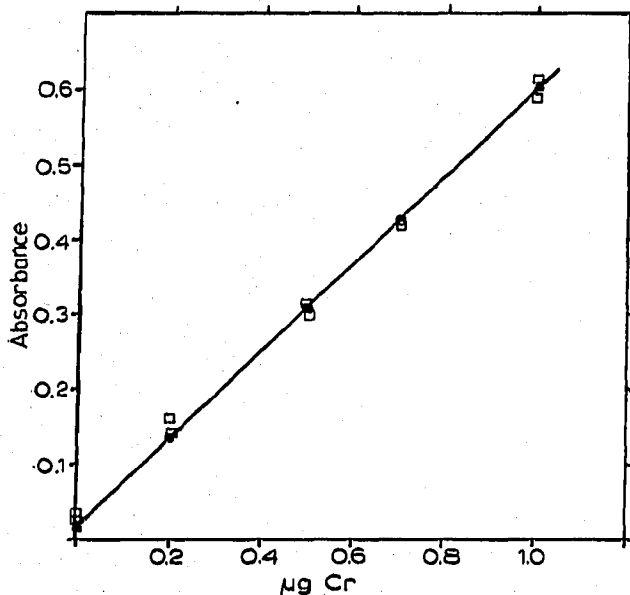


Fig. 3. Calibration curve for Cr analysis at 18,600 cm^{-1} . ● = Cr(VI) standard; □ = experimental results for $[\text{Cr}(\text{H}_2\text{O})_6]^{3+}$ on silica gel.

TABLE II

THIN-LAYER CHROMATOGRAPHY OF SOME REPRESENTATIVE Cr(III) COMPLEX IONS
Solvents: acidic, 0.06 M aqueous HClO_4 ; basic, 1.0 M sodium acetate.

Complex	Charge	R_F value			
		Silica gel ^a		Alumina ^b	
		Basic	Acidic	Basic	Acidic
$\text{Cr}(\text{NH}_3)_6$	3 ⁺	0.6 ^c	0.50	0.81	—
$\text{Cr}(\text{NH}_3)_5\text{H}_2\text{O}$	3 ⁺	0.9 ^c	0.43	0.0 ^d	—
$\text{Cr}(\text{H}_2\text{O})_6$	3 ⁺	0.8 ^c	0.51	0.0 ^d	0.0 ^d
$\text{Cr}(\text{NH}_3)_5\text{Cl}$	2 ⁺	0.6 ^c	0.61	—	0.0 ^d
$\text{Cr en ox}(\text{H}_2\text{O})_2$	1 ⁺	1.0	1.0	0.0 ^d	0.0 ^d
$\text{Cr}(\text{en})_2\text{ox}$	1 ⁺	0.75	0.43	0.94	0.0 ^d
$\text{Cr}(\text{dipyr})_2\text{Cl}_2$	1 ⁺	0.10	0.0	0.69	0.0 ^d
$\text{Cr}(\text{pyr})_3\text{Cl}_3$	0	0.0	0.0	0.0 ^d	0.0 ^d
<i>cis</i> - $\text{Cr}(\text{ox})_2(\text{H}_2\text{O})_2$	1 ⁻	0.98	0.96	0.0 ^d	0.0 ^d
<i>trans</i> - $\text{Cr}(\text{ox})_2(\text{H}_2\text{O})_2$	1 ⁻	0.94	0.96	0.0 ^d	0.0 ^d
$\text{Cr en}(\text{ox})_2$	1 ⁻	1.0	0.98	0.61	0.87
$\text{Cr dipyr}(\text{ox})_2$	1 ⁻	0.7 ^c	0.96	0.69	0.91
$\text{Cr phen}(\text{ox})_2$	1 ⁻	0.0	0.88	0.0 ^d	0.0 ^d
$\text{Cr}(\text{NH}_3)_2(\text{SCN})_4$	1 ⁻	0.67	0.82	0.56	0.92
$\text{Cr}(\text{ox})_3$	3 ⁻	1.0	0.96	0.92	1.0
$\text{Cr}(\text{SCN})_6$	3 ⁻	0.55	0.88	0.0 ^d	0.0 ^d

^a Activated by heating for 20 min at 100°.

^b Activated by heating for 20 min at 100°.

^c Badly tailed.

^d Most of the 0.0 values correspond to strong irreversible adsorption, often accompanied by decomposition.

Prompted by the success of the separation of $[\text{Cr}^{\text{III}}(\text{en})_2\text{ox}]^+$ from $[\text{Cr}^{\text{III}}\text{en ox}(\text{H}_2\text{O})_2]^+$, a number of Cr(III) complexes were run both on alumina and silica gel TLC sheets under acidic and basic conditions. The R_F values so obtained are shown in Table II, which shows that many separations are clearly effected. Behaviour on silica gel seems to be determined in a complex manner by the charge on the ion, the symmetry of its ligand field and the nature of the ligands. Where the R_F value of a complex changes with pH, it appears, in some cases, to be due to lowering of the charge on the complex due to acid base dissociation. The results on alumina are even more difficult to interpret and it appears that irreversible adsorption and/or decomposition occurs in some cases. A few runs were carried out on cellulose and some of the data are shown in Table III. Cellulose as an adsorbent clearly has utility in this context; however, silica gel appears to offer the greatest potential on the basis of the work carried out so far.

TABLE III

CELLULOSE THIN-LAYER CHROMATOGRAPHY DATA

(A) Eluent: MeOH-HClO₄-DMSO, in the volume ratio 100:2.5:2.5. (B) Eluent: MeOH-H₂O-DMSO-sodium acetate in the ratio 100 ml:10 ml:2.5 ml:5 g, and then adjusted to pH ~ 8 with NaOH.

	R_F values	
	A	B
$[\text{Cr}(\text{NH}_3)_6]^{3+}$	0.0	0.3
$[\text{Cr}(\text{urea})_6]^{3+}$	0.5	0.7
$[\text{Cr}(\text{NH}_3)_5\text{H}_2\text{O}]^{3+}$	0.0	0.2
$[\text{Cr}(\text{H}_2\text{O})_6]^{3+}$	0.6	0.7

Work is now proceeding on the separation of some of the less stable acid-ammine Cr(III) complexes and also on the separation of *cis/trans* isomers. For this latter purpose, ion-exchange impregnated paper as well as TLC sheets are being investigated. The authors feel that these preliminary studies have established the utility of combining TLC and reflectance spectroscopy in solving some of the difficult analytical problems attendant on photochemical and kinetic studies of Cr(III) complexes.

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