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# HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY IN THE STUDY OF SOME REACTIONS OF CHROMIUM(III) COMPLEXES

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## SUMMARY

The chemistry of a number of chromium(III) complex ions has been studied with the aid of a reversed-phase high-performance liquid chromatography system. These studies have been used to confirm and extend earlier work with the di(oxalato)diaquochromate(III) ion and to initiate a detailed study of the aquation of the bis(malonato)ethylenediaminechromate(III) ion.

## INTRODUCTION

Fundamental to the study of transition metal complexes is the ability to identify unequivocally the reaction products. Many reactions readily lend themselves to study by spectroscopic methods, but even when reactions give spectroscopically stable products, the spectra alone do not always provide conclusive evidence for the nature of the product(s).

We have been interested for some time in the use of conventional ion-exchange chromatography both for the identification of products in the reactions of complexes of chromium(III) and other metal ions and also for the kinetic investigation of reactions[1,2]. Whereas spectroscopic data are always derived from the absorbance values representing the sum, at the chosen wavelength, of the absorbance of each of the species present in solution, chromatographic separation allows for the independent identification and measurement of each species in solution. This facility has been particularly useful in the study of multi-step reactions such as the aquation of the ions $[Cr(ox)(en)_2]^+$ and $[Cr(ox)(NH_3)_4]^+$ and related systems[2–4]. The rates of the reactions of many chromium(III) complexes are compatible with this type of chromatographic study, nevertheless the method is time consuming and not well adapted for automatic data collection.

Chromatographic studies of transition metal complexes have included the use of thin-layer chromatography (TLC)[5], electrophoresis[6] and high-performance liquid chromatography (HPLC)[7,8]. Kinetic studies have been carried out using electro-

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phoretic separation with radioactive tracers to quantify the separated components[9]. Potential advantages of HPLC for the study of reaction kinetics of complexes are the ready, precise quantification of reactants and products by UV detection, rapid analysis time and ready availability of equipment.

Disadvantages have been noted as the possible reaction of the metal ions with stainless-steel tubing[8] and the fact that, unlike other chromatographic techniques, this technique does not normally lend itself to operation at sub-ambient temperature, which is necessary when separating complexes unstable at normal temperatures[6].

These disadvantages presented no problem in the current study. The chromium(III) complexes used are relatively stable at room temperature and show no reaction with stainless steel or with the methanol–water–ion-pairing eluents used. The reactions investigated, which are acid catalysed, are effectively frozen in the eluent at pH 4.

The first application of reversed-phase ion-pair HPLC to transition metal complexes appears to have been by Valenty and Behnken[10]. Bipyridyl derivatives of ruthenium(II) were separated using alkyl sulphonates as ion-pairing agents and UV detection. More recently cobalt(III) amino acid cationic complexes were separated using *p*-toluene sulphonate with detection in the visible region of the spectrum[11–14]. In the present work using a simple isocratic system with UV detection we have obtained rapid, clean, separation and quantitative determination of several chromium(III) complex cations and anions. We report here the application of this technique to the study of reactant purity and solution composition, solution equilibria and reaction kinetics. This appears to be the first kinetic investigation of chromium(III) complexes by HPLC.

## EXPERIMENTAL

### *Instrumentation*

A Pye-Unicam LC-3 pump with LC-UV detector was used throughout these studies. A flow-rate of 1.5 ml/min was normally used at pressures of 100–150 bar. The detector was set at 220 nm, at which wavelength all the complex species of interest give strong absorption (absorbance in the range 0.1 to 1.0 for 0.001 *M* solutions). Eluate flow could be switched to a flow cell set within a Pye-Unicam SP 8-250 spectrophotometer. Using this arrangement the eluate could be monitored at a second wavelength or the spectrum of a "trapped" peak obtained whilst column elution continued.

### *Columns and eluents*

Columns used (200 × 5 mm) were packed with either Partisil ODS 5 $\mu$m or Spherisorb ODS 5 $\mu$m, both giving good separations ($N$ = 2500–5000). Aqueous methanolic eluents were used containing, for anion separation, tetrabutylammonium hydroxide (0.25–1%), neutralized to pH 4.0 using phosphoric acid and, for cation separations, sodium laurylsulphate (0.25%), also adjusted to pH 4.0 using phosphoric acid.

Concentrations of the counter ions were chosen to give optimum separation and column life. Complex ion retention increased with eluent water content. Samples were injected using a 20-$\mu$l loop. Typical separations are shown in Figs. 1–4.

*Potassium di(oxalato)diaquochromate(III), K[Cr(ox)$_2$(H$_2$O)$_2$]*

The *cis* and *trans* isomers of this complex were prepared by published methods[15].

*Potassium bis(malonato)ethylendiaminechromate(III), K[Cr(mal)$_2$(en)]*

This complex was prepared by the reaction of 1,2-diaminoethane (ethylenediamine) with a concentrated solution of potassium di(malonato)diaquochromate(III)[16]. The solid obtained from this reactionn was dissolved in water and the solution passed through an anion-exchange column (Deacidite FF, $Cl^-$ form).

The ion, $[Cr(mal)_2(en)]^-$, was retained on the column and eluted using 1 *M* potassium chloride. On evaporation of the eluate, well formed crystals of the double salt, $K[Cr(mal)_2(en)] \cdot KCl \cdot 2H_2O$, were obtained contaminated only with a small amount (3%) of excess potassium chloride (Found: C, 19.9%; H, 3.5%; N, 5.5%; Cl, 8.5%; K, 18.1%. Calculated: C, 20.0%; H, 3.3%; N, 5.8%; Cl, 8.9%; K, 17.9%).

*Kinetic investigations.* Solutions of the complexes were prepared as required and held in a thermostat bath (±0.1°K). Samples were withdrawn at suitable time intervals (4–30 min), loaded into the injection loop and immediately injected onto the column. Sample time was taken as the time of injection. Component concentrations were directly proportional to peak heights. First order rate constants were obtained from plots of $\ln(A_t - A_\infty)$ *vs.* time, which were linear for at least two to three half-lives.

## RESULTS AND DISCUSSION

*Product analysis*

It has previously been shown that the standard method for the preparation of *cis*-$K[Cr(ox)_2(H_2O)_2] \cdot 2H_2O$ gives a material containing significant quantities of the ions, $Cr_2O_7^{2-}$, $1Cr(ox)(H_2O)_4]^+$ and $[Cr(ox)_3]^{3-}$ as impurities[17]. The compound obtained in the preparation can be shown to be spectroscopically impure but because of the balance between the quantities of the impurities their presence may not be directly shown by elemental analysis. The different ions can be separated by ion-exchange chromatography and each component analyzed independently, but the procedure is slow and indirect. The only other reported previous chromatographic investigations of this complex are by TLC[18,19].

Fig. 1 shows the separation of the four components of interest by direct injection of a mixed-ion solution using reversed-phase HPLC. A peak for the cation, $[Cr(ox)(H_2O)_4]^+$ is obtained at the head of the solvent front and the height of this peak, as of the other peaks, is directly proportional to the ion concentration. The presence of the other ions as impurities in the preparation of *cis*-$K[Cr(ox)_2(H_2O)_2] \cdot 2H_2O$ could clearly be seen and their concentrations measured down to the 1% level by reference to standard solutions. This method is direct and rapid and the results agree well with those we have previously obtained by classical analysis. Typical analyses gave results in the ranges: $[Cr(ox)_3]^{3-}$, 15–30%; $[Cr(ox)(H_2O)_4]^+$, 4–10%; $Cr_2O_7^{2-}$, 0–2%.

*Equilibrium studies*

We have previously shown that an estimate of the proportions of the *cis* and

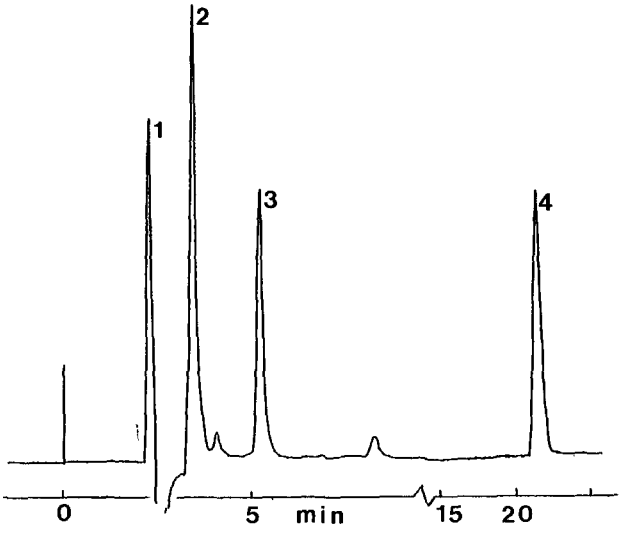


Fig. 1. Separations of anions. Eluent, 0.5% tetrabutylammonium phosphate (pH 4.0) in water–methanol (1:1). Flow-rate, 1.3 ml/min; absorbance, 0.32 a.u.f.s. Peak identification: 1 = $[Cr(ox)(H_2O)_4]^+$, 0.00032 *M* (2.2 min); 2 = $[Cr(ox)_2(H_2O)_2]^-$, 0.00017 *M* (3.3 min, *cis* and *trans* isomers unresolved); 3 = $Cr_2O_7^{2-}$, 0.00008 *M* (5.0 min); 4 = $[Cr(ox)_3]^{3-}$, 0.00059 *M* (23 min).

*trans* isomers of the $[Cr(ox)_2(H_2O)_2]^-$ ion present together in solution can be obtained from the spectra of the separated isomers and of their equilibrated solution[17]. However, since it is not possible entirely to suppress the equilibration reaction in solution, the exact values of the extinction coefficients required for the calculation remain in some doubt. The two isomers are readily separated using water–methanol (6:1) as eluent. In the course of the study of the equilibration of these ions, the only

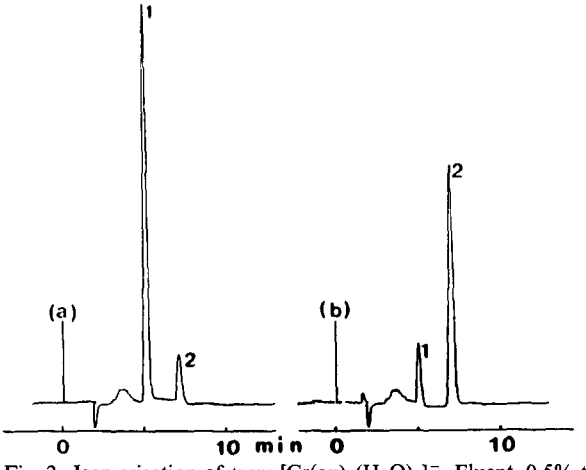


Fig. 2. Isomerisation of *trans*-$[Cr(ox)_2(H_2O)_2]^-$. Eluent, 0.5% tetrabutylammonium phosphate (pH 4.0) in water–methanol (6:1). Flow-rate, 1.5 ml/min; Absorbance, 0.32 a.u.f.s. Peak identification: 1 = *trans*-isomer (5.0 min); 2 = *cis* isomer (7.0 min); a = fresh solution of *trans* isomer, 0.00067 *M*, in 0.01 *M* perchloric acid; b = same solution after equilibration (room temperature, 2 days).

chromatographic peaks observed are those for the two isomers. The absolute decrease in the height of one peak (*trans*) is always in a fixed proportion to the growth of the other peak (*cis*). Fig. 2. The ratio of these changes gives a direct measure of the relative chromatographic sensitivity of the two isomers. It follows that the ratio of the concentrations of the two isomers in the equilibrated solution can be calculated from the peak heights at equilibrium. The value obtained in this way, [*trans*]/[*cis*] = 0.16 ± 0.01, is somewhat higher than our previous estimate (0.10 ± 0.01). The ratio was not found to change significantly with temperature (278–298°K) or acid concentration [0.002–0.5 *M* perchloric acid ($HClO_4$)].

*Kinetic studies, diaquadi(oxalato)chromate(III)*

The *trans–cis* isomerisation of this ion was followed in order to obtain data parallel with that obtained spectrophotometrically[17]. At low acidity (less than 0.05 *M* $HClO_4$) the growth of the *cis* peak gave a first order plot matching that for the decay of the *trans* isomer. At higher acid concentrations the initial rate of *cis* isomer formation was markedly less than that of *trans* loss. During the course of the isomerisation there was no significant formation of the $[Cr(ox)(H_2O)_4]^+$ ion showing that hydrolysis of the isomers was negligible under the conditions used. This pattern of behaviour could not have been deduced from the spectroscopic data but is consistent with the mechanism previously suggested[17] whereby isomerisation proceeds by means of a five -coordinated protonated intermediate. The absolute values of peak heights of the *cis* isomer in the equilibrated solution do decrease with increase in acid concentration. Whilst this could be a solute effect (such effect do increase with acid concentration), it could also be accounted for by the presence of the neutral protonated species. No peak in the chromatograms could be identified directly as due to such a neutral species but failure to observe a peak for a neutral species does not rule out its formation. Experience with these systems has shown that neutral species tend to appear within the solvent front where they can be masked by other solute effects. There has been one report in the literature of the isolation of a similar protonated intermediate by conventional ion-exchange chromatography[20] but this work does not appear to have been repeated. More recent work has suggested that the more stable form of these transient protonated species is six-coordinate with a single bridging carboxylate group[21]. It is hoped that more information on these species might be obtained using HPLC.

Table I summarizes the kinetic data obtained by chromatographic analysis

TABLE I

COMPARISON OF KINETIC PARAMETERS FOR THE ISOMERISATION OF *trans*-DI(OXALATO)DIAQUOCHROMATE(III) ION AT 298°K

| | $k(obs)$, 0.1 *M* $HClO_4$ ($sec^{-1}$) | $k(o)$* ($sec^{-1}$) | $k(H)$** ($sec^{-1}\,M^{-1}$) | $\Delta H(o)$* (kJ $mol^{-1}$) | $\Delta S(o)$* (J $mol^{-1}$ $°K^{-1}$) | $\Delta H(H)$** (kJ $mol^{-1}$) | $\Delta S(H)$** (J $mol^{-1}$ $°K^{-1}$) |
|---|---|---|---|---|---|---|---|
| Spectrometric[17] | $7.0 \cdot 10^{-4}$ | $4.5 \cdot 10^{-4}$ | $2.5 \cdot 10^{-3}$ | 75 | −54 | – | – |
| Chromatographic | $7.3 \cdot 10^{-4}$ | $4.3 \cdot 10^{-4}$ | $3.0 \cdot 10^{-3}$ | 79 | −44 | 65 | −73 |

* Non-acid-catalysed path.
** Acid-catalysed path.

together with corresponding spectroscopic data, the two sets of results can be seen to be in good agreement.

*Kinetic studies, bis(malonato)ethylenediaminechromate(III)*

The aquation of the ion, $[Cr(mal)_2(en)]^-$, (I), was chosen for this study, both for its intrinsic interest and because of the type of ionic products expected from its aquation. There appears to be no other previous chromatographic investigation of this ion. The major reactions expected are:

$$[Cr(mal)_2(en)]^- \text{ (I)} + H_3O^+ \underset{k_{-1}}{\overset{k}{\rightleftharpoons}} [Cr(mal)(malH)(H_2O)(en)] \text{ (Ia)} \quad (1)$$

$$\text{Ia} + H_2O \xrightarrow{k_2} [Cr(mal)(H_2O)_2(en)]^+ \text{ (II)} + malH^- \quad (2a)$$

$$\text{Ia} + H_3O^+ \xrightarrow{k_2'} \text{II} + malH_2 \quad (2b)$$

$$\text{I} + H_3O^+ \xrightarrow{k_3} [Cr(mal)_2(H_2O)(enH)] \text{ (III)} \quad (3)$$

$$\text{III} + H_2O \xrightarrow{k_4} [Cr(mal)_2(H_2O)_2]^- \text{ (IV)} + enH^+ \quad (4)$$

It was expected that reaction 1 would be fast and that the unstable, protonated intermediate (Ia) would be present only in low concentration. Using the anion-separation system (tetrabutylammonium ion), well separated peaks corresponding with ions I and IV were obtained (Fig. 3). The extinction coefficient for the malonate ion

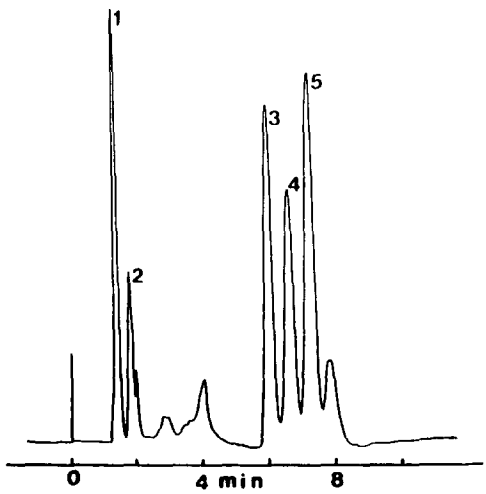


Fig. 3. Separation of species formed in the aquation of $[Cr(mal)_2(en)]^-$. Eluent, 1% tetrabutylammonium phosphate (pH 4.0) in water–methanol (9:1). Flow-rate, 1.5 ml/min; Absorbance, 0.16 a.u.f.s. Peak identification: 1 = $[Cr(mal)(en)(H_2O)_2]^+$, 0.0001 *M* (1.4 min); 2 = $[Cr(mal)_2(enH)(H_2O)]$, (1.8 min, not confirmed or quantified); 3 = *trans*-$[Cr(mal)_2(H_2O)_2]^-$, 0.0002 *M* (6.1 min); 4 = $[Cr(mal)_2(en)]^-$, 0.0001 *M* (6.8 min); 5 = *cis*-$[Cr(mal)_2(H_2O)_2]^-$, 0.0002 *M* (7.4 min).

at 220 nm is only some 6% of that for the complex ions, a small peak for this ion appears much later on the chromatogram than for the two mono-negative complex ions. The *cis* and *trans* forms of ion IV were also clearly separated so that as the aquation reaction proceeded the later formation of the *trans* isomer from the initial *cis* product was observable. Ion II was the only significant cation expected to be present and the peak for this ion appeared at the head of the solvent front, the peak height for this cation was directly proportional to its concentration.

The formation of cation II was also followed as a separated peak using the

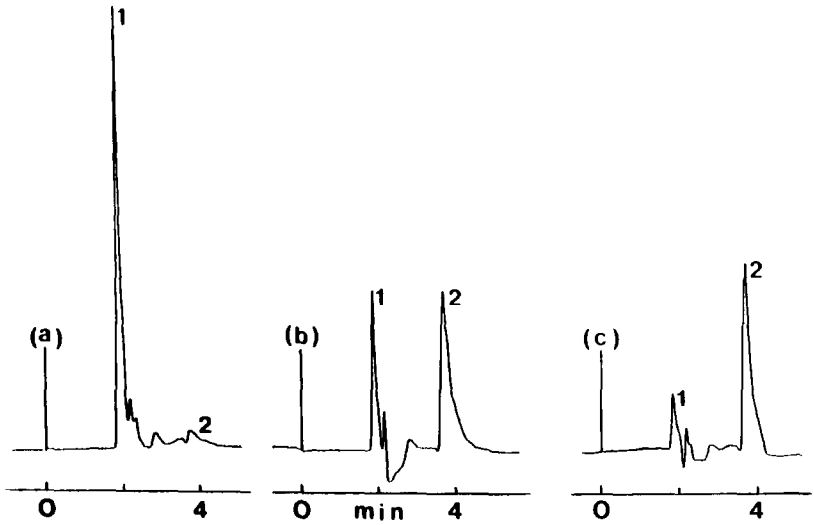


Fig. 4. Separation of $[Cr(mal)(en)(H_2O)_2]^+$ from the aquation of $[Cr(mal)_2(en)]^-$ (cation-exchange system). Eluent 0.25% sodium laurylsulphate (pH 4.0) in water–methanol (1:4). Flow-rate: 1.5 ml/min; Absorbance, 0.16 a.u.f.s. Peak identification: $1 = [Cr(mal)_2(en)]^-$ (1.9 min); $2 = [Cr(mal)(en)(H_2O)_2]^+$ (3.7 min); other anionic species also occur about the solvent front (2–3 min). a = fresh solution, 0.0004 *M*, in 0.01 *M* perchloric acid; b = solution after 60 min at 343°K; c = after 120 min at 343°K.

cation-separation system (laurylsulphate). Under these conditions all the anions appeared together at the head of the solvent front (Fig. 4).

The most obvious reaction sequence shown by the chromatograms was the decay of I and the growth of II. Both reactions were markedly dependent on hydrogen ion concentration. The acid dependence of the observed $k$, $k$(obs), is shown in Table II. The first order rate constants derived from the decay of I, $k$(d), differ significantly from those derived from the growth of II, $k$(g). This discrepancy gives evidence that the reaction I→II proceeds by a series of steps, such as 1, 2a and 2b, and not directly.

At room temperature it was possible to observe the growth on the chromatograms of a small peak within the solvent front which could be equated with intermediate Ia. It subsequently decayed at the same rate as the main reactant peak I. At hydrogen ion concentrations less than 0.02 *M* a second transient peak appeared at the head of the solvent front and this was followed by the growth of the anion peak corresponding with the product, IV. Although not fully characterised, this second

TABLE III

CALCULATED AND OBSERVED RATE CONSTANTS FOR THE AQUATION OF $[Cr(mal)_2(en)]^-$ ION AT 323°K

Values of $k$(d) and $k$(g) are for reactions followed using the anion-exchange system (results using cation-exchange system in brackets). Values of $k$(calc) were obtained using trial values of $k_3$, $k_a$, $k_b$ and $k_c$ to obtain the best least squares fit of data. These values were then related to the corresponding terms in the original rate equation as follows: $k_3 = 5.4 \cdot 10^{-6}$ sec$^{-1}$; $k_a = 4.5 \cdot 10^{-3}$ sec$^{-1}$ $M^{-1}$; $k_b = 5$ sec$^{-1}$ $M^{-1}$; $k_c = 0.54$ sec$^{-1}$ $M^{-2}$; $k_1 = 0.11$ sec$^{-1}$ $M^{-1}$; $k_2/k_{-1} = 0.0041$; $k'_2/k_{-1} = 4.9$ $M^{-1}$.

| $[H^+]/M$ | $k(d) \cdot 10^5/sec^{-1}$ | $k(g) \cdot 10^5/sec^{-1}$ | $k(calc) \cdot 10^5/sec^{-1}$ |
|---|---|---|---|
| 0.002 | 1.72 | 2.9 | 1.65 |
| 0.004 | 2.90 | 4.0 | 3.15 |
| 0.006 | 5.4 (5.6) | 6.3 (5.5) | 5.05 |
| 0.008 | 7.1 (7.3) | 10.9 (11.5) | 7.32 |
| 0.01 | 9.2 (10.0) | 12.5 (12.3) | 9.96 |
| 0.02 | 28.3 (26) | 32.0 (32.8) | 28.3 |
| 0.03 | 55.5 (56.4) | 57.2 (59.2) | 54.5 |
| 0.04 | 107 (93) | 82 (92) | 87.5 |
| 0.05 | 121 | 114 (115) | 126.5 |

transient peak at the head of the solvent front most likely corresponds with the neutral species, III. The magnitude of these peaks increased with decrease in hydrogen ion concentration and the final size of the cation peak, II, decreased. From the relative sizes of the peaks corresponding with cation II and anion IV an estimate of the extent of reaction by means of chromium–nitrogen bond fission at low hydrogen ion concentration could be made.

The chromatographic separations thus provide good evidence for the proposed reaction scheme. This scheme gives rise to the rate law:

$$k(\text{obs}) = k_3 + \frac{k_1 k_2[\text{H}^+]}{k_{-1} + k_2 + k'_2[\text{H}^+]} + \frac{k_1 k'_2[\text{H}^+]^2}{k_{-1} + k_2 + k'_2[\text{H}^+]} \tag{5}$$

which can be written in the form:

$$k(\text{obs}) = k_3 + \frac{k_a[\text{H}^+]}{1 + k_b[\text{H}^+]} + \frac{k_c[\text{H}^+]^2}{1 + k_b[\text{H}^+]} \tag{6}$$

The fit of calculated values, $k$(calc) which can be obtained using this relationship and the experimental rate constants, $k$(d) at 323°K are shown in Table II.

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