

**Effects of Low POC/DOC on Metal Speciation
In Lake Erie**

A Report to the Lake Erie Protection Fund

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Abstract

A method was developed for the of a separation and detection of species of chromium. Both complexes of Cr(III) and Cr(VI) as dichromate were separated and quantified simultaneously using cation exchange high performance liquid chromatography (HPLC) with atomic absorption spectroscopy (AAS) detection. Standard curve Cr(III) was linear between 0.1 and 1 mM with detection level approximately 0.005mM. A standard curve using dichromate was not as linear, with sensitivity somewhat less than that of Cr(III) and its complexes. Recovery of Cr(III) complexes appeared to be complete indicating that different complexes posed no problem to the detection of Cr. Kinetic and equilibrium studies of the formation of Cr complexes of the model compounds oxalate, fluoride and EDTA were easily accomplished with the HPLC-AAS method. All indications are that the use of HPLC-ICP-MS would improve the detection limits and flexibility of this method so that non only Cr, but also other toxic metals of interest could be separated and quantified. This work suggests that studies of the equilibria and kinetics of other metals and metal species of environmental interest could be determined by cation exchange HPLC using either AAS or ICP-MS.

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Introduction

Scope of Project

The purpose of this research was twofold: 1) preliminary investigation of the possibility of using HPLC-ICP-MS to determine toxic metal speciation in Lake Erie, and 2) preliminary investigation of release of various toxic metal species into Lake Erie from sediments or particulate matter. The investigation was designed first to examine several toxic metals to determine if their complexes would be stable to analysis by High Performance Liquid Chromatography (HPLC). Second, a single metal determined to have stable complexes would then be used to develop an HPLC procedure to quantitatively determine the concentration of the various species. It was anticipated that an HPLC procedure devised to separate a transition metal and its known complexes would be valuable in determining the concentration of a variety of environmentally significant metals and their naturally occurring complexes. Finally, using a combination of HPLC with ICP-MS, a preliminary study was to involve the release of a single toxic metal species from sediments in the Cuyahoga River into Lake Erie.

Metal Speciation

Virtually all metal ions exist naturally in many different forms or *species*. Frequently metal speciation refers to differences resulting from variation in oxidation state. For example, chromium exists commonly in the non-toxic Cr (III) form as well as in the carcinogenic Cr (VI) form. Many metals in turn have pH dependent hydrolysis species. Thus Cr (III) varies from Cr^{+3} to $\text{Cr}(\text{OH})^{+2}$ to $\text{Cr}(\text{OH})_2^+$ to $\text{Cr}(\text{OH})_3$ (solid or dissolved) to $\text{Cr}(\text{OH})_4^-$ as pH increases from 3 to 10 (Richard and Bourg, 1991; Vercoutere and Cornelis, 1995). Hydrolysis species are very quickly interconverted in most metals as the pH changes. Additionally, metals in certain oxidation states form complexes with electron pair donating ligands such as oxalate and fluoride, bonding singly or multiply depending on the metal, ligand, pH and other solution factors. Finally, metals themselves may form polymers (oxy or hydroxy dimers, trimers and higher) depending largely on their concentration and pH.

The environmental interest in speciation of metals evolves primarily from the species variability in toxicity and mobility. Once again using chromium as an example, Cr(III) is either essential or benign to metabolism (Herald and Fitzgerald, 1994) while Cr (VI) is both mutagenic and carcinogenic (Flessel *et al.*, 1980). Cr(III) adheres to soil and sediment while Cr (VI), usually as the chromate (CrO_4^-) or dichromate (Cr_2O_7^-) is mobile (Richard and Bourg, 1991). Because of the differences in toxicity, bioavailability and mobility, determination of total metal concentration does not give adequate information about the potential environmental problems posed by a particular metal species (Campanella, 1996; Vercoutere and Cornelis, 1995). This is reflected in the methods for determination of metal species of mercury and chromium recommended by the US EPA (EPA, 1991).

Investigation of Colorimetric Ligands of Toxic Metals

The metals of interest proposed for this investigation included mercury (Hg), cadmium (Cd), chromium (Cr), lead (Pb) and arsenic (As). An extensive literature investigation was undertaken to find ligands that would form colorimetric complexes in appropriate concentrations and pH values that would serve as model compounds for this investigation. Several colorimetric reagents are able to complex with these metals to give responses adequate to be detected spectrophotometrically (a minimum Absorbance of 0.1 using a 1 cm cell). Table 1 shows the metals of interest and possible ligands. Initially these metal complexes were tested for their stability toward dilution. It was anticipated that dilution that occurs during HPLC might result in dissociation of the complexes and therefore erroneous quantification of species concentrations. Complexes that survived the stability test (see Results and Discussion) were then further investigated.

Table 1. Ligands and Metals investigated

Ligand	Metal	Reference
PAR	Cd, Pb, Hg, Cr(III)	Gomez <i>et al.</i> 1992
Eriochrome Black T	Cd	Hobbs, <i>et al.</i> 1983
Tiron	Cd, Pb, Hg, Cr(III)	Dionex, 1982
Xylenol Orange	Cd, Pb, Hg	Martel <i>et al.</i> , 1993
Molybdate, reduced	As	Standard Methods, 1995

Investigation of HPLC as a tool for speciation

HPLC has been demonstrated to be an effective means of quantifying aluminum species especially in acid impacted lake samples (Sutheimer and Cabaniss, 1995b and 1997). In lakes with pH values less than 5, the more toxic Al^{3+} is present along with other Al species generally thought to be less toxic. However, HPLC speciation has not been studied for other environmentally significant metals. In fact, HPLC has been questioned as useful in metal speciation analysis because of the potential for dissociation of the complex or otherwise alteration of the species during HPLC. Sutheimer and Cabaniss (1995b) showed that this was not true for complexes of aluminum analyzed by HPLC, and that complexes remained in tact.

One of the problems associated with HPLC of metal species is the need for a detection system specific for a particular metal. For the aluminum speciation study of Sutheimer and Cabaniss (1995b), the addition of the fluorescent reagent lumogallion post column, provided a quantitative, sensitive, specific detection system for aluminum species. It is difficult, however, to find similarly specific and sensitive reagents for the variety of metals implicated in environmental toxicity. A relatively new development has been the use of HPLC coupled to an Inductively Coupled Plasma-Mass Spectrometry

(ICP-MS) detection system. In these systems, the eluent containing the species of interest is aspirated through a nebulizer, which converts the liquid into a fine aerosol. The aerosol is then introduced into a high temperature argon plasma "torch" which ionizes transition metals for introduction into the mass spectrometer. The MS acts as a detector for a specific mass, often the atomic mass of the metal of interest. Thus any species from the HPLC with the mass of interest is detected, but other metals are opaque to the system. With most ICP-MS instrument, multiple metals can be detected simultaneously, producing individual chromatograms for each metal.

Detection for the HPLC-ICP-MS of chromium has been reported to be 40 nmol l^{-1} ($\sim 0.2 \text{ ppb}$) (Byrdey *et al.*, 1995). This is comparable to the detection limits achieved for the aluminum speciation method. Typical river water concentrations of chromium range from 0 to 2200 nmol l^{-1} (~ 0 to 110 ppb), although polluted rivers may have as high as $27,000 \text{ nmol l}^{-1}$ (1400 ppb) (Richard and Bourg, 1991). Cr(VI) was found in the Grand River in May of 1998 at a concentration of 4.9 mg kg^{-1} of sediment (personal communication, Ohio EPA). Since chromium may leech from sediment, one can easily see how the sensitivity and selectivity of the HPLC-ICP-MS method might be very useful for studying the toxicity and mobility in toxic metal impacted systems.

Investigation of Release of Toxic Forms of Metals into Lakes

The systematic decreases in phosphate addition to Lake Erie from waste streams as well as the introduction of the Zebra Mussel have drastically altered the Lakes chemical ecology. Decreases in phosphate, the limiting nutrient for algal growth, has enabled Lake Erie to become significantly less productive. One chemical change in the lake that happened concurrently with the decrease in productivity was the decrease in organics, both dissolved (DOM) and particulate (POM). Although the sources of these heterogeneous, complex compounds are numerous, significant amounts originate from algal and animal exudates and decomposition products. Thus the decrease in algae resulted in a decrease in DOM. Further decreases especially in POM have occurred due to the filter feeding of Zebra Mussels. While the combination of these processes has improved the appearance and health of Lake Erie, a new question arises as to the fate of toxic metals deposited into the Lake either as dissolved species or as metals which are sorbed onto particles. Thus, detoxification of metals simply by chelating to algae and algal byproducts or by adhering to sedimenting particles, may now be reduced. Moreover, some metals which may be chelated to naturally occurring or anthropogenic ligands, may equilibrate to their more toxic forms when they reach the "cleaner" Lake Erie. Changes in the toxic/non-toxic forms of some metals may also occur through a change in their oxidation states, such as in the reduction of Cr(VI) to Cr(III) by humic substances (Richard and Bourg, 1991) or oxidation of Cr(III) by Mn oxides (Bartlett and James, 1979; Rai and Zacharra, 1986) but generally these processes occur slowly in natural systems although the rate decreases as organic matter decreases. One could argue that the decrease in organic matter in Lake Erie would result in a higher concentrations of the toxic Cr(VI) form.

The development of an analytical system for monitoring specific species of toxic metals and the changes of metal species as they leave tributaries of Lake Erie are the thrust of this research. The development of a working analytical method for the determination of metal species concentrations relied on two premises. First, the species must be separable by HPLC and second, the species can be detected by ICP-MS. This preliminary investigation

- 1) determined likely metal candidates to study the stability of ligand-toxic metal complexes to HPLC,
- 2) extensively investigated the analytical speciation of a single metal, chromium, and a preliminary investigated the changes in chromium in Cuyahoga River water and from Grand River sediment in so far as possible with the detector system which was available.

Results and Discussion

Analytical Problems Associated with Trace Metal Speciation

Questions that arise concerning the analytical determination of metal species by HPLC frequently center on changes in the species during chromatography. When complexes are involved, the dissociation of the complex while passing through the chromatographic column could result in smaller concentrations of some species, and larger concentrations of others. One probable method of dissociation of complexes during analysis occurs when a complex is diluted with the HPLC eluent and dissociates. This rapid hydrolysis requires readjustment to the new equilibrium situation. Since this represents a kinetically labile complex, such complexes are expected to be inappropriate for further study by HPLC.

Investigation of changes in complexes with dilution

Each pair of metal/ligand complexes listed in Table 1 was investigated for kinetic lability to a ten-fold dilution. Changes in spectra with dilution for kinetically stable complexes should be one purely of less intensity (10% of the original absorbance) for all portions of a spectrum (except solvent). If, however, complexes are kinetically labile, shifts in the spectral intensity at different wavelengths will result.

An extensive literature search was first performed to locate metal-ligand complexes that would be appropriate for the dilution study. Ligand-metal complexes needed to have K values high enough at near neutral pH (to mimic natural waters) that low concentrations of metal and ligand would form detectable amounts of complex. Complexes also had to have high molar extinction coefficient (ϵ) so that they could be detected spectrophotometrically.

For most of the metal-ligand complexes indicated on Table 1, a formation constant is available from the literature (Martel *et al.* 1993). Using these formation constants calculations were performed to determine if the complex would form in sufficient concentration for analysis by HPLC when the concentration of the metal and ligand were 0.1 mM. Metal concentrations was somewhat higher than normally found in the environment, but are relevant for this study in that they are not so large that the results from the experiments could not be extrapolated to environmental situations. Calculations were done using TITRATOR (Cabaniss, 1987). TITRATOR is computer program for calculating the concentration of individual species given the total concentration of the metal and ligands, pH and ionic strength. Examples of TITRATOR calculations for Pb-PAR at 1 and 0.1 mM levels is shown in Appendix B-1. The arseno-molybdate complex is not well characterized and therefore deemed to be unacceptable for these experiments.

For metal-ligand pairs that resulted in estimated complex concentrations greater than 50% of the original ligand concentration, and for which the molar absorptivity (ϵ) was sufficiently high enough to obtain spectra, a series of visible spectra were recorded. Spectra were obtained for the metal alone, the ligand alone and the metal ligand complex. Solutions were either buffered in HEPES or used deionized water. Each spectrum was recorded as part of the data set for that particular metal-ligand pair.

The results of these studies indicated that the metal-ligand complex most likely to remain intact during HPLC was the Cr(III)-PAR complex. Further investigation of chromium complexes in general indicated that they were slow forming and relatively stable. However, a limited amount of information was available for these complexes (Nieboer and Jusys, 1988) including formation constants (Smith and Martell, 1976; Martell and Smith, 1977; Martel *et al.* 1993).

Investigation of Chromium-PAR Complex by HPLC

In order to investigate the separation of Cr(III) from Cr(III)-PAR and PAR both ion chromatography and reverse phase chromatography were employed. The first experiments were performed to establish HPLC conditions using a SynChrome® S300 cation ion exchange column to separate Cr(III) and PAR. Cr was found to elute from the column at times dependent upon the concentration of Ca in the eluent as expected. PAR, on the other hand, did not elute from the column at all despite changes in pH and cation concentrations (Ca^{+2}) of the eluent. This result was not anticipated and was repeated in many experiments to verify the results.

Subsequently, experiments were performed to chromatographically separate Cr(III) and the Cr-PAR complex. Cr(III) could be detected at 228 nm, while the complex could be detected at 448 nm. Consequently, the two could not be viewed in the same chromatogram because the equipment's (LKB) HPLC detector is capable of only single wavelength detection. As a solution to this problem, a flow cell was purchased for the Hewlett Packard UV/Visible, diode array spectrometer which is available in the laboratory. Preliminary results show that the combined HPLC-UV/Visible spectrometer as the detector was able to see a larger spectrum of eluting components, but because of inadequate software, it was difficult to coordinate with the elution times of the chromatogram with the spectra.

Another attempt was made to separate Cr(III) and the Cr-PAR complex utilizing an LKB detector. Problems were encountered with the available LKB HPLC equipment, especially the eluent programming, but investigations are continuing along these lines. Both cation exchange and reverse phase columns were utilized. The use of organic/water mixtures for eluents in reverse phase chromatography may lessen the dissociative effects on complexes as compared to that of ions in the eluents used for cation exchange chromatography.

Investigation of Cr-organic complexes using Atomic Absorption Detection

When determining the potential for dissociation of known concentrations of metal-ligand complexes on an HPLC column, the following must be known:

1. the formation constant of the complex (assuming only one complex is formed)
2. any related formation constants such as for hydrolysis of the metal or the acidity (K_a) of the ligand
3. the starting concentration of the metal and ligand
4. the pH and ionic strength of the solution
5. the concentration of any two of the following in the final, equilibrated solution: metal, ligand, or complex (the third being available by subtraction)

The formation of more than one complex necessitates knowing correspondingly more information.

The original intent of these experiments was to determine the concentration of the complex and the ligand in the final solution using HPLC with spectrophotometric detection since ligands and complexes could be chosen that had high ($> 10^4$) values of the molar extinction coefficient ϵ . However it became increasingly apparent that differences in λ_{\max} and ϵ values for ligands and complexes were going to make the quantification difficult. The final approach was instead to use atomic absorption spectroscopy (AAS) as the detection system.

AAS is an analytical technique that more closely resembles ICP-MS in that it is specific for a metal as opposed to detecting a range of visible absorptions. By using AAS as the detection system, any peak indicates the presence a specific metal ion dictated by the AAS operating conditions (lamp, wavelength) (Skoog *et al.*,1988). Thus in an equilibrium solution of metal, ligand and complex, only the ligand would not be detected. Furthermore, metals other than the one of interest would not be detected, and the detection sensitivity of the different species of a metal is usually similar if not identical. In this case, only AAS detection for chromium was utilized. The change to AAS also eliminated the requirement that spectroscopically visible ligands and complexes be used. However, detection limits for HPLC-AAS of Cr(III) are approximately 0.005 mM, considerably higher than for HPLC-ICP-MS.

Reverse Phase HPLC using AAS Detection

The first investigations involved the use of a reverse phase HPLC column and a variety of solvents and conditions. Solutions of Cr (5 parts, pH 3.16) were investigated using 1 mM ligands (1 part) oxalate, pyrocatechol violet (PCV), EDTA and ascorbate with solution pH values between 3 and 4. Representative results are shown on Table 2. All samples were seven days old. Copies of chromatograms can be found in Appendix B 2.

Table 2. Reverse Phase HPLC separation of Cr Complexes

Ligand	pH of ligand solution	Peak Retention Time (min)	% of Total Peak Area	Tentative ID
EDTA	3.59	1.55	0.72	
		1.98	82.2	Cr(III)
		2.3	15.2	Cr(III)-EDTA (?)
Ascorbate	3.54	1.69	8.6	Cr-Ascorbate
		2.07	79.5	Cr(III)
		2.49	11.9	(?)
PCV	3.16	1.65	15.5	Cr(III)-PCV
		2.01	80.1	Cr(III)
		2.56	4.4	(?)
Oxalate	3.17	2.00	100	Cr(III) + Cr-oxalate

While several reasonable separations of Cr species were achieved, baseline separation was not. Although cation exchange HPLC might be a reasonable alternative to reverse phase, the relatively high concentrations of salts in the eluent was expected to cause problems with the AAS detector. Furthermore, the use of salts with an ICP-MS detector might also be a problem. However, to achieve baseline separation the change to cation exchange seemed necessary.

Cation Exchange HPLC using AAS Detection

Cation exchange chromatography using gradient elution (CaCl_2) produced excellent separation of Cr(III) complexes from free Cr(III). Furthermore, Cr(VI) as dichromate was also separated from free and complexed Cr(III), although the peak shape was somewhat skewed (Figure 2). To determine the linearity of the Cr(III) determination by HPLC-AAS, standard solutions of $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$, pH 3.5, were chromatographed. The results indicated excellent linearity between 0.1 and 1 mM, with an r^2 of 0.999 for four points (see Appendix B-3). A standard curve prepared using $\text{K}_2\text{Cr}_2\text{O}_7$ was linear (0.999) between 0.5 and 2 mM, but a 0.1 mM sample was not detected and the retention time appeared to change with concentration. The HPLC of dichromate may require more stringent pH control, as the dichromate - chromate equilibrium is pH dependent (Vercoutere and Cornelis, 1995).

Solutions chromatographed shortly after preparation of the $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ solution indicated three peaks at 1.3, 2.6 and 9.8 min (Figure 1). This was unanticipated since CrCl_3 , a relatively weak complex, was expected to form free Cr(III) soon after dissolution. The peak at 9.8 min was identified as the free Cr(III) species through development of the chromatographic method and by comparison to similar chromatography of Al(III) (Sutheimer and Cabaniss, 1995b and 1997). The other two

peaks were tentatively identified as the combined di- and trichlorinated species CrCl_2^+ and CrCl_3 at 1.3 min, and the monochlorinated CrCl^{+2} at 2.6 min. (Figure 1a)

After one day, the representative amounts of the three peaks had changed (Figure 1b) and by 2 days, the sample showed virtually 100% free Cr(III) (Figure 1c). This change was interpreted as being the gradual loss of chloride from the chromium chloride species over time to produce the ligand free Cr(III).

Figure 1. Chromatograms of CrCl_3 at different time intervals. The peak at 1.30 min is probably CrCl_3 plus CrCl_2^{+1} and decreases over two days to virtually zero. The peak at 2.60 min is probably CrCl^{+2} . The final peak is free Cr(III). pH is 3.47.

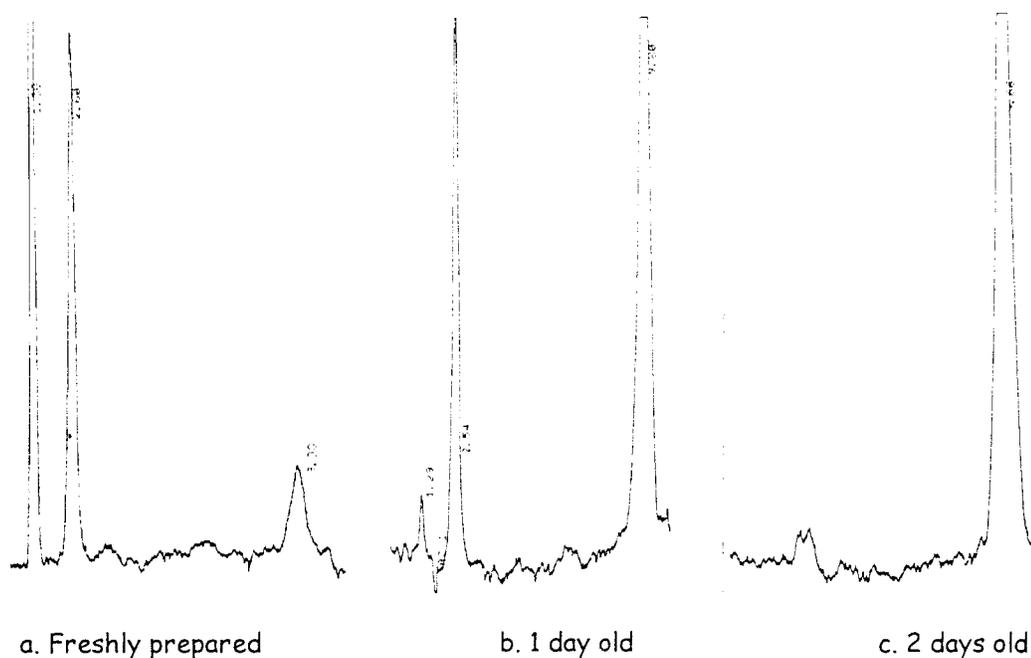
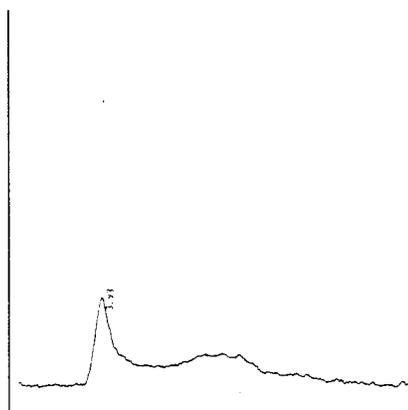


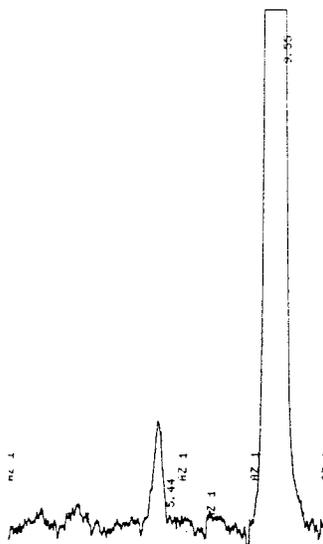
Figure 2. 0.5 mM dichromate. The retention time is 3.9 min



It should be noted that Cr(III)-6H₂O exists with different numbers of protons depending on the pH of the solution. Thus, reference to free Cr(III) here always includes all possible monomeric hydrolysis species, that is all of the pH dependent species containing one Cr atom and only water or hydroxide ligands.

After four months, chromatography of the same CrCl₃ sample produced the chromatogram shown in Figure 3. The peak at 5.63 min has been tentatively identified as the Cr dimer, Cr₂(OH)₂(H₂O)₄⁺⁴ because it was not present in any of the earlier chromatograms (0-2 da) of the same sample. The peak also appears in samples of Cr(III) equilibrated for five months with fluoride and with very low concentrations of oxalate (see below). Calculations (see Appendix B-1) estimate the concentration of the dimer at 0.2 mM at equilibrium (Rai and Zachara, 1986). This chromatogram indicates a concentration of approximately 0.006 mM. It is possible that if this is the dimer, it has not yet reached equilibrium. However, since the dimer is expected to bear a +4 charge, one would expect it to appear after the Cr(III) peak, i.e. much later in the chromatogram. Further study, possibly using ICP-MS, is need to identify this peak.

Figure 3. Chromatogram of four month old 1 mM CrCl₃. The peak at 5.44 is tentatively identified as the Cr dimer. The peak at 9.55 is free Cr(III).



Kinetic studies of Cr complexes

Once it became apparent that the slow approach to equilibria of Cr complexes could be monitored by HPLC-AAS, an experiment was performed to observe the formation of several Cr complexes over an extended period. A 1 mM solution of CrCl₃·6H₂O was prepared and allowed to stand for 8 days. To aliquots of this solution was added either sodium fluoride, sodium oxalate or disodium EDTA so as to obtain a final concentration of 1mM with pH values between 3 and 4. Fluoride and oxalate were

chosen because they occur naturally in fresh waters. EDTA is an anthropogenic ligand that occurs in relatively high concentrations in some industrial areas. These solutions were chromatographed at 1, 18 and 48 hours, then periodically for five months. Retention times varied slightly over the five-month period. Complete data is contained in Appendix B-4. Graphs of kinetic results as well as chromatograms of samples containing 1mM ligand plus 1mM Cr(III) after five months equilibration are shown in Figures 4.5 and 6.

As can be seen in Figure 4, species in solutions containing 1mM Cr(III) and 1mM EDTA (final pH 2.86) changed significantly over the five month period. While only tentative identifications can be made, the data suggest that the peak at 4.1 min was Cr(III)-EDTA which has a large formation constant ($\log K = 23.4$) for free Cr(III) and EDTA, and is the species in which represents approximately 95% of Cr after five months (Martel *et al.* 1993). The peak near 9.8 min, assumed to be free Cr(III) decreases from 100% to less than 3% during the same period. It is difficult to assign species to other peaks. The peak at 1.9 min that increases then decreases in concentration is intriguing and may represent an intermediate form such as Cr-EDTA₂ which forms quickly but then dissipates as equilibrium is approached. The peak at 1.4 min is an unknown Cr species which shows a remarkably consistent concentration over the duration of the experiment.

The solution containing 1mM each Cr(III) and oxalate (final pH 3.14) also contains several species over the five month period (Figure 5). Once again assignments are difficult. One would expect the CrOx⁺ species to form first and may be represented by the peak at 1.4 min. The peaks at 2.1 and 3.8 min may be species complexed with additional oxalate, but the charges of the complexes are difficult to interpret. Further investigation is needed to clarify if the species bear more oxalate or possibly additional protons, thus giving them their cation exchange characteristics. Little has been reported about the equilibrium between Cr(III) and oxalate.

Figure 4. Chromatograph and kinetic study results of 1mM Cr(III) and 1mM EDTA, pH 2.86. The legend indicates retention time in min

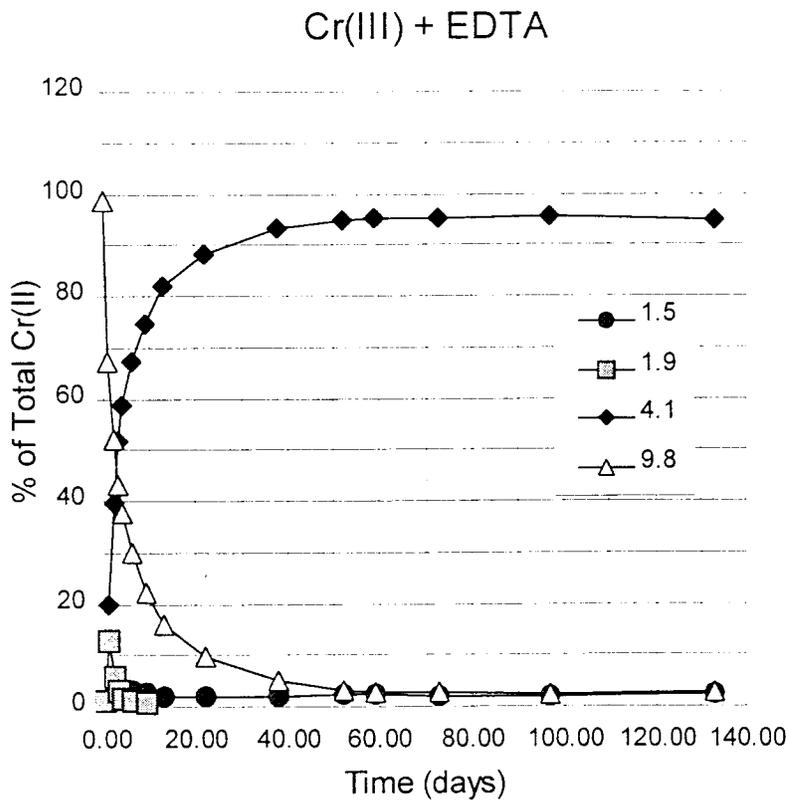
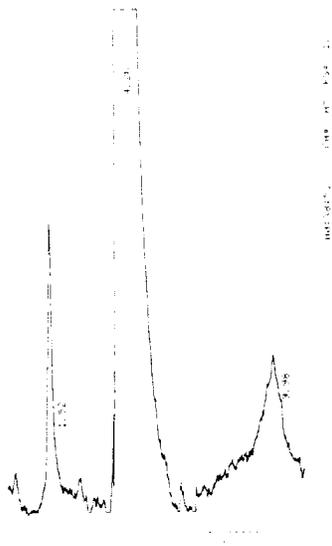


Figure 5. Chromatograph and kinetic study of 1mM Cr(III) and 1mM Oxalate, pH 3.14. The legend indicates retention time in min

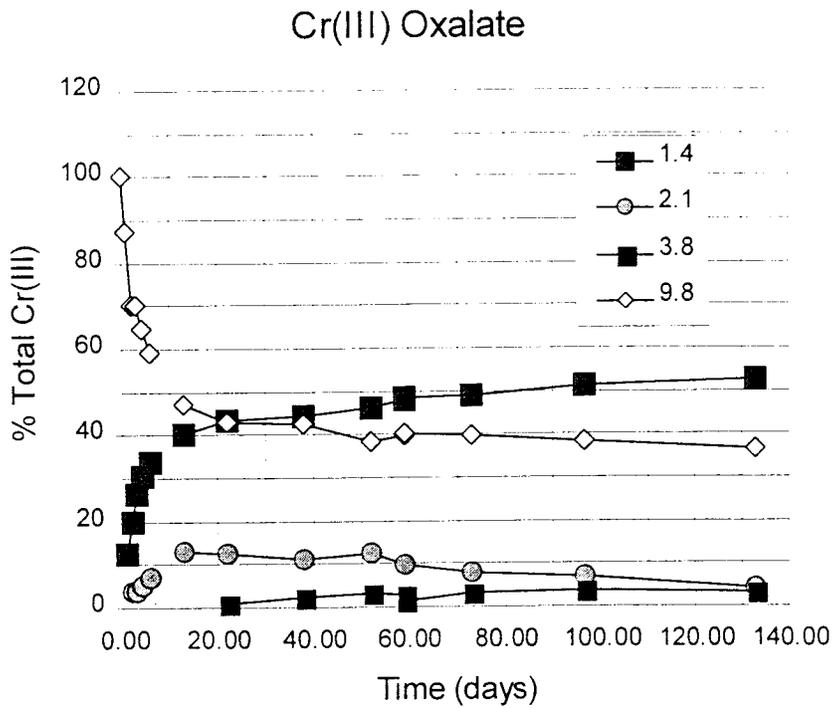
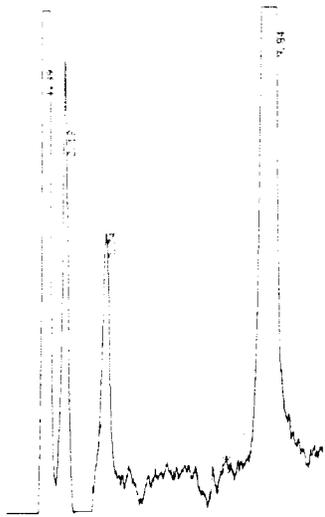
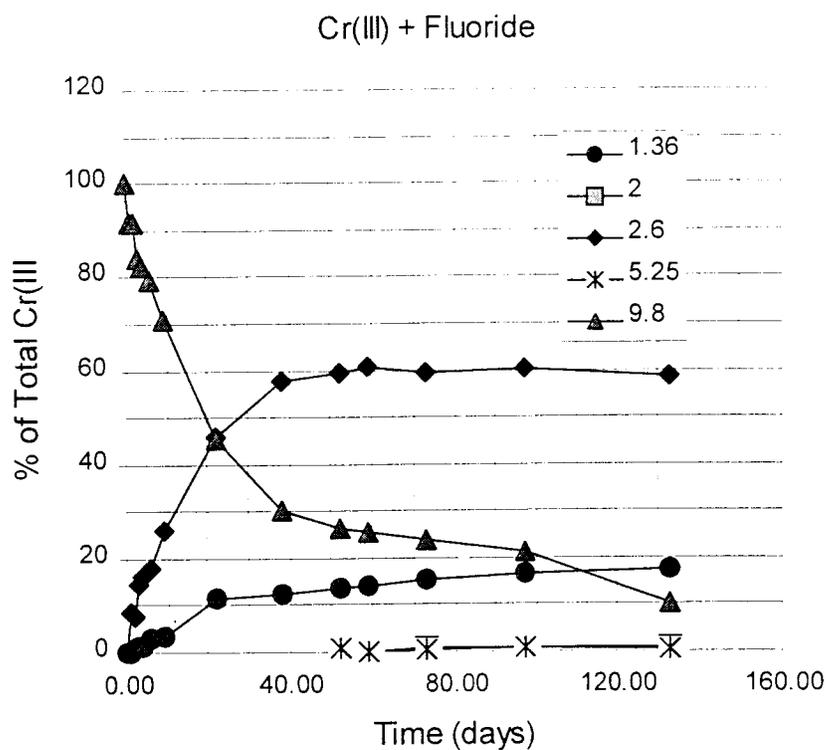
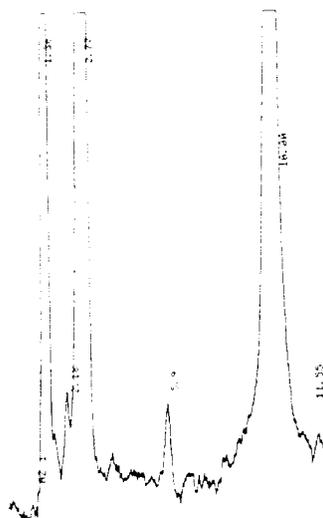


Figure 6. Chromatograph and kinetic study of 1mM Cr(III) and 1mM F⁻, pH 3.51. The legend indicates retention time in min



The solution containing 1mM each Cr(III) and fluoride (pH 3.51) likewise shows several peaks (Figure 6). The formation constants of Cr-F species are better characterized, however, than those of oxalate. This equilibrium mixture is anticipated to contain 0.18 mM of free Cr(III), 0.66 mM of CrF^{+2} , 0.15 mM of Cr_2F^+ and 0.001 mM of CrF_3 . Results after four months (tentative identifications) show 0.21 mM Cr(III) free (9.8 min), 0.60 mM of CrF^{+2} (2.6 min), and 0.17 mM of Cr_2F^+ and CrF_3 combined in a single peak (1.4 min). The peak at 5.25 min is suspected to be the Cr dimer, as it did not begin to appear until 52 days and is at the time seen in Figure 3.

Significance of chromium speciation

The ability to detect individual species of chromium, including complexes and possibly polynuclear species, even with the relatively crude AAS detector is truly exciting. Although the separation of Cr(III) and Cr(VI) has been reported in the literature (Byrdy *et al.*, 1995; Pansar-Kallio and Manninen, 1996; Urasa and Nam, 1989) only Milacic and Stupar (1994) have reported some success in separating and detecting complexes, although not in a concerted process (that is post column eluent aliquots were collected and determined by FAAS). Zoorob and Caruso (1997) have recently had some success detecting chromium dyes by HPLC using ICP-MS. These dyes, however, are very large ligands compared to oxalate and fluoride. No one has reported the ability to follow the formation and loss of chromium species.

Since Cr(III) may be changed to toxic Cr(VI) in the environment (Rai and Zachara, 1986), the ability to carefully monitor and/or estimate these species is critically important. In so far as environmentally relevant systems are concerned, the present separation and quantitation is an important step forward in environmental speciation of metals.

Equilibrium Studies of chromium complexes

In order to confirm the validity of the present chromatographic technique, a study was undertaken to match mathematical estimates of complex concentrations with analytically determined values. Estimates were determined using the formation constants for fluoride and EDTA species of Cr(III) (Martel *et al.* 1993) with ionic strength corrected using the extended Debye-Huckel equation (Skoog *et al.*, 1988) and are listed in Appendix B-6. As indicated from the kinetic studies, several species of each ligand were determined by HPLC. A series of solutions containing 1 mM Cr(III) and various concentrations of the fluoride, oxalate and EDTA were prepared and allowed to equilibrate at 25 degrees for five months. Complete analytical results are listed in Appendix B-5.

Graphs comparing calculated estimates (lines) to analytical results (marks) are shown in Figure 7. Figure 7a (fluoride) and 7b (EDTA) show that estimates and actual analytical values match well, an indication that the technique produces valid results. No formation constants were available for oxalate, so that the lines in Figure 7c are meant to indicate trends and not calculated estimates. It is difficult to assign species identification to the oxalate complexes, as little appears in the literature regarding these species.

Figure 7 a. Calculated (line) and experimental (marks) for 1 mM Cr(III) and varying amounts of fluoride.

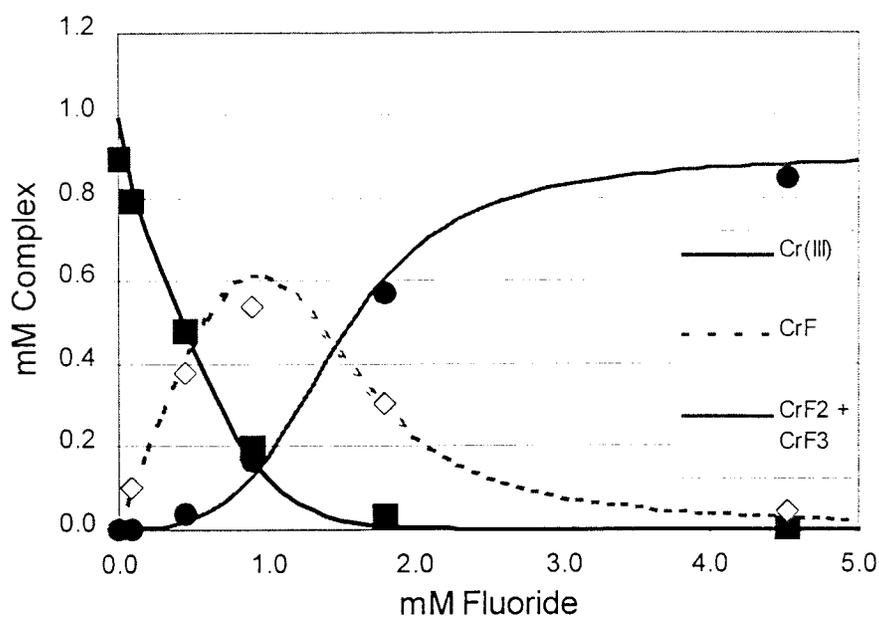


Figure 7 b. Calculated (line) and experimental (marks) for 1 mM Cr(III) and varying amounts of EDTA.

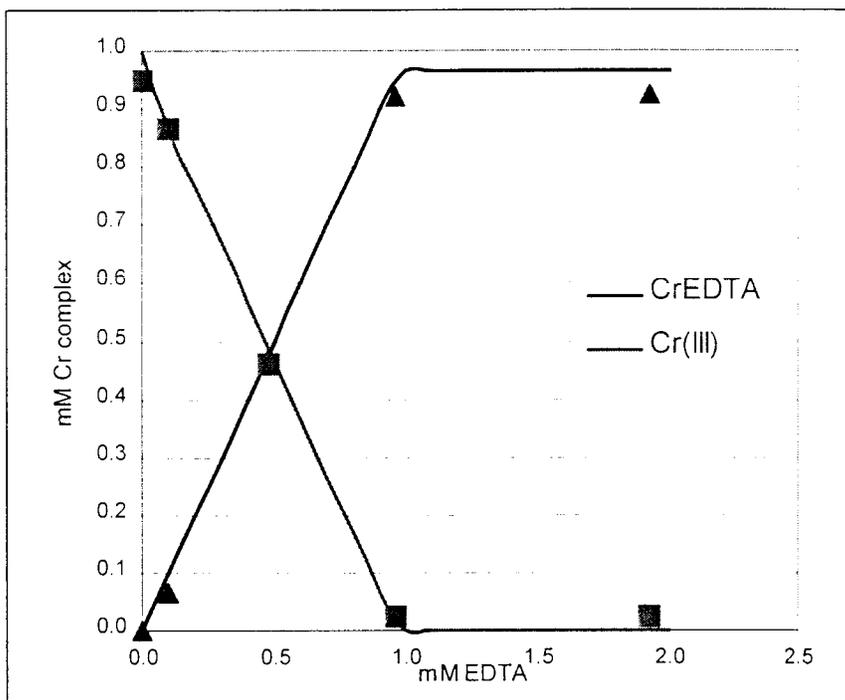
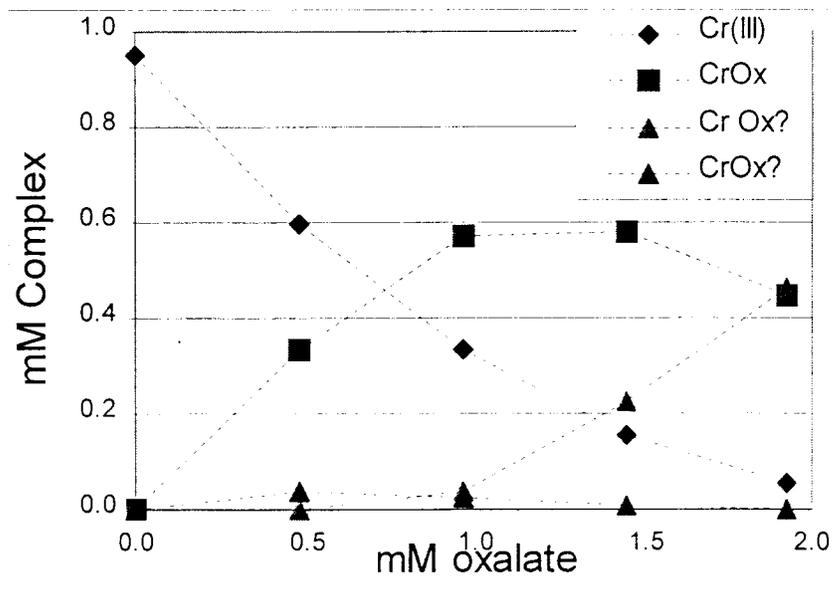


Figure 7c. Experimental (marks) for 1 mM Cr(III) and varying amounts of oxalate. No calculated values are available for oxalate. Dashed lines are for clarity only.



The percent relative standard deviation of the total peak area for a series of chromatograms having 1 mM Cr and concentrations of ligand (F. EDTA) between .1 and 5 mM was 3.4%. This indicates that the detection system shows no bias in the detection of different complexes and thus total peak area can be easily related to the total Cr concentration.

Using ICP/MS detection

One of the main purposes of this research was to determine if speciation of metal complexes could be performed using HPLC-ICP-MS. The three components required to accomplish this would be 1) the proper separation by HPLC, 2) the proper quantification by HPLC, and 3) the detection by ICP-MS. The original design of this project was to devise the HPLC separation and quantification as reported above, then to collect aliquots of the eluent in which the injected sample concentration was far less than detectable by AAS but large enough to be detected by ICP-MS. The sample aliquots would then be submitted to ICP-MS analysis at John Carroll University by Michael Ketterer, a co-investigator on this project. Unfortunately, after the time that the above research was completed, Dr. Ketterer left John Carroll, and the ICP-MS was no longer available for this project.

The other possible approach to estimating the possibility of using ICP-MS with this system was to review the current literature on ICP-MS detection of Cr. Most of this information deals with the separation and detection of Cr(VI) from Cr(III), but provides useful insight into the possibilities.

Byrdy *et al.* (1995) reported the ability to separate Cr(III) and Cr(VI) using an anion -exchange HPLC column using single ion monitoring a m/z of 53 and ammonium sulfate and ammonium hydroxide eluent. The m/z isotope of 52 is more abundant, but the sulfate produced high background at this m/z. Absolute detection was 40 pg for Cr(III) and 200 ng for Cr(VI). Using the NIST-SRM for trace elements in water, they detected 20 ppb of Cr(III). EDTA was used to "stabilize" Cr(III) from oxidation to Cr(VI), however no complexes were determined.

A review of ICP-MS methods by Byrdy *et al.* (1995) for Cr until that time, reveals that carbon in organic eluents, which are commonly used with reverse phase HPLC, interfered with detection. Other possible interferences are the use of dissolved salts in concentrations greater than 0.2%, and $^{40}\text{Ar}^{12}\text{C}^+$ from use of organic mobile phases and Ar used to generate the ICP flame.

Subsequently, Zoorob and Caruso (1997) investigated the separation and detection of chromium dyes using HPLC-ICP-MS. Once again using m/z of 53, they were able to detect as little as 1 ng of Cr dye using either anion exchange or reverse phase HPLC. The eluent for anion exchange was 50mM ammonium sulfate.

Finally Pansar-Kallio and Manninen (1996) used ICP-MS to determine both Cr(III) and Cr(VI) in waste water following coupled anion cation exchange HPLC. The eluent, nitric acid, did not pose any interference to the ICP-MS at m/z of 52. Interferences in the wastewater including chlorine and sulfur were eliminated in the HPLC step. Detection limits were about 0.5 ppb with relative standard deviation of replicates between 1 and 2.5 %. No mention was made of interferences that might be caused by Ca, a component in the eluent of this report. Since wastewater would likely be a significant component of any water samples, it is apparently inconsequential.

Although the actual ICP-MS work was unable to be performed for this report, one can conclude that HPLC-ICP-MS would be very feasible for chromium determinations. The detection limits of about 0.5 ppb in waste water, a factor of ten better than attainable by AAS. Concentrations in surface waters in the low ppb level, usually between 0.3 and 6 ppb (Sperling *et al.* 1992).

Does low POC or DOC exacerbate the concentration of toxic chromium in lake water?

One of the goals of this work was to perform preliminary investigations into the release of various toxic metal species into Lake Erie from sediments or particulate matter. Although the separation of metal species by HPLC was very acceptable, the limitations in detection by AAS, which would not be encountered using ICP-MS detection, rather large concentrations of Cr(III) had to be used for these experiments.

The first of these experiments was designed to determine if Cr(III) was adsorbed onto particles or complexed by river water. Water from the Cuyahoga River was collected on a day in which high rains had caused high sediment flow. To water that was unchanged (whole water), centrifuged (water with colloids) or filtered (water with molecules < 0.45 μm) was added various concentrations of Cr(III). Chromatograms of water to which no Cr(III) was added, showed no indication of Cr(III).

Unfortunately, because of the high concentrations needed for detection by HPLC-AAS, in most samples added Cr(OH)₃(s) precipitated. Consequently no detectable Cr appeared in the chromatograms and there was no way to separate precipitated Cr from sediment in order to analyse Cr adsorbed to sediment. This experiment would work better if the Cr concentrations that were added to the water were much lower since at the pH of the river water, Cr has very low solubility. Lower concentrations could have been used if ICP-MS had been available.

A second experiment was designed to determine if Cr from sediment in the Grand River would leech into water from Lake Erie. High Cr(VI) levels from Grand River sediments near the old chromium plant were known to show high levels of Cr(VI). Originally, the water from Lake Erie showed undetectable Cr. Sediment samples (15g) and Lake water (40 ml) were rotated for one month before the water was again analyzed for Cr. No Cr was detected in these water samples using the AAS as a detector.

Since Cr(III) is practically insoluble at the pH of lake water, it is likely that very little free Cr(III) is dissolved in it. Ligands that bind to Cr(III) would afford more solubility, but the low DOC of Lake Erie makes this unlikely. However, little information is available on the environmental changes of Cr(III) to toxic Cr(VI) except by manganese (Rai and Zacharra, 1986).

Future Research

The method developed in this project would be ideal for studying the complexation and speciation of a variety of metals and ligands of environmental importance. Ligands, including humic substance, proteins, lipids and many others could be studied by this method especially using ICP-MS detection. Further study on the species that were detected in the experiments with oxalate, EDTA and fluoride but not identified may be an important new step in identifying and characterizing their equilibria. On a more practical plane, the toxicity and transport of species in the environment is now not only possible, but simple. Finally, studies on the changes of Cr(III) to Cr(VI) may be possible with some preliminary work on the chromatography of chromate and dichromate.

Conclusion

The primary outcome of this work was the development of a separation and detection method for species of chromium. Both complexes of Cr(III) and Cr(VI) as dichromate were separated and quantified. Standard curve Cr(III) was linear over the range from 1 to 0.1 mM, with detection level approximately 5 ppb. A standard curve using dichromate was not as linear, with sensitivity somewhat less than that of Cr(III) and its complexes. Recovery of Cr(III) complexes appeared to be nearly complete indicating that complexes posed no problem to the detection of Cr.

Kinetic and equilibrium studies of the formation of Cr complexes of the model ligands oxalate, fluoride and EDTA were easily accomplished with the HPLC-AAS method. Kinetic studies indicated that equilibration was achieved in about five months. Equilibrium studies validated the analytical method by showing excellent comparison between analytically determined and calculated values. All indications are that the use of HPLC-ICP-MS would improve the detection limits and flexibility of this method so that not only Cr, but also other toxic metals of interest could be separated and quantified. Unfortunately the anticipated work on ICP-MS could not be undertaken.

With the method for chromium established, the development of methods for speciation of other metals is an important next step. The critical need to determine speciation of metals so as to understand toxicity, mobility and reactions in environmental systems is an important part of the understanding of aquatic system. The development of this method and the probable extrapolation of the method to include ICP-MS detection of many metals is anticipated to open new doors in the study of the environmentally relevant kinetics and equilibria of a variety of toxic metals.

Acknowledgements

The author greatly appreciates the funding of the Lake Erie Protection Fund for this project. Additionally, Notre Dame College of Ohio has graciously provided equipment and supplies. Thanks also go to Drs. Stephen Cabaniss and Patricia Maurice of Kent State University for their continued technical assistance. Students contributing to the completion of this project include Lena Golovinchits and Jennifer Lehman.

Appendix A

Methods and Materials

All chemicals were reagent grade unless otherwise indicated. Nitric acid (Aldrich, 99.99%) was used for adjusting pH. All glassware was acid washed overnight in 10% HNO₃ then rinsed several times with deionized water. Solutions were made and stored in Nalgene bottles or polypropylene tubes.

UV-Vis

Spectroscopy was performed on a HP Model 8452A UV-Visible spectrometer equipped with a diode array detector and HP 8452 Data System using a 1 cm quartz cuvette. Stock solutions (1 mM) of metal salts were prepared using Pb(NO₃)₂, Cd(NO₃)₂·4H₂O, HgCl₂, and Cr(NO₃)₃·9H₂O in deionized water. Stock solutions (1 mM) of colorimetric ligands including Tiron, 4-(pyridyl-2-azo) resorcinol (PAR), Xylenol Orange, Eriochrome Black T were also prepared in deionized water. In the case of Cr, the solutions were heated at 80° for 24 to 48 hours before spectroscopic analysis.

For analysis, an aqueous solution containing 0.1 mM each colorimetric ligand and metal was prepared in polypropylene test tubes. Spectra of the colored solutions were then recorded between 200 and 800 nm. To obtain a solution for stability studies, a 100ml aliquot of this solution was then diluted with 900µl of water in a quartz cuvette to give a 0.01mM solution. This diluted solution was then immediately capped, mixed and placed in the spectrometer to record any changes at the λ_{max} over time. Blanks containing 0.01 mM metal or 0.01 mM ligand were also prepared and spectra recorded. Spectra were also taken after the timed stability run was completed, usually at about 30 min.

HPLC using UV-Visible Detection

A HPLC system using a flow cell fitted to the HP 8452A UV-Visible spectrometer was used initially for the separation and detection of the PAR complex of Cr(III). The complex was prepared by heating a solution of 0.1 mM Cr(III) and 0.1 mM PAR in water at 90° for 10 min at which time a significant color change was noted. Several columns were studied including a Cation-R (Wescan., cation exchange) and S-300 (Synchrom, strong cation exchange). Also, a variety of eluents were used, but chiefly aqueous CaCl₂ solutions of various concentrations and gradients were employed. Eluents containing methanol and acetonitrile were also used with the S-300 column.

HPLC using Atomic Absorption Spectroscopy Detection

HPLC was performed using a Varian Model 2150 HPLC Pump, Model 2151 Variable Wavelength Monitor, Model 2152 HPLC Controller and a Perkin Elmer Model 503 Atomic Absorption Spectrometer (AA). Both reverse phase (PRP-1, 250µl injections) and cation exchange (PRP X-200, 50 µl injections) were employed using a

variety of eluents and solvent programs. All data was transferred to an Excel spreadsheet for calculations.

The AAS inlet was attached to the exit of the HPLC column with an intervening T that permitted water or air to be aspirated as needed. The inlet flow to the spectrometer was about 3 ml min^{-1} while the flow from the HPLC was 1 ml min^{-1} . Initially water was used as make-up, but eventually the use of air eliminated this dilution factor and did not seem to alter the detection in any negative manner. The fuel for the AAS was an air/acetylene mix, and detection using a Cr lamp was at 357.9 nm.

Cr(III)-ligand solutions for reverse phase were prepared as follows. 1.0 mM solutions of oxalic acid, L-ascorbic acid, pyrocatechol violet, disodium EDTA and $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ were prepared in glass volumetric flasks. The pH of the solutions were measured and adjusted if necessary with HCl to less than 4.0 (see Table 2). 1 ml of ligand solution was then mixed with 5 ml of Cr(III) solution (pH 4.01) in a polypropylene test tube and rotated for 1 to 7 days at room temperature. 20 ml samples were injected using an eluent (50/50 methanol/water) flow of 1 ml min^{-1} .

Final Chromatographic Method for Chromium Speciation by Cation Exchange

Fifty microliter samples were analyzed using a PRP-X200 (Hamilton) column and a flow of 1 ml min^{-1} . Two eluents, A: 0.1M CaCl_2 , pH 2.9, and B: deionized water adjusted to pH 3.0 with HNO_3 , were used with the following program:

initial: 5% A, 95%B
0 to 2.5 min: increase linearly to 10%A, 90%B
2.5 to 5.0 min: increase linearly to 30%A, 70%B
5.0 min: hold at 30%A, 70%B

Eluent flowed directly from the column to the AA for detection, with addition of air as needed to make up the difference in flow between the HPLC and the AA.

Cr(III) solutions for cation exchange were either prepared by weighing the appropriate amount of ligand or $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ into an acid washed Nalgene bottle, then diluting with 100.0 ml of deionized ultra filtered water (Fisher). Dilutions for standard curves were made in polypropylene test tubes or bottles using deionized water adjusted to pH 4.0 with HNO_3 . Ligand solutions were prepared in acid washed glass volumetric flasks. All Cr(III)-ligand solutions were prepared in acid washed Nalgene bottles.

Dichromate solutions were prepared from reference standard grade $\text{K}_2\text{Cr}_2\text{O}_7$ in deionized ultra filtered water (Fisher). The pH was not adjusted. Dilutions were made from a 5 mM stock standard.

Natural Water Samples

Samples of Cuyahoga River water was collected in Nalgene bottles on August 25, 1998, from the shoreline near the Canal Visitors Center of CVNRA. The pH of the water was 7.4 and dissolved oxygen 7.43 mg L⁻¹. Subsamples of the original (whole water) were filtered through 0.45m filters (filtered samples), or centrifuged (centrifuged samples) before being further treated with sufficient 0.1M Cr(III) to give final concentrations of 5, 1, 0.5 or 0 (blank) mM Cr(III). In all cases this addition lowered the pH although not significantly enough to prevent the eventual formation of precipitated chromium. Samples were analyzed according to the "final" HPLC protocol (below).

Samples of sediment were collected from the Grand River in Painseville and stored in plastic containers. Lake Erie water samples were also collected in Nalgene, from the beach at Headlands State Park. The Grand River and Lake Erie samples were collected on December 15, 1998. Filtered (0.45m) pore water and Lake Erie water were analyzed by HPLC. Samples were analyzed according to the "final" HPLC protocol (below).

Appendix B

Data

B-1. Example of TITRATOR calculation

0.1mM of Pb and PAR

1:10 dilution (0.01 mM Pb and 0.01 mM PAR)

CrCl₃ at pH 3.5 showing dimer

Chemical Components: Pb PAR

#	Name	Cl	Chge	Total	Initial	Log Free	Error	Std. Dev.
1	Pb+2	1	2	1.0000E-04	-5.000	-5.251	7.2370E-13	0.0000E+00
2	PAR	1	-3	1.0000E-04	-5.000	-11.873	3.0462E-13	0.0000E+00
3	CO3=	2	-2	0.0000E+00	-5.660	-5.660	2.1878E-06	0.0000E+00
4	H+	2	1	0.0000E+00	-8.000	-8.000	8.4028E-05	0.0000E+00

Ionic Strength = 0.00000
 Convergence achieved in 21 iterations.

Equilibrium Species: Pb PAR

#	Name	Cl	Log K	Std. Dev.	Molarity	P b +	P A R	C O 3 =	H +
1	Pb(OH)-	1	-7.600	0.000	1.409E-05	1	0	0	-1
2	Pb(OH)2	1	-17.100	0.000	4.456E-07	1	0	0	-2
3	Pb(OH)3	1	-28.100	0.000	4.456E-10	1	0	0	-3
4	OH-	1	-14.000	0.000	1.000E-06	0	0	0	-1
5	HPAR	1	12.300	0.000	2.675E-08	0	1	0	1
6	H2 PAR	1	17.840	0.000	9.276E-11	0	1	0	2
7	H3 PAR	1	20.530	0.000	4.543E-16	0	1	0	3
8	Pb H PAR	1	20.900	0.000	5.974E-05	1	1	0	1
9	Pb2HPAR	1	40.300	0.000	2.012E-05	1	2	0	2
10	Pb(OH)2	2	-12.800	0.000	0.000E+00	1	0	0	-2
11	PbCO3	2	-13.100	0.000	0.000E+00	1	0	1	0

Chemical Components: Pb PAR

#	Name	Cl	Chge	Total	Initial	Log Free	Error	Std. Dev.
1	Pb+2	1	2	1.0000E-03	-5.000	-4.251	4.4917E-10	0.0000E+00
2	PAR	1	-3	1.0000E-03	-5.000	-11.872	5.3183E-10	0.0000E+00
3	CO3=	2	-2	0.0000E+00	-5.660	-5.660	2.1878E-06	0.0000E+00
4	H+	2	1	0.0000E+00	-8.000	-8.000	8.4928E-04	0.0000E+00

Ionic Strength = 0.00000
 Convergence achieved in 19 iterations.

Equilibrium Species: Pb PAR

#	Name	Cl	Log K	Std. Dev.	Molarity	P b +	P A R	C O 3 =	H +
1	Pb(OH)-	1	-7.600	0.000	1.408E-04	1	0	0	-1
2	Pb(OH)2	1	-17.100	0.000	4.453E-06	1	0	0	-2
3	Pb(OH)3	1	-28.100	0.000	4.453E-09	1	0	0	-3
4	OH-	1	-14.000	0.000	1.000E-06	0	0	0	-1
5	HPAR	1	12.300	0.000	2.677E-08	0	1	0	1
6	H2 PAR	1	17.840	0.000	9.281E-11	0	1	0	2
7	H3 PAR	1	20.530	0.000	4.546E-16	0	1	0	3
8	Pb H PAR	1	20.900	0.000	5.974E-04	1	1	0	1
9	Pb2HPAR	1	40.300	0.000	2.013E-04	1	2	0	2
10	Pb(OH)2	2	-12.800	0.000	0.000E+00	1	0	0	-2
11	PbCO3	2	-13.100	0.000	0.000E+00	1	0	1	0

Chemical Components: Cr OH Cl

#	Name	Cl	Chge	Total	Initial	Log Free	Error	Std. Dev.
1	Cr+3	1	3	1.0000E-03	-7.000	-3.330	8.7394E-09	0.0000E+00
2	Cl-	1	-1	3.0000E-03	-4.000	-2.523	-2.6991E-12	0.0000E+00
3	OH-	2	-1	0.0000E+00	-10.500	-10.500	5.3164E-04	0.0000E+00

Ionic Strength = 0.00000
 Convergence achieved in 5 iterations.

Equilibrium Species: Cr OH Cl

#	Name	Cl	Log K	Std. Dev.	Molarity	C r +	C l -	O H -
1	Cr(OH)+2	1	9.800	0.000	9.338E-05	1	0	1
2	Cr(OH)2+	1	17.300	0.000	9.338E-08	1	0	2
3	Cr2(OH)2	1	24.000	0.000	2.190E-04	2	0	2
4	CrCl+2	1	-0.500	0.000	4.439E-07	1	1	0

Cr OH Cl

16:57 January 16, 1999

Cr with OH - at IS = 0

B-2. Examples of Chromatograms using reverse phase HPLC and AAS detection

Cr-EDTA
Cr-PCV
Cr-Ascorbate

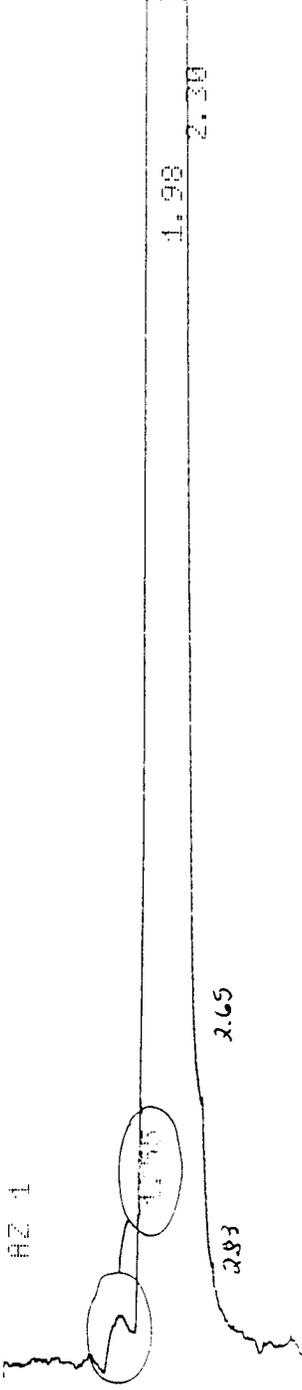
EDTA + CuCl_2

5:1

Att = 64

(fid)

CHANNEL A INJECT 00:41:09



00:41:09 CH= "A" PS= 1.

FILE 1. METHOD 0. RUN 9 INDEX 9

PEAK#	AREA%	RT	AREA	BC
1	0.717	1.55	90010	02
2	82.217	1.98	10316117	02
3	15.152	2.3	1901169	02
4	1.098	2.65	137745	02
5	0.816	2.83	102413	03

TOTAL 100.

*

2

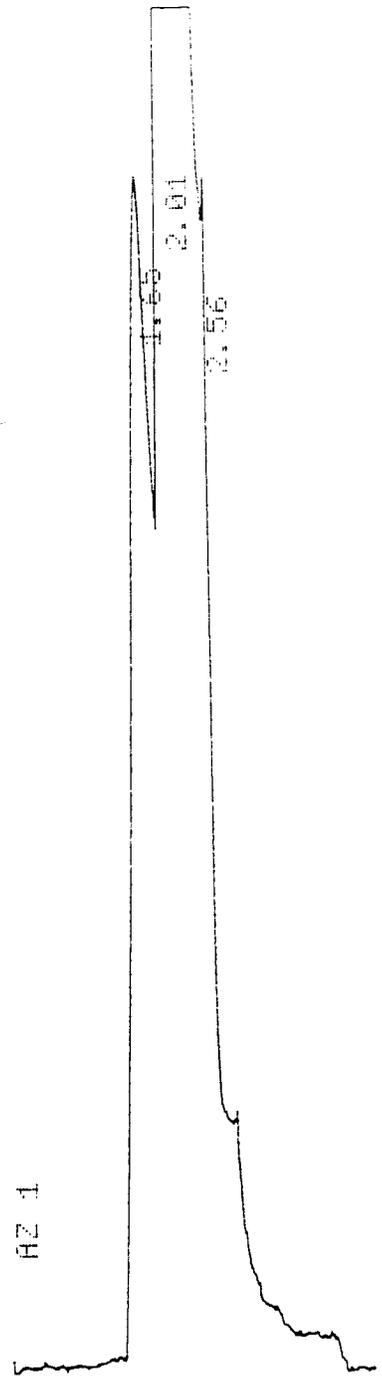
PCV+OxCl3
5:1
At = 64

*

89

CHANNEL A INJECT 00:26:48

AZ 1



00:26:48 CH= "A" PS= 1

FILE 1. METHOD 0. RUN 6 INDEX 6

PEAK#	AREA	RT	AREA BC
1	15.461	1.65	1732758 02
2	80.105	2.01	8977713 02
3	4.435	2.56	497004 03

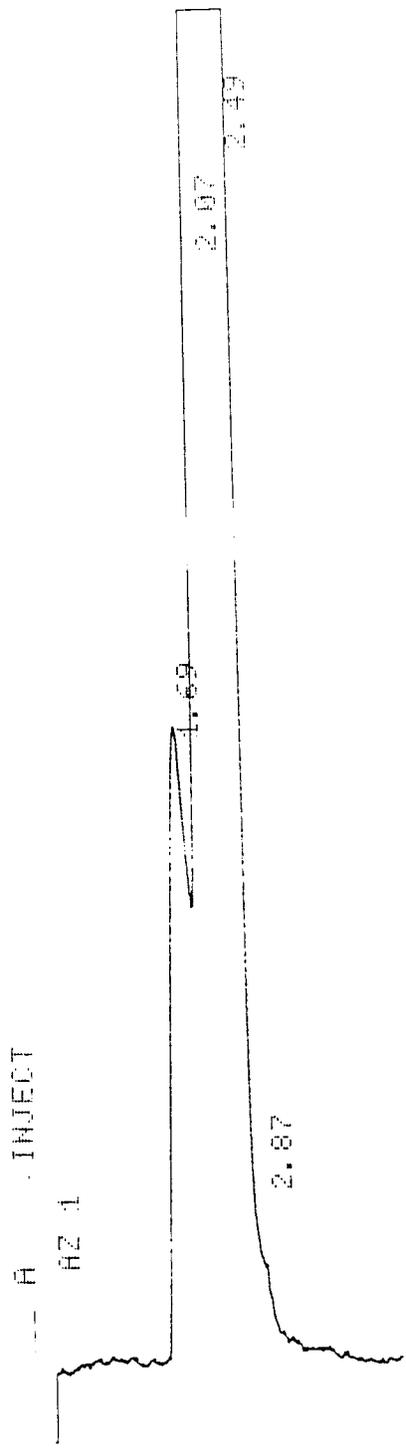
11/26/04 mple

Ascorbic Acid

5:1

(1)

*



00:03:42 CH= "A" PS= 1.

FILE 1. METHOD 0. RUN 2 INDEX 2

PEAK#	AREA%	RT	AREA	BC
1	8.568	1.69	910740	02
2	79.5	2.07	8450088	02
3	11.932	2.49	1267233	08
4	0.009	2.87	963	05

TOTAL / 100.

B-3. Standard Curves

Chromium (III)

Chromium (VI) as dichromate

Chromium (III) standard curve
Aug 3, 1998

mM Cr	area in millions	calc
1.047	1.7	1.71
0.524	0.874	0.865
0.209	0.369	0.357
0.105	0.174	0.19

Regression Output:

Constant	20964.46
Std Err of Y Est	16138.22
R Squared	0.999626
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	1609094
Std Err of Coef.	22018.81

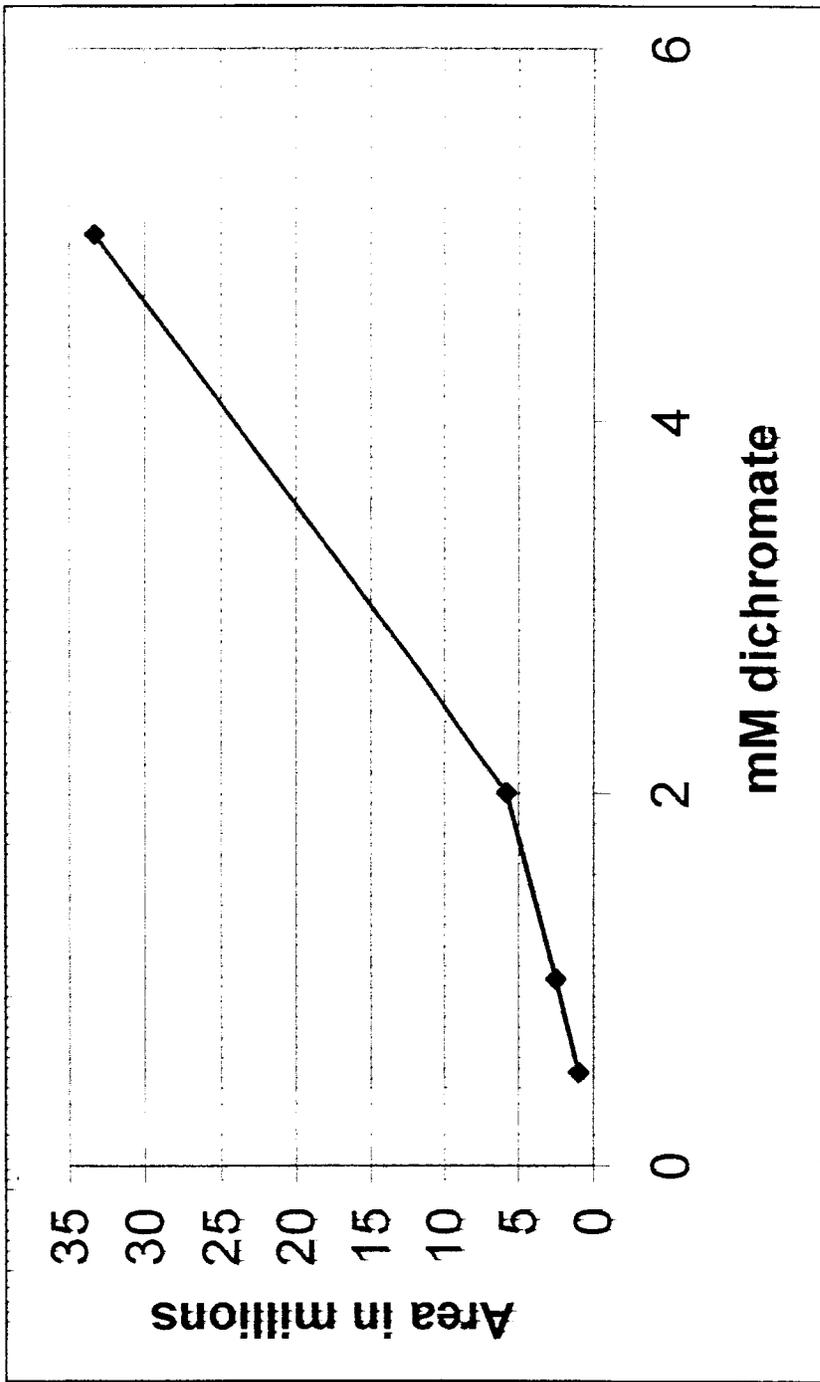
Dichromate Standard Curve

1/23/99

mM dichromate	area
	$\times 10^6$
5	33.31
2	5.768
1	2.524
0.5	1

For 3 lowest points

slope	0.313604
int	-0.622
r2	0.999884



B-4. Data from kinetic studies

Cr-EDTA
Cr-Oxalate
Cr-F

Data for the reaction of 1mM Cr(III) with three ligands

Starting Reaction, August 20, 1998 at noon (approximately)

Cr(III) from CrCl CrCl3-6H2O 0.0010206 M 100 ml in all cases
 prepared August 11, 1998 2 ml acidified water in all cases
 Final Concentration of Cr(III) 0.0009814 Molar 2 ml 50 mM ligand in all cases

EDTA

Na2EDTA 0.0499919 M prepared Aug 20, 1998
 Final Concentration of Na2EDTA 0.0009614 M

Table II: % Total Cr(III) present as different species over time (1mM each Cr(III) & EDTA-)

Ret time Tentative ID Date	time(da)	1.52	1.9	4.14	9.79	comment
				Cr-EDTA	Cr+3	
8/20	0.04		1.27			98.7
8/21	0.75		12.9	20		67.05
8/22	2.00	2.1	6	39.6		52.1
8/23	3.00	1.7	3.3	51.7		43.3
8/24	4.00	2	1.6	58.8		37.6
8/26	6.00	3	1	67	30	
8/29	9.00	2.7	0.6	74.5	22.2	
9/2	13.00	1.9		81.9	16.1	
9/11	22.00	2.1		88.2	9.73	
9/27	38.00	2		93.1	4.94	
10/11	52.00	2.23		94.6	3.2	
10/18	59.00	2.15		95.04	2.8	
11/3	73.00	2		95	2.8	
11/27	97.00	2.12		95.4	2.47	pH 2.86
1/13/99	133	2.44		94.7	2.88	

Data for the reaction of 1mM Cr(III) with three ligands

Starting Reaction, August 20, 1998 at noon (approximately)

Cr(III) from CrCl CrCl3·6H2O 0.0010206 M 100 ml in all cases
 prepared August 11, 1998 2 ml acidified water in all cases
 Final Concentration of Cr(III) 0.0009814 Molar 2 ml 50 mM ligand in all cases

Oxalate

oxalic acid 0.0500444 M prepared Aug 12, 1998 pH 3.45 at t=0
 Final Concentration of oxalate 0.0009624 M

Table III: % Total Cr(III) present as different species over time (1mM each Cr(III) & Oxalate)

Ret time Tentative ID Date	1.4 CrOx +1	2.1 CrOx2	3.8 CrOx3	9.8 Cr +3	comment
8/20	0.04			100	
8/21	0.75	12.6		87.4	
8/22	2.00	19.8	3.7	70	
8/23	3.00	26.2	3.7	70	
8/24	4.00	30.3	5.3	64.4	
8/26	6.00	33.8	6.9	59.3	
8/29	9.00				
9/2	13.00	40	13.1	46.9	
9/11	22.00	43.6	12.5	43.1	0.78
9/27	38.00	44.2	11.3	42.3	2.52
10/11	52.00	46.3	12.3	38.1	3.24
10/18	59.00	47.8	9.53	39.9	2.79
10/18	59.00	48.5	9.78	40.3	1.4
11/3	73.00	49.1	7.92	39.8	3.2
11/27	97.00	51.4	6.76	38.5	3.9
1/13/99	133	52.4	4.24	36.3	3.29

Data for the reaction of 1mM Cr(III) with three ligands

Starting Reaction, August 20, 1998 at noon (approximately)

Cr(III) from CrCl₃·6H₂O 0.0010206 M 100 ml in all cases
 prepared August 11, 1998 2 ml acidified water in all cases
 Final Concentration of Cr(III) 0.0009814 Molar 2 ml 50 mM ligand in all cases

Fluoride

NaF 0.049619048 M prepared Aug 12, 1998
 Final Concentration of NaF 0.0009542 M

Table I: % Total Cr(III) present as different species over time (1mM each Cr(III) & F⁻)

Ret time Tentative ID Date	time (da)	1.36 CrF ₂ ⁺ + CrF ₃ ⁻ ?	2 CrF ⁺²	2.6 ? dimer	5.25 Cr ⁺³	9.8	comment
8/20	0.04	0		0		100	1 hr
8/21	0.75	0		8.5		91.5	18 hr
8/22	2.00	0.67		7.55		91.8	48 hr
8/23	3.00	1.45		14.4		84.1	
8/24	4.00	1.26		16.3		82.4	
8/26	6.00	2.8		18		79.2	
8/29	9.00	3.4		25.9		70.7	
9/11	22.00	11.5		45.8		45.5	
9/27	38.00	12.41		57.73		29.9	
10/11	52.00	13.76	0	59.17	0.64	26.15	trace at 2.0
10/18	59.00	13.98	0	60.78	0	25.24	trace at 2.0 and 5.25
11/3	73.00	15.3	0.92	59.36	0.5	23.94	
11/27	97.00	16.6	0.68	60.13	0.93	21.08	pH 3.51
1/13/99	133	17.5	0.64	58.7	0.62	10	

B-5. Data from equilibrium studies

Cr-EDTA
Cr-Oxalate
Cr-F

Cr-EDTA Equilibrium study

conc EDTA standard 0.0499919 M
 conc Cr standard 0.0010038 M

The label refers to the mM EDTA

label	ml EDTA	ml Cr	total vol	mM EDTA	mM Cr
0	0	25	26	0.00	0.97
0.1	0.05	25	26	0.10	0.97
0.5	0.25	25	26	0.48	0.97
1	2	100	104	0.96	0.97
2	1	25	26	1.92	0.97

Area percents of peaks on chromatogram at different retention times

label/RT->	1.52	2.61	4.14	5.33	9.79	total area	mean area
0	0.99	0	6.78	0.21	98.4	1.01E+07	1.03E+07
0.1	2.13	0	48.7	0.93	89.9	9.76E+06	
0.5	2.61	1.04	95.4		47.6	1.04E+07	%rsd
1	2.03		95.8		2.63	1.02E+07	3.438404
2	1.79			1.66	2.4	1.05E+07	
std 1 mM Cr					97.4	1.08E+07	

Concentrations of monomeric Cr species in mM at different retention times

label/RT->	1.52	2.61	4.14	5.33	9.79
0	0.00	0.01	0.00	0.00	0.95
0.1	0.02	0.00	0.07	0.01	0.87
0.5	0.03	0.01	0.47	0.00	0.46
1	0.02	0.00	0.92	0.00	0.03
2	0.02	0.00	0.92	0.00	0.02

Concentrations calculated by assuming 100% of the Cr in solution was equivalent to 100% of the total area

Cr-Oxalate Equilibrium study

conc oxalate standard 0.0500444 M
 conc Cr standard 0.0010038 M
 pH 3.14

The label refers to the mM oxalate

label	ml oxalate	ml Cr	total vol	mM ox	mM Cr
0	0	25	26	0.00	0.97
0.5	0.25	25	26	0.48	0.97
1	2	100	104	0.96	0.97
1.5	0.75	25	26	1.44	0.97
2	1	25	26	1.92	0.97

Area percents of peaks on chromatogram at different retention times

label/RT->	1.4	2.1	3.8	5.33	9.8	total area
0	0	0	0	1.41	98.59	8.64E+06
0.5	34.73	0	3.7	0	61.58	9.52E+06
1	59.00	3.75	2.56	0.00	34.69	8.37E+06
1.5	60.16	23.11	0.742	0	15.99	8.79E+06
2	46.26	48.04	0	0	5.7	8.50E+06

std 1 mM Cr

Concentrations of monomeric Cr species in mM at different retention times

label/RT->	1.52	2.61	4.14	5.33	9.79
0	0.00	0.00	0.00	0.01	0.95
0.5	0.34	0.00	0.04	0.00	0.59
1	0.57	0.04	0.02	0.00	0.33
1.5	0.58	0.22	0.01	0.00	0.15
2	0.45	0.46	0.00	0.00	0.06

Concentrations calculated by assuming 100% of the Cr in solution was equivalent to 100% of the total area

Cr - Fluoride Equilibrium Study

conc F standard 0.050 M
 conc Cr standard 0.001 M

The label refers to the mM Cr

label	ml F	ml Cr	total vol	mM F	mM Cr
0.00	0.00	25.00	27.50	0.00	0.91
0.10	0.05	25.00	27.50	0.09	0.91
0.50	0.25	25.00	27.50	0.45	0.91
1.00	2.00	100.00	110.00	0.90	0.91
2.00	1.00	25.00	27.50	1.81	0.91
5.00	2.50	25.00	27.50	4.52	0.91

Area percents of peaks on chromatogram at different retention times

label/RT->	1.36	2.00	2.60	5.25	9.80
0.00	0.00	0.00	0.00	1.84	98.05
0.10	0.00	0.00	11.00	1.95	87.05
0.50	4.09	0.00	41.77	1.55	52.59
1.00	17.83	0.59	59.24	0.67	21.66
2.00	62.41	0.20	33.15	0.00	3.59
5.00	93.16	0.00	4.80	0.00	0.00
std 1 mM Cr				1.84	98.05

Concentrations of monomeric Cr species in mM

at different retention times

label/RT->	1.36	2.00	2.60	5.25	9.80
0.00	0.000	0.000	0.000	0.017	0.895
0.10	0.000	0.000	0.100	0.018	0.794
0.50	0.037	0.000	0.381	0.014	0.480
1.00	0.163	0.005	0.541	0.006	0.198
2.00	0.569	0.002	0.302	0.000	0.033
5.00	0.850	0.000	0.044	0.000	0.000

Concentrations calculated by assuming 100% of the Cr in solution was equivalent to 100% of the total are

B-6. Equilibrium Calculations

TITRATOR calculations

Cr with fluoride

Cr with EDTA

Chemical Components: Cr + F- using H

#	Name	Cl	Chge	Total	Initial	Log Free	Error	Std. Dev.
1	Cr(III)	1	3	9.1000E-04	-5.000	-3.091	-1.1698E-09	0.0000E+00
2	F-	1	-1	1.0000E-04	-6.000	-6.121	-5.5790E-10	0.0000E+00
3	H+	2	1	0.0000E+00	-3.510	-3.510	3.0937E-04	0.0000E+00

Ionic Strength = 0.00000
 Convergence achieved in 4 iterations.

Equilibrium Species: Cr + F- using H

#	Name	Cl	Log K	Std. Dev.	Molarity	C r (I	F -	H +
1	Cr(OH)+2	1	-9.800	0.000	4.164E-10	1	0	-1
2	Cr(OH) 2+	1	-18.180	0.000	5.617E-15	1	0	-2
3	OH-	1	-14.000	0.000	3.236E-11	0	0	-1
4	CrF+2	1	5.200	0.000	9.742E-05	1	1	0
5	CrF2 +	1	9.200	0.000	7.376E-07	1	2	0
6	CrF3	1	11.820	0.000	2.328E-10	1	3	0
7	HF	1	3.169	0.000	3.453E-07	0	1	1
8	Cr(OH) 3	2	-30.920	0.000	0.000E+00	1	0	3

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#	F-	Titration Results:			Cr(III)
		CrF+2	CrF2 +	CrF3	
1	1.000E-04	9.792E-05	6.484E-07	1.557E-10	-3.091E+00
2	2.000E-04	1.926E-04	2.859E-06	1.545E-09	-3.146E+00
3	3.000E-04	2.828E-04	7.134E-06	6.575E-09	-3.208E+00
4	4.000E-04	3.671E-04	1.416E-05	2.006E-08	-3.277E+00
5	5.000E-04	4.435E-04	2.486E-05	5.137E-08	-3.355E+00
6	6.000E-04	5.094E-04	4.039E-05	1.186E-07	-3.444E+00
7	7.000E-04	5.617E-04	6.212E-05	2.556E-07	-3.544E+00
8	8.000E-04	5.972E-04	9.132E-05	5.216E-07	-3.656E+00
9	9.000E-04	6.139E-04	1.287E-04	1.012E-06	-3.779E+00
10	1.000E-03	6.116E-04	1.739E-04	1.864E-06	-3.911E+00
11	1.100E-03	5.922E-04	2.255E-04	3.247E-06	-4.050E+00
12	1.200E-03	5.596E-04	2.809E-04	5.351E-06	-4.193E+00
13	1.300E-03	5.183E-04	3.376E-04	8.379E-06	-4.338E+00
14	1.400E-03	4.718E-04	3.929E-04	1.251E-05	-4.485E+00
15	1.500E-03	4.238E-04	4.448E-04	1.790E-05	-4.630E+00
16	1.600E-03	3.768E-04	4.917E-04	2.466E-05	-4.775E+00
17	1.700E-03	3.325E-04	5.327E-04	3.283E-05	-4.917E+00
18	1.800E-03	2.925E-04	5.664E-04	4.234E-05	-5.054E+00
19	1.900E-03	2.568E-04	5.936E-04	5.306E-05	-5.187E+00
20	2.000E-03	2.256E-04	6.146E-04	6.484E-05	-5.314E+00

#	F-	Titration Results:			Cr(III)
		CrF+2	CrF2 +	CrF3	
21	2.100E-03	1.987E-04	6.301E-04	7.745E-05	-5.434E+00
22	2.200E-03	1.757E-04	6.409E-04	9.069E-05	-5.548E+00
23	2.300E-03	1.560E-04	6.475E-04	1.043E-04	-5.656E+00
24	2.400E-03	1.392E-04	6.508E-04	1.182E-04	-5.757E+00
25	2.500E-03	1.248E-04	6.516E-04	1.322E-04	-5.853E+00
26	2.600E-03	1.124E-04	6.503E-04	1.462E-04	-5.942E+00
27	2.700E-03	1.017E-04	6.473E-04	1.601E-04	-6.027E+00
28	2.800E-03	9.236E-05	6.430E-04	1.738E-04	-6.108E+00
29	2.900E-03	8.425E-05	6.376E-04	1.874E-04	-6.184E+00
30	3.000E-03	7.714E-05	6.315E-04	2.007E-04	-6.257E+00
31	3.100E-03	7.088E-05	6.248E-04	2.137E-04	-6.326E+00
32	3.200E-03	6.533E-05	6.177E-04	2.265E-04	-6.392E+00
33	3.300E-03	6.041E-05	6.102E-04	2.390E-04	-6.455E+00
34	3.400E-03	5.601E-05	6.025E-04	2.512E-04	-6.515E+00
35	3.500E-03	5.206E-05	5.947E-04	2.630E-04	-6.573E+00
36	3.600E-03	4.850E-05	5.866E-04	2.745E-04	-6.629E+00
37	3.700E-03	4.530E-05	5.785E-04	2.858E-04	-6.683E+00
38	3.800E-03	4.240E-05	5.705E-04	2.967E-04	-6.734E+00
39	3.900E-03	3.977E-05	5.626E-04	3.074E-04	-6.784E+00
40	4.000E-03	3.737E-05	5.546E-04	3.178E-04	-6.832E+00

#	F-	Titration Results:			Cr(III)
		CrF+2	CrF2 +	CrF3	
41	4.100E-03	3.518E-05	5.468E-04	3.279E-04	-6.879E+00
42	4.200E-03	3.316E-05	5.389E-04	3.377E-04	-6.924E+00
43	4.300E-03	3.131E-05	5.312E-04	3.473E-04	-6.968E+00

44	4.400E-03	2.961E-05	5.237E-04	3.565E-04	-7.011E+00
45	4.500E-03	2.804E-05	5.162E-04	3.656E-04	-7.052E+00
46	4.600E-03	2.659E-05	5.089E-04	3.744E-04	-7.092E+00
47	4.700E-03	2.525E-05	5.017E-04	3.829E-04	-7.131E+00
48	4.800E-03	2.400E-05	4.946E-04	3.912E-04	-7.169E+00
49	4.900E-03	2.284E-05	4.876E-04	3.993E-04	-7.207E+00
50	5.000E-03	2.176E-05	4.808E-04	4.072E-04	-7.243E+00

Chemical Components: Cr EDTA

#	Name	Cl	Chge	Total	Initial	Log Free	Error	Std. Dev.
1	Cr(III)	1	3	9.6500E-04	-6.000	-3.063	9.6981E-16	0.0000E+00
2	EDTA	1	-4	1.0000E-04	-6.000	-24.337	8.6931E-16	0.0000E+00
3	H+	2	1	0.0000E+00	-3.510	-3.510	3.0903E-04	0.0000E+00

Ionic Strength = 0.00000
 Convergence achieved in 13 iterations.

Equilibrium Species: Cr EDTA

#	Name	Cl	Log K	Std. Dev.	Molarity	C r (I	E D T A	H +
1	Cr(OH)+2	1	-9.800	0.000	4.436E-10	1	0	-1
2	Cr(OH)2+	1	-18.180	0.000	5.984E-15	1	0	-2
3	OH-	1	-14.000	0.000	3.236E-11	0	0	-1
4	HL	1	11.014	0.000	1.469E-17	0	1	1
5	H2 L	1	6.320	0.000	9.183E-26	0	1	2
6	H3 L	1	2.690	0.000	6.652E-33	0	1	3
7	H4 L	1	2.000	0.000	4.197E-37	0	1	4
8	CrEDTA	1	23.400	0.000	1.000E-04	1	1	0
9	CrH EDTA	1	2.000	0.000	1.230E-29	1	1	1

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