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Liquid chromatographic separation of aqueous species of Cr(VI) and Cr(III)

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Abstract

Although chromium can be present in aqueous solutions as Cr(VI) or as any of various kinetically stable forms of Cr(III), whose distribution depends on the chemical history of the aqueous sample, most chromium speciation procedures in the literature consider only Cr(VI) and Cr(III) (as the hexaaquo species). The present paper gives examples of characterizations involving Cr(VI) and several typical forms of Cr(III) using a cation-HPLC separation scheme with spectrophotometric or radiometric detection. These results are compared with those obtained with open column cation-exchange chromatography using radiometric detection. © 1997 Elsevier Science B.V.

Keywords: Chromium; Inorganic cations; Inorganic anions

1. Introduction

Chromium is an important constituent of modern metallic alloys and has played a key role in many major technological developments. The wide utilization of chromium and its compounds by modern industry results in the discharging of large quantities of this element into the environment. In view of its paradoxical roles as an essential micronutrient in human and animal nutrition at low concentrations [as Cr(III)] and as a known carcinogen at elevated levels [as Cr(VI)], there is now a growing concern about the fate and effects of chromium in the environment [1]. Thus, chromium speciation is an important analytical operation for laboratories which determine chromium in natural waters, in drinking waters or in other consumables. Such speciation, in most cases, is taken to mean the determination of two categories of aqueous chromium: anionic [Cr(VI)] and cationic [Cr(III)]. The importance of this two-category determination lies in the greater toxicity of Cr(VI) [2].

The analytical chemistry of chromium has undergone considerable change in the last decade, with new analytical techniques that permit both speciation and an increase in the sensitivity of the chromium determination being developed and implemented. In environmental analysis, some theoretical models [3,4] predict, from both thermodynamic and kinetic considerations, that chromium(III) as $[Cr(H_2O)_4(OH)_2]^+$ and chromium(VI) as CrO_4^{2-} are

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the dominant species found in natural waters. In well aerated waters, these calculations show that the stable form is Cr(VI). However, these conclusions do not agree with most analytical data. The suggested causes [5,6] of these discrepancies are: (1) extremely slow oxidation kinetics; (2) the absence of data relative to the existence of either Cr(III) complexes or colloidal forms of Cr(III); (3) Cr(VI) reduction, induced or not; (4) loss of either (or both) Cr(VI) and Cr(III) by absorption processes and (5) the great variety of speciation techniques which complicate direct comparisons.

There are many published procedures for speciation involving only the separation of Cr(VI) and Cr(III) in aqueous samples. Some methods involve preconcentration or derivatization, while others do not. Some of these methods involve the separation of one species and the calculation of the other by the difference between the total chromium concentration and that of the measured species. Other methods separate and directly measure both Cr(VI) and Cr(III). These methods include selective adsorption [5], selective volatilization [7], selective electrochemical determination [8], precipitation [9–11], solvent extraction [12–16] and various forms of chromatography.

Planar chromatography has frequently been used to determine the radiochemical purity of $Na_2^{51}CrO_4$ [17–20], the best stationary phase being alumina with solutions of Na_2CO_3 [17] or KNO_3 [20] as the developing solvent.

Open column (gravity flow) chromatography has often been used for Cr(VI)–Cr(III) speciation. Cr(VI) is retained by activated alumina [21–24], zirconium(IV) oxide modified silica [25] or several anion-exchange resins [18,26,27] while Cr(III) is retained by several chelating resins [28–30] and cation-exchange resins [31]. Cr(VI)–Cr(III) separations using coupled anionic and cationic columns [32] and uncharged dextrans (Sephadex G) [33,34] have also been reported.

Usually only the chromium content of the non-retained species and total chromium are determined, although some methods involve elution of the retained fraction. Others perform direct determination of the chromium content on the stationary phase. Many procedures also disregard the chromium that is complexed or adsorbed to organic ligands and/or colloids. However, Cr(III), Cr(VI) and the total dissolved Cr have been measured and the organic/coloidal Cr fraction calculated by difference [35]. Recently, a method has been described [36] that can be used to isolate and measure these three dissolved forms of chromium. This technique was optimized [37] to process large sample volumes and to avoid preservation problems, such as the Cr(VI) reduction under acidic conditions or the liberation of Cr(III) from the complexed/adsorbed fractions.

High-performance liquid chromatography (HPLC) has also been used for Cr(VI)–Cr(III) speciation. Cr(III) retention on a cationic column [38–40], Cr(VI) on an anionic column [38,41–50], or both on paired anionic and cationic columns [45,46,51,52] have been reported. Many HPLC speciation studies involve the use of ion-pair chromatography on reversed-phase columns, with either cation pairing reagents [42,53–58] or anion pairing reagents, with Cr(III) eluting in the void peak [54,59–63] or complexed to form an anion [64–67]. Pre-column derivatization of Cr(III) species with lipophilic reagents for retention on reversed-phase columns has also been reported [69–74]. Detection using visible spectrophotometry, [41,67–70,72], sometimes with post-column derivatization [46,53,60], atomic emission spectrometry [58], atomic absorption spectrometry [52,59,61], inductively coupled plasma (ICP) atomic emission spectrometry (AES) [40,42–44,54–56,64], ICP-MS [45,46,49,50], amperometry [71,72], conductivity [38] and chemiluminesce [61] have all been described.

Although most of these procedures for Cr(VI)–Cr(III) speciation assume that the only significant species are $Cr(H_2O)_6^{3+}$ and CrO_4^{2-}, as well as their simple hydrolysis forms, such as $Cr(H_2O)_5(OH)^{2+}$ and $Cr(H_2O)_4(OH)^{2+}$ for Cr(III) and $HCrO_4^-$ and $Cr_2O_7^{2-}$ for Cr(VI), whose concentrations depend on factors such as pH, concentration of the Cr species, storage time, etc., many other Cr(III) species may be present in aqueous solution. For example, neutral and slightly basic solutions of Cr(III) often contain dimeric and higher polymeric forms of Cr(III), while solutions containing possible complexing agents, such as Cl^-, Br^-, SO_4^{2-}, SCN^-, etc., can have various distributions of these complexed species, such as $Cr(H_2O)_5Cl^{2+}$, $Cr(H_2O)_4Cl_2^+$, $Cr(H_2O)_4SO_4^+$, $Cr(H_2O)_2(SO_4)_2^-$ and $Cr(SCN)_n(H_2O)_{6-n}^{(3-n)+}$. These complexes constitute, in many cases, kinetically stable species that have potential

significance for an accurate description of the aqueous solution. Since some of these complexes have either no charge or negative charges, they can give totally erroneous determinations of Cr(III) in some simple Cr(VI)–Cr(III) speciation procedures, such as solvent extraction or adsorption chromatography.

Recognizing this, a number of liquid chromatographic methods have been proposed to separate the various chromium species which may be present. For separating the several Cr(III) species with open column chromatography, cation-exchange is the most used procedure [75–82], although some separations have used either charged [83] or uncharged dextrans [33]. Table 1 summarizes some of these open column systems used for separating the positively charged Cr(III) complexes, $Cr(X)_n(H_2O)_{6-n}^{(3-n)+}$

Table 1
Open column cation-exchange chromatographic separations of Cr(VI) and various Cr(III) species

Stationary phase	Eluent	Eluted species	Detection	Ref.
2 ml of Bio-Rad AG50W-X8, H^+ form, 100–200 mesh	H_2O	Anionic and neutral	Radioactivity	[79]
	0.15 mol l^{-1} $HClO_4$	$[Cr(H_2O)_4(NCS)_2]^{1+}$		
	1 mol l^{-1} $HClO_4$	$[Cr(H_2O)_5NCS]^{2+}$		
	4 mol l^{-1} $HClO_4$	$[Cr(H_2O)_6]^{3+}$		
AG50W-X4 (5×1 cm), H^+ form, 200–400 mesh + Toyopearl HW-40 (fine) (1×8+1×3 cm) (in series)	0.2 mol l^{-1} $NaClO_4$ pH 2	NCS^-	UV–Vis	[82]
		trans$[Cr(NCS)_2(H_2O)_4]^{1+}$		
		cis$[Cr(NCS)_2(H_2O)_4]^{1+}$		
		mer$[Cr(NCS)_3(H_2O)_3]$		
		fac$[Cr(NCS)_3(H_2O)_3]$		
		$[Cr(NCS)(H_2O)_5]^{2+}$		
	1 mol l^{-1} $NaClO_4$ pH 2	$[Cr(H_2O)_6]^{3+}$		
2 ml of Bio-Rad AG50W-X8, H^+ form, 100–200 mesh	0.05 mol l^{-1} $HClO_4$	Cr(VI)	Radioactivity	[31]
	4 mol l^{-1} $HClO_4$	$[Cr(H_2O)_6]^{3+}$		
	5 mol l^{-1} HCl	$[Cr_2(OH)_2]^{4+}$		
	Oxidation with basic H_2O_2	Polymers		
Dowex 50-X8, H^+ form, 100–200 mesh	0.1 or 0.2 mol l^{-1} $HClO_4$	$[Cr(Ox)_3]$	Radioactivity	[75]
	1.0 mol l^{-1} $HClO_4$	Lower charged cations		
	4 mol l^{-1} $HClO_4$	$[Cr(H_2O)_5Br]^{2+}$		
	5 mol l^{-1} HCl	$[Cr(H_2O)_6]^{3+}$		
	Not eluted	$[Cr_2(OH)_2]^{4+}$		
		Polymers		
1 ml of Bio-Rad AG50W-X8, Na^+ form, 100–200 mesh	0.01 mol l^{-1} $HClO_4$	Cr(VI) and $[Cr(H_2O)_3Cl_3]^0$	UV-Vis and radioactivity	[80]
	0.1 mol l^{-1} $HClO_4$	$[Cr(H_2O)_4Cl_2]^{1+}$		
	1 mol l^{-1} $HClO_4$	$[Cr(H_2O)_5Cl]^{2+}$		
	0.25 mol l^{-1} $Ca(ClO_4)_2$ pH 2	$[Cr(H_2O)_6]^{3+}$		
	0.50 mol l^{-1} $Ca(ClO_4)_2$ pH 2	$[Cr_2(OH)_2]^{4+}$		
	1 mol l^{-1} $Ca(ClO_4)_2$ pH 2	$[Cr_3(OH)_4]^{5+}$		
	0.5 mol l^{-1} $La(ClO_4)_3$ pH 2	$[Cr_4(OH)_6]^{6+}$		
Dowex 50W-X4, H^+ form, 200–400 mesh	Displacement with 1.2 *M* $Th(ClO_4)_4$	$[Cr(H_2O)_5Cl]^{2+}$	UV–Vis	[77]
		$[Cr(H_2O)_6]^{3+}$		
		$[Cr_2(OH)_2]^{4+}$		
		$[Cr_2OH]^{5+}$		
Sephadex SP C_{25} 8×1 cm column	0.5 mol l^{-1} $NaClO_4$ pH 2	$[Cr(H_2O)_6]^{3+}$	UV–Vis	[83]
	1.0 mol l^{-1} $NaClO_4$ pH 2	$[Cr_2(OH)_2]^{4+}$		
	2.0 mol l^{-1} $NaClO_4$ pH 2	$[Cr_3(OH)_4]^{5+}$		
	4.0 mol l^{-1} $NaClO_4$ pH 2	$[Cr_4(OH)_6]^{6+}$		

(where $n=0$–3 and X is a singly charged anion) and the hydrolysis dimer, trimer and higher polymers.

The stable, negatively charged $Cr(SCN)_n(H_2O)_{6-n}^{(3-n)+}$ ($n=3$–6) complexes have been separated by open column chromatography on Selectacel-DEAE [79] or by low-pressure chromatography using Sephadex G25 and AG50W-X4 cation resin columns in series [82].

The HPLC separation of $Cr(H_2O)_3F_3$, $Cr(H_2O)_4F_2^+$, $Cr(H_2O)_5F^{2+}$ and $Cr(H_2O)_6^{3+}$ on a cationic column using an eluent containing hydrochloric acid and 2,3-diaminopropionic acid, with post-column derivatization with 2,6-pyridinedicarboxylic acid followed by spectrophotometric detection at 235 nm has recently been reported [84]. To our knowledge this is the only published HPLC separation of the monomeric Cr(III) complexes while apparently no HPLC separations of the Cr(III) polymeric species have been reported.

We describe in this paper an HPLC cation-exchange procedure for the separation of Cr(VI) and various species of Cr(III), comparing the results to those from open column cation chromatography. The procedure has been used both in the fraction collection mode, with radiometric detection of ^{51}Cr labelled species, and in the on-line mode, with spectrophotometric detection of Cr(VI) and the several separated Cr(III) species.

2. Experimental

2.1. Reagents

All solutions were prepared with deionized water (Nanopure, Barnstead, Dubuque, IA, USA or Milli-Q, Millipore, Bedford, MA, USA) and analytical-grade reagents. The eluents for HPLC were passed through 0.45 μm HA type membrane filters (Millipore) and degassed by ultrasound and vacuum.

2.2. Radioactive material

Some $^{51}Cr(VI)$ was prepared from aqueous $^{51}CrCl_3$ (CNEN-IPEN, São Paulo, SP, Brazil) by oxidation with peroxide in basic medium [85]. Other portions were obtained as aqueous solutions of $Na_2^{51}CrO_4$ (Amersham, Little Chalfort, UK). Specific activities ranged from 6000 to 31 000 MBq mg^{-1}. All samples were submitted to radionuclidic and radiochemical analysis (precipitation as $PbCrO_4$ [9] and open column cation-exchange chromatography [80]) to show that ^{51}Cr was the only radionuclide present and that at least 98% of the ^{51}Cr was initially present as $^{51}Cr(VI)$. $^{51}Cr(III)$ was prepared by reduction of $^{51}Cr(VI)$ in acid solutions, with or without added hydrogen peroxide. In the latter case, excess peroxide was destroyed on powdered platinum black [85].

2.3. Radioactivity measurements

The 0.320 MeV γ rays from the decay of ^{51}Cr in the collected fractions were measured using a well type NaI(Tl) detector in a modular single channel γ analyser (EG and G Ortec, Oak Ridge, TN, USA and Hewlett-Packard, Palo Alto, CA, USA).

2.4. Chromium species separation by open column ion-exchange chromatography

Cation-exchange columns were prepared by placing 0.5 ml of pretreated [31] resin (Bio-Rad AG50W-X8, 100–200 or 200–400 mesh, Na^+ form, Bio-Rad, Hercules, CA, USA) in a glass column tube (40–80×5 mm) having a small (5 ml) open reservoir at the top and a porous polyethylene support disk at the exit. Flow was controlled by a small PTFE stopcock [86]. Just prior to use, the resin bed (20 mm high) was treated with 0.5 ml of a 0.02 mol l^{-1} $Na_2Cr_2O_7$ solution (to eliminate possible reducing impurities) and washed with 6 ml of 0.01 mol l^{-1} $HClO_4$ and 10 ml of deionized water. Then, 0.01 to 1 ml of sample solution containing a few μmol of the ionic species was carefully placed on the top of the column and was allowed to begin passage through the resin. When the solution to be analyzed had an acid concentration or ionic strength greater than 0.01 mol l^{-1}, the sample solution was added to an appropriate quantity of deionized water contained in the open reservoir. After the liquid level reached the top of the column, a small portion of the first eluent was used to carefully rinse the walls.

Elution was carried out using the sequence of

eluents: 0.01, 0.1 and 1 mol l^{-1} $HClO_4$; 0.25, 0.5 and 1 mol l^{-1} $Ca(ClO_4)_2$ or $Ca(NO_3)_2$ at pH 2 (adjusted with the acid), and 0.5 mol l^{-1} $La(ClO_4)_3$ or $La(NO_3)_3$ at pH 2 (Table 2). 2 ml fractions (ISCO 328 fraction collector, ISCO, Lincoln, NB, USA) of the eluate were collected for radioactivity measurements. The resin was also counted to estimate non-eluted species.

Anion-exchange columns of 0.5 ml of Bio Rad AG1-X8 resin (200–400 mesh, Cl^- form) were prepared in similar glass columns. After extensive conditioning with water and 0.01 mol l^{-1} $HClO_4$, the sample (0.01–1 ml) was placed on the column. Elution was carried out with 8 ml of 0.01 mol l^{-1} $HClO_4$, followed by 12 ml of deionized water and the resin was also counted after elution.

2.5. Chromium species separation by cation-HPLC

Several different modular HPLC systems have been used, although most of the work has been carried out with either a LDC 396 pump with pulse dampener (Milton Roy, Riviera, FL, USA) or a Waters 510 pump (Milford, MA, USA), Rheodyne 5012 solvent selection valve and Rheodyne 7010 or 8125 injector valves (Rheodyne, Cotati, CA, USA). Schoeffel 770 (Kratos, Ramsey, NJ, USA) or LDC variable wavelength detectors were used ''on-line'', while fractions were collected for ''off-line'' radiochemical detection. 80×5.0 mm, 120×4.0 mm or 230×4.0 mm stainless-steel columns were packed at 300 bar with Partisil SCX (10 μm) or Nucleosil 5SA (5 μm) (Alltech, Deerfield, IL, USA). The suspension solvent was carbon tetrachloride and the pressurizing solvent was methanol. The elution sequence (Table 3) was similar to that of the open column chromatography while the flow-rate was maintained at 1.2 ml min^{-1}. The direct on-line spectrophotometric detection of Cr(III) species was at 415 nm for concentrations above 10^{-4} mol l^{-1}. Other separations used post-column derivatization to increase the spectrophotometric signal by oxidation of the Cr(III) with Ce(IV) followed by complexation with diphenylcarbazide in a nitric acid media [87], with detection at 540 nm. The detection limit with derivatization was 10^{-6} mol l^{-1}.

2.6. Preparation of monomeric Cr(III) complexes

To a 5 ml screw-capped Erlenmeyer flask were added 1.0 ml of acid and 100 μl of high specific activity $^{51}Cr(VI)$. The system was homogenized and maintained at room temperature (19.6–20.6°C). After appropriate time periods, 10 μl-aliquots were

Table 2
Eluents and corresponding species eluted from open column cation-exchange chromatography

Eluent	ID code[1]	Volume (ml)	Eluted species[2]	ID code[1]
0.01 mol l^{-1} $HClO_4$	A	12	Anionic and/or neutral	1
0.1 mol l^{-1} $HClO_4$	B	20	$[Cr(H_2O)_4X_2]^{1+}$	2
1.0 mol l^{-1} $HClO_4$	C	20	$[Cr(H_2O)_5X]^{2+}$	3
0.25 mol l^{-1} $Ca(ClO_4)_2$ in 0.01 mol l^{-1} $HClO_4$	E	24	$[Cr(H_2O)_6]^{3+}$	4
0.5 mol l^{-1} $Ca(ClO_4)_2$ in 0.01 mol l^{-1} $HClO_4$	F	20	$[Cr_2(OH)_2(H_2O)_8]^{4+}$	5
1.0 mol l^{-1} $Ca(ClO_4)_2$ in 0.01 mol l^{-1} $HClO_4$	G	20	$[Cr_3(OH)_4(H_2O)_{10}]^{5+}$	6
0.5 mol l^{-1} $La(ClO_4)_3$ in 0.01 mol l^{-1} $HClO_4$	H	20	Higher polymer	7
Resin				R

[1] As used in the Figures.
[2] X represents a monovalent anion appropriate to the mixture being analyzed.

Table 3
Eluents and corresponding species eluted from cation-HPLC

Eluent	ID code[1]	Volume (ml)	Eluted species[2]	ID code[1]
0.01 mol l^{-1} $HClO_4$	A	12	Anionic and/or neutral	1
0.1 mol l^{-1} $HClO_4$	B	20	$[Cr(H_2O)_4X_2]^{1+}$ and	2
			$[Cr(H_2O)_5X]^{2+}$	3
1.0 mol l^{-1} $HClO_4$	C			
or		20	$[Cr(H_2O)_6]^{3+}$	4
0.1 mol l^{-1} $Ca(ClO_4)_2$ in				
0.01 mol l^{-1} $HClO_4$	D			
0.25 mol l^{-1} $Ca(ClO_4)_2$ in			$[Cr_2(OH)_2(H_2O)_8]^{4+}$	5
0.01 mol l^{-1} $HClO_4$	E	24	and	
			$[Cr_3(OH)_4(H_2O)_{10}]^{5+}$	6
0.5 mol l^{-1} $Ca(ClO_4)_2$ in				
0.01 mol l^{-1} $HClO_4$	F	20–40	Higher polymers	7–10
1.0 mol l^{-1} $Ca(ClO_4)_2$ in				
0.01 mol l^{-1} $HClO_4$	G	20	Higher polymers	

[1] As used in the Figures.
[2] X represents a monovalent anion appropriate to the mixture being analyzed.

taken and immediately analyzed or diluted in water for later analysis (''aquation solutions''). For the open tube chromatographic analyses, aliquots from concentrated acids were placed into the column reservoir containing 4 ml of water above the resin level. For cation-HPLC analyses, these aliquots were rapidly diluted in 2 ml of deionized water and 613 μl were injected.

Solutions of $CrX_n(H_2O)_{6-n}^{(3-n)+}$ were also prepared (with and without radiolabel) by dissolving a Cr(III) salt in deionized water or the appropriate dilute (0.01 mol l^{-1}) acid.

2.7. Preparation of Cr(III) polymers

Mixtures of Cr(III) hydrolysis products were obtained by extended stirring of 0.05 mol l^{-1} chromic perchlorate or nitrate solutions (radiolabelled or not) at room temperature [83] or at 50°C [77]. Dimer-rich Cr(III) solutions were obtained by oxidation of Cr(II) produced by a Jones reductor [88].

3. Results

Typical separations of ^{51}Cr labelled Cr(VI) and Cr(III) species by open column chromatography are shown in Figs. 1 and 2. The elution sequence is indicated in Table 2. The ^{51}Cr(III) species were generated by placing ^{51}Cr(VI) into an acid solution.

Fig. 1 shows the products formed when 10^{-6} mol l^{-1} ^{51}Cr(VI) is placed in 0.1 mol l^{-1} HNO_3 and stored for 24 h. The peak eluted with 0.01 mol l^{-1} $HClO_4$ (eluent A) is ^{51}Cr(VI) while peak 4 is

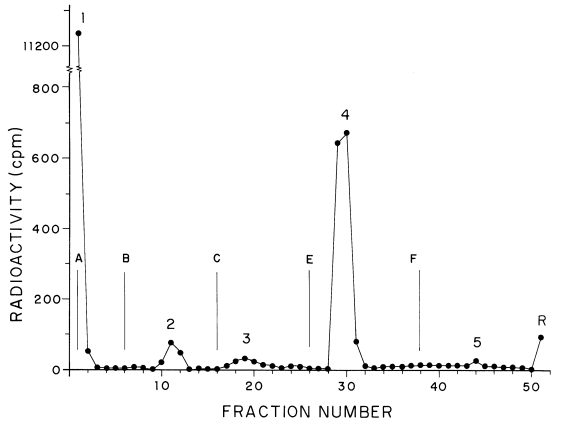

Fig. 1. Open column cation-exchange chromatogram of ^{51}Cr(VI) and several ^{51}Cr(III) products from the reaction of ^{51}Cr(VI) in 0.1 mol l^{-1} HNO_3 (24 h). 0.5 ml AG50W-X8, 200–400 mesh, Na^+ form resin in a 5 mm I.D. tube. Radiometric detection on 2 ml fractions. Eluents and peak identifications as in Table 2.

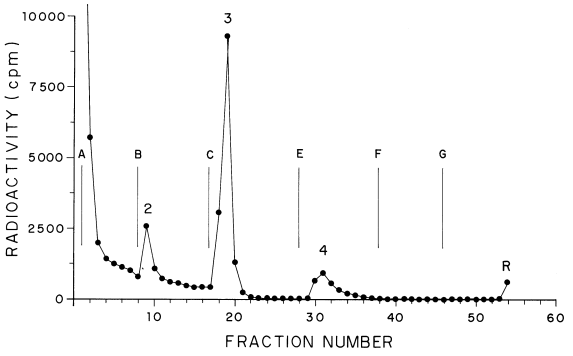

Fig. 2. Open column cation-exchange chromatogram of the $^{51}Cr(III)$ products from the reaction of $^{51}Cr(VI)$ in 37% HCl (15 min). 0.5 ml AG50W-X8, 100–200 mesh, Na^+ form resin, in a 5 mm I.D. tube. Radiometric detection on 2 ml fractions. Eluents and peak identifications as in Table 2.

$^{51}Cr(H_2O)_6^{3+}$, both identified by comparison of their elution behavior with authentic, non radioactive samples. Only small amounts of radioactivity appear in the fractions eluted with eluents B and C. These small peaks were identified as the nitrato complexes of Cr(III) by analogy to the elution sequence of the well known chloride [80] and thiocyanate [79] substituted complexes.

In the case of the reaction products from 37% HCl with 10^{-2} mol l^{-1} $^{51}Cr(VI)$ (Fig. 2), 60% of the radioactivity elutes with the first eluate, smaller amounts appearing in subsequent fractions. As indicated in Table 2, the first eluent (0.01 mol l^{-1} $HClO_4$) washes anionic and neutral species from cation-exchange column, used in the Na^+ form to prevent possible reduction of trace Cr(VI) by the H^+ form [89]. To verify if the eluate is anionic or neutral, this eluate, or the individual collected fractions, are passed through a second column containing anion-exchange resin (Cl^- form). Anionic species are retained while the neutral species elute in (less than) 8 ml of 0.01 mol l^{-1} $HClO_4$. When the first eluate from the reaction of $^{51}Cr(VI)$ and HCl is thus analyzed, all the radioactivity also elutes from the anion-exchange column, indicating that the species obtained is $Cr(H_2O)_3Cl_3$. In addition, when the reaction product mixture is stored in 0.05 mol l^{-1} HCl, the composition of the mixture slowly changes, indicating aquation of the Cr(III)–chloride complexes. Similar chromatograms and aquation reactions are seen with the reaction products of 40% HF or 37% HCl with 10^{-5} mol l^{-1} $^{51}Cr(VI)$, as well as when $Cr(H_2O)_4Cl_2^+$ is dissolved in deionized water to produce a 0.1 mol l^{-1} solution.

When 10^{-5} mol l^{-1} $^{51}Cr(VI)$ is reacted with 98% H_2SO_4, a significant quantity elutes from the cation-exchange column with the first eluent but is retained by the anion-exchange column. However, this product is identified as $Cr(H_2O)_2(SO_4)_2^-$ since the subsequent aquation process produces, first, a significant portion of the radioactivity eluting as a +1 species, $[Cr(SO_4)(H_2O)_4]^+$, and, after 4 days, the $Cr(H_2O)_6^{3+}$ species.

Fig. 3 shows the cation-HPLC separation of the Cr(III) complexes formed when 10^{-5} mol l^{-1} $^{51}Cr(VI)$ reacts with 37% HCl. Aquation studies similar to those described above indicate that peak 1, eluted with 0.01 mol l^{-1} $HClO_4$, is $Cr(H_2O)_3Cl_3$, and does not contain anionic species of either Cr(VI) or Cr(III). The two peaks eluted with 0.1 mol l^{-1} $HClO_4$ (peaks 2 and 3) correspond, by comparison of their radioactivity contents with those of a separation of the same solution with open column cation-exchange (not shown), to $Cr(H_2O)_4Cl_2^+$ and $Cr(H_2O)_5Cl^{2+}$ while a similar comparison identifies peak 4, eluted with 1.0 mol l^{-1} $HClO_4$, as $Cr(H_2O)_6^{3+}$. These attributions were confirmed by elution of an authentic mixture of these chloro complexes produced by dissolving $CrCl_3$ in dilute acid. No further radioactive species were encountered using eluents E and F.

Fig. 4 shows a similar cation separation of the

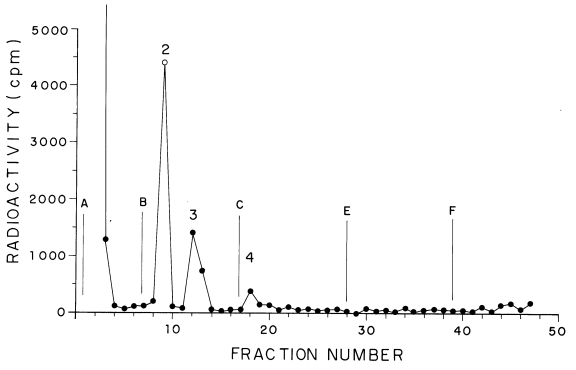

Fig. 3. Cation-HPLC chromatogram of the $^{51}Cr(III)$ products from the reaction of $^{51}Cr(VI)$ with 37% HCl (60 min). Partisil SCX, 10 μm, in a 120×4.6 mm column. Injected volume 613 μl. Radiometric detection on 2 ml fractions. Eluents and peak identifications as in Table 3.

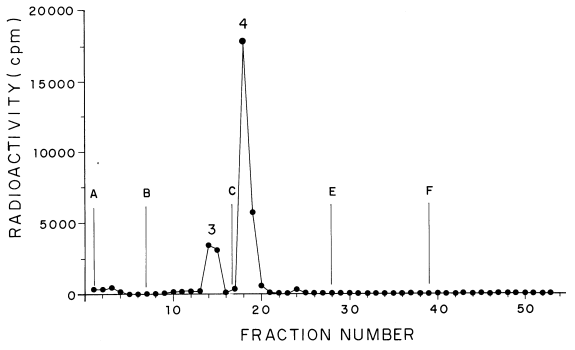

Fig. 4. Cation-HPLC chromatogram of the $^{51}Cr(III)$ products from the reaction of $^{51}Cr(VI)$ with 70% $HClO_4$ (15 min). Partisil SCX, 10 μm, in a 120×4.6 mm column. Injected volume 613 μl. Radiometric detection on 2 ml fractions. Eluents and peak identifications as in Table 3.

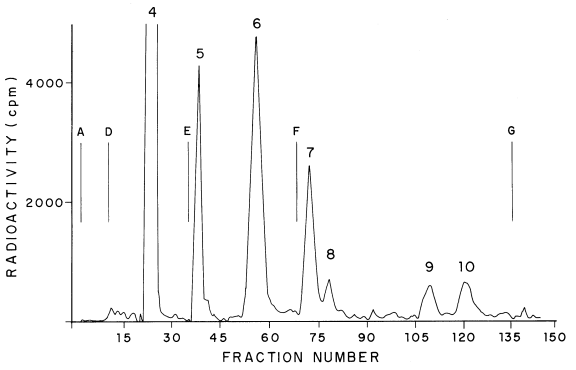

Fig. 6. Cation-HPLC chromatogram of $^{51}Cr(III)$ monomer and several hydrolysis polymers. Nucleosil 5SA, 5 μm, in an 80×5 mm column. Injected volume 15 μl. Radiometric detection on 0.5 ml fractions. Eluents and peak identifications as in Table 3.

radioactive products obtained by reacting 70% $HClO_4$ with 10^{-5} mol l^{-1} $^{51}Cr(VI)$. Identification of peak 3 as the monoperchlorato complex, $Cr(H_2O)_5(ClO_4)^{2+}$, was made through the similarity of its behavior to that of the monochloro complex in both open column and HPLC separations.

Elution of the Cr(III) monomeric complexes with ''weaker'' eluents is attributed to the lower concentrations of cation-exchanger groups on the silica-based stationary phases, in comparison to the strongly acidic polymeric resins used in open column chromatography.

Fig. 5 shows the open column cation-exchange separation of $^{51}Cr(III)$ monomer and several polymeric species of $^{51}Cr(III)$. Attribution of each peak to a distinct polymer has previously been made [80]. Figs. 6 and 7 show the separation of $^{51}Cr(III)$ monomer and several polymeric species using cation-HPLC, with radiometric detection (Fig. 6) or post-column derivatization and spectrophotometric detection at 540 nm (Fig. 7). For these separations, a $Ca(ClO_4)_2$ solution was used to elute the monomeric Cr(III), instead of 1 mol l^{-1} $HClO_4$. However, both eluents show the same elution behavior for $Cr(H_2O)_6^{3+}$ and may be used interchangeably. Note also that, for the elutions shown in Figs. 6 and 7,

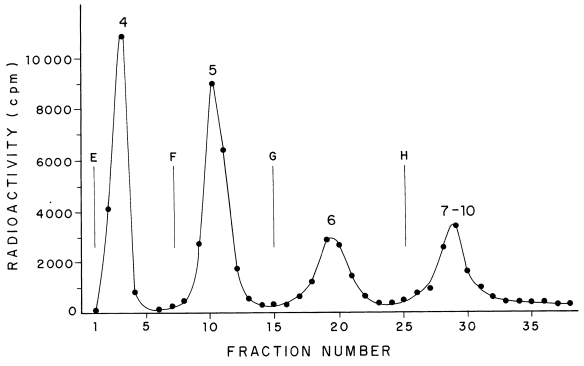

Fig. 5. Open column cation-exchange chromatogram of $^{51}Cr(III)$ monomer and several hydrolysis polymers. 0.5 ml AG50W-X8, 200–400 mesh, Na^+ form resin, in a 5 mm I.D. tube. Radiometric detection on 2 ml fractions. Eluents and peak identifications as in Table 2.

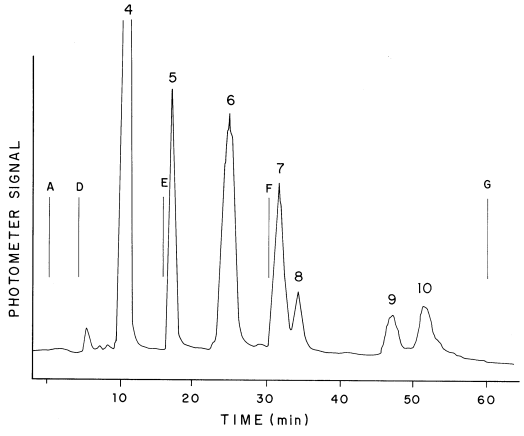

Fig. 7. Cation-HPLC chromatogram of $^{51}Cr(III)$ monomer and several hydrolysis polymers. Nucleosil 5SA, 5 μm, in an 80×5 mm column. Injected volume 15 μl. Post-column oxidation and derivitization for spectrophotometric detection at 540 nm. Eluents and peak identifications as in Table 3.

eluent B (Table 3) was omitted as no other monomeric species was present in the solution.

When the sample from the radiometric detection of cation-HPLC is analyzed by the open column method, the major peak (4) is equivalent to $Cr(H_2O)_6^{3+}$, while the next peaks (5 and 6) are equivalent to the dimeric and trimeric species. When the contents of peaks 7 and 8 of Fig. 6 are placed on an open column, they elute with $La(NO_3)_3$, suggesting the presence of tetramers [80]. A similar experiment indicates that a significant fraction of the last peaks (9 and 10) also elutes with $La(NO_3)_3$, while a portion remains on the resin. Thus, the cation-HPLC separation reveals more detail about the composition of a polymeric Cr(III) mixture than does the open column procedure. A summary of the eluants and suggested attributions of peak identities are presented in Table 3.

4. Conclusions

A cation-HPLC separation of Cr(VI) and several Cr(III) species using a step-wise gradient obtained by means of a solvent selection valve can conveniently separate all $Cr(H_2O)_n(X)_{6-n}^{(3-n)+}$ $(n=3–6)$ species as well as separate monomeric, dimeric and several higher hydrolysis polymers in a single run, as previously obtained with an open column [80], using cation-exchange stationary phases. Successful separations have been obtained with both Partisil SCX and Nucleosil 5SA with column lengths from 80 to 250 mm. The shorter columns (80 mm for 5 μm particles and 120 mm for 10 μm particles) proved to be adequate and required a shorter analysis time.

Peak attributions were made by comparison of the eluted ^{51}Cr labelled fractions using both open column cation-exchange and cation-HPLC with the same sample.

Detection of very low concentrations of the eluted species can be carried out by measuring their ^{51}Cr radioactivity, when present. Cr(III) concentrations as low as 10^{-6} mol l^{-1} can also be detected by use of post-column derivatization with diphenylcarbazide or an equivalent reagent while, due to their low molar absortivity, only higher concentrations of Cr(III) ($>10^{-4}$ mol l^{-1}) can be determined spectrophotometrically at 415 nm without derivatization.

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