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## SIMULTANEOUS DETERMINATION OF Cr(III) AND Cr(VI) AT ULTRA-TRACE LEVELS USING ION CHROMATOGRAPHY WITH CHEMILUMINESCENCE DETECTION

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### SUMMARY

An ion chromatography system was developed to determine both trivalent and hexavalent chromium. The separation system involved both anion and cation exchange columns connected in parallel with aqueous potassium sulphate as the mobile phase. Determination of Cr(III) and Cr(VI) was performed simultaneously without the need for sample pretreatment. Very-high-sensitivity detection was achieved by post-column reaction with luminol chemiluminescence. The calibration graphs for both Cr(III) and Cr(VI) were linear over at least three orders of magnitude down to the sub  $\mu\text{g l}^{-1}$  range. Detection limits found for Cr(III) and Cr(VI) were 0.1 and 0.3  $\mu\text{g l}^{-1}$  respectively. The chromium content of a simulated fresh water certified reference material was determined giving good agreement with the certificate value.

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### INTRODUCTION

Chromium exists in the environment predominantly in two oxidation states Cr(III) and Cr(VI). The hexavalent state of chromium has been shown to be more toxic than the trivalent state in animal experiments<sup>1,2</sup> and the trivalent state is necessary for the maintenance of the normal glucose tolerance factor. There is, therefore, a need for information on speciation and ion exchange chromatography would seem the most appropriate system for study.

Several workers<sup>3,4</sup> have used high-performance liquid chromatography for the simultaneous determination of Cr(III) and Cr(VI). In each case a substantial amount of sample handling and pretreatment was required which is undesirable from a trace analysis viewpoint, and also considerably lengthens the analysis time.

The post-column chemiluminescence detection of Co after ion chromatography has been shown by Jones *et al.*<sup>5</sup> to be an attractive technique due to the extremely high sensitivities obtained, coupled with the freedom of interferences and improvement in reproducibility that results when using a chromatographic technique.

Cr(III) is known to act in a similar way to cobalt in the catalysis of the luminol CL reaction<sup>5</sup> but Cr(VI) does not produce any chemiluminescence. However, Cr(VI) is easily reduced to Cr(III) and so it was considered that a useful approach would be to reduce Cr(VI) post-column just before mixing with the luminol reagent.

## EXPERIMENTAL

*Instrumentation*

The four-pump liquid chromatography system used is shown in Fig. 1. A high-pressure titanium pump (Model 2150, LKB, Bromma, Sweden) was used for the elution of Cr(VI) and a high-pressure inert plastic pump (Dionex gradient pump, Dionex, Sunnyvale, CA, U.S.A.) for the Cr(III). The reduction system employed a high-pressure stainless-steel pump (Model 6000A, Waters Assoc., MA, U.S.A.) as did the post-column reagent pump (Knauer, Bad Homburg, F.R.G.). All connections were achieved with either titanium or PTFE tubing. A sample was loaded into two sample loops, 200  $\mu\text{l}$  for Cr(III) and 100  $\mu\text{l}$  for Cr(VI), using a ten-port zirconium valve (Valco, Schenkon, Switzerland) and injected simultaneously onto two separate columns connected in parallel. The separations of Cr(III) and Cr(VI) were carried out on a Dionex HPLC CG2 cation-exchange column (50 mm  $\times$  4.6 mm I.D.) and a Dionex HPLC AG4A anion-exchange column (50 mm  $\times$  4.6 mm I.D.) respectively. The eluents and reducing agent were connected and mixed by means of a cross-piece and the resulting stream was then mixed with the luminol post-column reagent with the aid of a T-piece. This resulting solution was passed through a PTFE coiled flow cell (volume 300  $\mu\text{l}$ ) which was mounted directly onto the photomultiplier tube of a fluorescence detector (950 Floromat, Kratos, Westwood, NJ, U.S.A.) which was operated with the lamp removed.

*Reagents and standards*

Analytical-reagent grade chemicals except luminol and potassium sulphite were used throughout. Solutions were prepared with high-quality deionized water from a MilliQ system (Millipore). Potassium hydroxide, 30% hydrogen peroxide, potassium sulphate and boric acid were obtained from BDH (Poole, U.K.). Luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) was obtained from Sigma (Poole, U.K.).

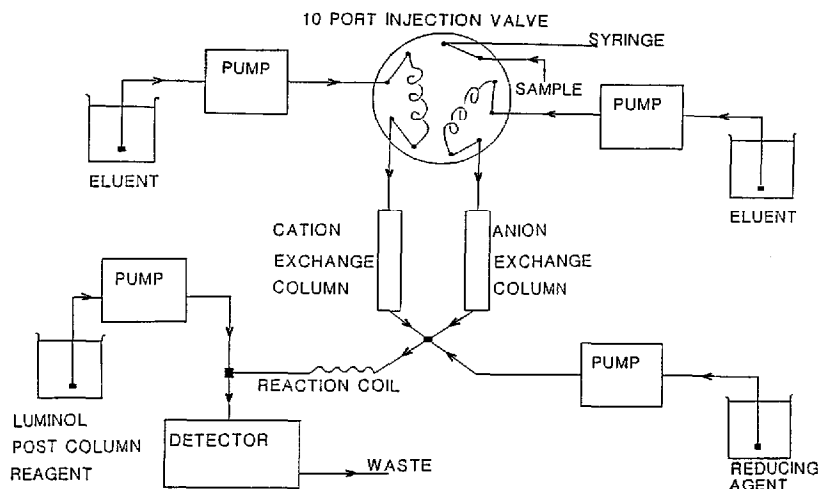


Fig. 1. The HPLC system employed for simultaneous Cr(III) and Cr(VI) determinations.

Metal standards were prepared by sequential dilution of 1000  $\mu\text{g ml}^{-1}$  Spectrosol stock solution (BDH).

#### *Eluent solutions*

The eluent used for the Cr(III) separation was prepared by diluting potassium sulphate to 0.085 *M* and the solution was adjusted to pH 3.0 with nitric acid.

The eluent used for the Cr(VI) separation was also potassium sulphate pH 3.0 but at a lower concentration of 0.003 *M*.

#### *Reduction system*

The reducing agent used was potassium sulphite 0.015 *M* which was freshly prepared daily and adjusted to pH 3.0 with nitric acid.

#### *Post-column reagent*

A 1-l volume of solution was prepared by adding 0.06 g of luminol, 1 ml of 30% hydrogen peroxide and 6.0 g of boric acid to deionized water. The post-column reagent was adjusted to pH 11.5 with concentrated aqueous potassium hydroxide.

### RESULTS AND DISCUSSION

#### *Cr(III) only*

Early attempts at the determination of Cr(III) using luminol chemiluminescence after separation on low capacity resins with lactate eluents gave very poor peak shapes. These poor peak shapes were presumably due to the slow kinetics of Cr(III)-chelate systems. The Cr(III)-chelate system was abandoned in favour of a potassium sulphate eluent which has been proved to be a useful elution system for Al(III) determinations<sup>6</sup>. As with Al(III) the strong retention of Cr(III) warranted the use of a short column.

*Detector performance.* Having optimized the luminol chemiluminescence system for Cr(III) detection, in terms of the best signal-to-noise ratio, an interference study was carried out to ascertain whether there was overlap of other metals on the Cr(III) signal. None of the 2<sup>+</sup> metals that were tested, Fe<sup>2+</sup>, Cu<sup>2+</sup>, Co<sup>2+</sup>, Mn<sup>2+</sup>, Ni<sup>2+</sup> and Zn<sup>2+</sup>, were found to interfere even at high levels [10  $\mu\text{g ml}^{-1}$  metal<sup>2+</sup> co-injected with 0.1  $\mu\text{g ml}^{-1}$  Cr(III)]. Fe<sup>3+</sup> and Al<sup>3+</sup> were also tested for interference properties and were only found to have an effect at relatively high levels [40  $\mu\text{g ml}^{-1}$  metal<sup>3+</sup> co-injected with 0.1  $\mu\text{g ml}^{-1}$  Cr(III) almost completely suppressed the Cr chemiluminescence signal].

*Linear range detection limit.* Linear calibrations were obtained covering approximately three orders of magnitude from 0.5  $\mu\text{g l}^{-1}$  to 1 mg l<sup>-1</sup>. The calibration plot of these data showed excellent linearity ( $r = 0.9999$ ). The reproducibility was acceptable at the 100  $\mu\text{g l}^{-1}$  level where a relative standard deviation of 3.3% was found for twelve replicate injections. The detection limit defined as three standard deviations of the blank signal was 0.1 ng.

#### *Cr(VI) only*

Hexavalent chromium itself will not catalyse the luminol chemiluminescence reaction. Since it has been shown that Cr(III) is an efficient catalyst, Cr(VI) was reduced post-column by an aqueous stream of potassium sulphite. The Cr(III) thus

produced was then free to catalyse the luminol chemiluminescence reaction. Other reduction systems using on-line columns packed with various reducing metals such as Cd, Zn and Sn were tried but proved unsatisfactory. The main problem with these on-line reductors were that the packing materials were not of good quality and so (a) the chromatography of the Cr(III) and Cr(VI) was affected and (b) the noise level increased to a much higher level.

*Detector performance.* Having optimized the reduction conditions, an interference trial was set up to see if there was any suppression or enhancement from overlap of the chromate signal with other anions. Phosphate, vanadate and molybdate were all tested and found not to interfere even at relatively high concentrations ( $10 \mu\text{g ml}^{-1}$  anion co-injected with  $0.1 \mu\text{g ml}^{-1}$  chromate).

*Linear range and detection limit.* A linear range of at least three orders of magnitude was established ( $1 \mu\text{g l}^{-1}$  to  $1 \text{mg l}^{-1}$ ) although the absolute upper limit was not determined. The calibration plot from these data showed good linearity ( $r = 0.999$ ). The reproducibility was good at the  $150 \mu\text{g l}^{-1}$  level where a relative standard deviation of 2.1% was obtained for seven replicate trials.

The detection limit ( $3\sigma$  the blank signal) was found to be 0.3 ng. It is felt by the authors that the detection limit could be improved somewhat if a higher purity reduction system were employed.

#### *Simultaneous determination of Cr(III) and Cr(VI)*

Using the ten-port injection valve with two sample loops the system was set up to load two replicate samples from the same standard solution. When switched to inject,

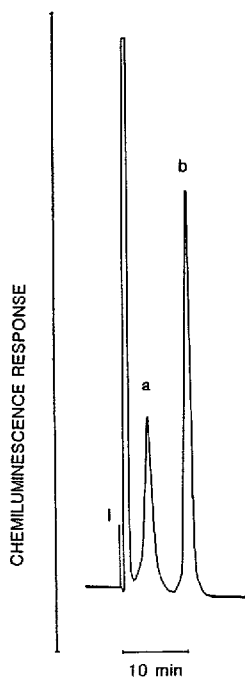


Fig. 2. A typical separation of (a) Cr(VI) and (b) Cr(III) with chemiluminescence detection.

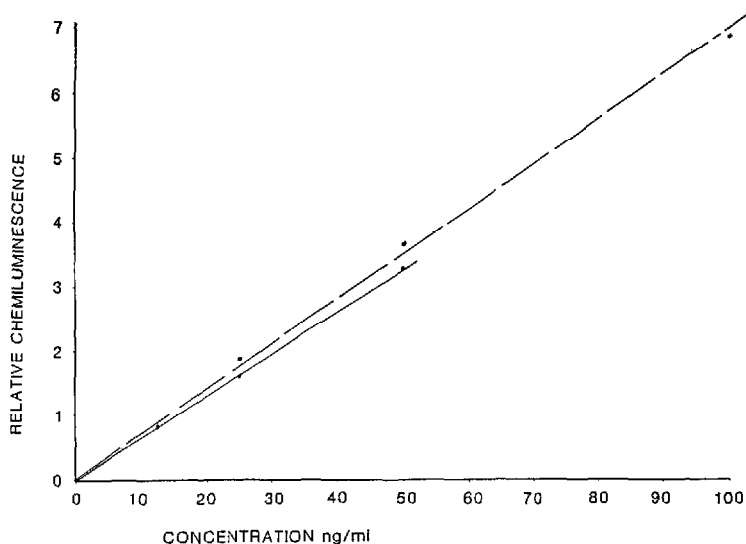


Fig. 3. Simultaneous calibration of (—) Cr(III) and (---) Cr(VI).

one sample was injected onto the cation-exchange column whilst the other was injected onto the anion-exchange column. Fig. 2 shows a typical trace of this simultaneous determination, both analytes being  $150 \mu\text{g l}^{-1}$  in the standard. Of the sample that is injected onto the cation-exchange column any Cr(VI) that is present will have no attraction for the column and will elute on the solvent front. Similarly any Cr(III) that is present in the sample that is injected onto the anion exchange column will also elute on the solvent front. It can be seen from Fig. 2 that these two solvent fronts combine to produce a signal.

The conditions are such that Cr(VI) elutes from the anion-exchange column after 5 min whilst the Cr(III) is retained on the cation-exchange column for well over 10

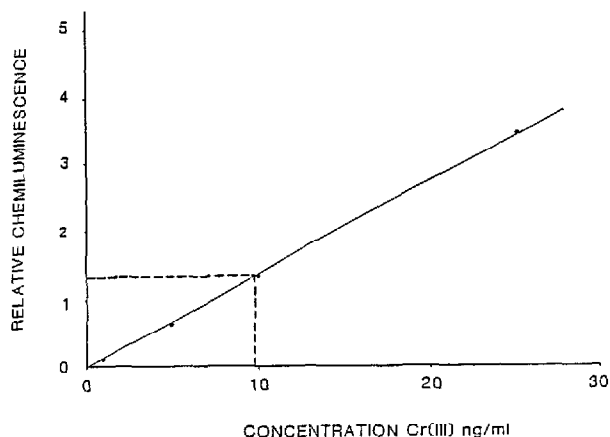


Fig. 4. The determination of Cr(III) from a certified fresh water sample IAEA4.

min thus ensuring complete separation of the two species. Fig. 3 shows a simultaneous calibration of the two species at the lower end of the linear range.

#### *Sample analysis*

To evaluate the quantitative performance and accuracy of the system a sample of low chromium (III) content was chosen. Unfortunately no certified reference material with both Cr(III) and Cr(VI) present was available so a simulated fresh water sample IAEA/W4 with a Cr(III) content of  $9.9 \text{ ng ml}^{-1}$  was selected.

The analysis was performed by simply injecting a  $200\text{-}\mu\text{l}$  sample into the system, followed by the appropriate blank solution. Certificate value Cr(III),  $9.9 \text{ ng ml}^{-1}$ ; confidence level,  $9.0\text{--}10.5 \mu\text{g ml}^{-1}$ ; found value ( $\bar{x}$ ) on seven trials,  $9.9 \text{ ng ml}^{-1}$ ; R.S.D. on seven trials, 3.3% (see Fig. 4).

#### CONCLUSION

The simultaneous determination of the two chromium species without any sample pretreatment has been achieved and described. Good sensitivity is obtainable with chemiluminescence detection. Even better detection limits are possible with an improved reduction step for Cr(VI) to Cr(III). The use of a good quality on-line reductor *e.g.* a cadmium metal immobilized short column, would eliminate one of the pumps and therefore reduce the complexity of the system. However problems were found when using this type of set up and so this on-line reduction system was abandoned in favour of the less troublesome four pump aqueous reduction system.

The near absence of any interferences and the successful determination of Cr(III) in a certified reference material combined with the excellent sensitivity and linearity of the system may possibly make it an attractive method for chromium analyses in real samples.

#### REFERENCES

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