Speciation analysis of Cr(III)–Cr(VI) using flow injection analysis with fluorimetric detection

Evangelos K. Paleologos, Spyros I. Lafs, Stella M. Tzouwara-Karayannis and Miltiades I. Karayannis*
Department of Chemistry, University of Ioannina, 45110 Ioannina, Greece

A relatively simple, sensitive, selective, automatic fluorimetric method for the simultaneous determination of chromium(III) and chromium(VI) by flow injection analysis (FIA) was developed. The method is based on the selective oxidation of the non-fluorescing reagent 2-(α-pyridyl)thioquinaldinamide (PTQA), which with Cr(III) yields an intensely fluorescent product ($\lambda_{ex} = 360$ nm; $\lambda_{em} = 500$ nm). Chromium(III) is oxidized on-line to Cr( VI) with sodium metaperiodate and the Cr(VI) is subsequently treated with PTQA. Fluorescence due to the sum of Cr(III) and Cr(VI) is measured and Cr(III) is determined from the difference in fluorescence values. The effects of various analytical parameters, such as acidity, flow rate, sample volume, temperature, reagent concentration and interfering species, were studied. Kinetic studies using both the stopped-flow technique and the FIA procedure were utilized in order to study and optimise the oxidation conditions for Cr(III) on the basis of its oxidation efficiency. The calibration graphs were rectilinear in the ranges 0.1–10 $\mu$g ml$^{-1}$ for Cr(III) and 0.1–1.0 $\mu$g ml$^{-1}$ for Cr(VI). The method was successfully verified by performing recovery experiments of Cr in several standard reference materials (peach leaves, sediments and tea), and it was applied to the speciation analysis of Cr(III)–Cr(VI) in environmental waters (mineral, tap and distilled water), a food sample (tomato juice) and synthetic mixtures. Up to 30 samples per hour can be analyzed with a relative standard deviation of about 0.1–2%.

Keywords: Flow injection; fluorimetry; chromium speciation; 2-(α-pyridyl)thioquinaldinamide; on-line oxidation

Chromium is an important trace element in nutrition, playing an essential role in the activation of insulin (resulting in a decrease in glucose levels in the blood) and as a component of the glucose tolerance factor (GTF). At the same time it is a toxic heavy metal, its toxicity depending on its oxidation state, Cr(III) being significantly more toxic than Cr(VI) owing to its mobility in aqueous solutions. It is also a carcinogen even at trace levels. Therefore, speciative determination of chromium is of particular interest.

The increasing production of Cr(III) due to oxidation of Cr(VI) in industrial plants and from spontaneous oxidation in soils due to the presence of strong oxidants such as permanganate, poses a great danger of Cr(III) accumulation in the environment with the possibility of it being taken up by plants.4–9

In view of the above, and taking into account the nutrient role of Cr(VI), the accurate determination of chromium, even at trace levels, using simple, sensitive and rapid methods becomes of great importance.

Although molecular fluorimetry is one of the most powerful trace analysis techniques, it has rarely been applied to the determination of Cr(VI) owing to the character of the metal ion, i.e., it is easily reducible and forms colored ions. Moreover, its strong oxidative effect on organic reagents used as ligands could well destroy them. Consequently, only a few direct fluorimetric methods, such as that using Safranine T in a non-aqueous solvent (dichloroethane), have been reported.10 No direct fluorimetric method is available that is suitable for the determination of Cr(VI) in aqueous solution. Element-specific trace analytical techniques, e.g., AAS and ICP-AES,11–15 can only be applied to the determination of total chromium, or for the metal ‘speciation’ only after the separation or isolation of Cr(VI) from Cr(III) in their mixtures.

The aim of this study was to develop a simple flow injection analysis (FIA) system for the simultaneous determination of Cr(III) and Cr(VI) using an alkaline solution of sodium metaperiodate to effect the on-line oxidation of Cr(III) to Cr(VI). PTQA has been reported as a spectrophotometric reagent but, to our knowledge, it has not been used previously for the simultaneous determination of Cr(III) and Cr(VI) in an FIA system.16–19 This paper reports its use in a very sensitive, highly specific spectrophotometric method for simultaneous determination of Cr(III) and Cr(VI). The method is based on the selective oxidation of the non-fluorescing reagent PTQA by Cr(VI) to produce an intensely fluorescing product in aqueous solution at room temperature. All reactions involved in the procedure are fast and no extraction or separation is required. With suitable masking, the reaction can be made highly selective.

Experimental

Apparatus

The FIA manifold for the simultaneous determination of Cr(III) and Cr(VI) was made of Teflon and poly(vinyl chloride) (PVC) tubing of various diameters, and a linear dual connector was used (Fig. 1). The system consisted of a four-way pneumatically actuated injection valve (Type 50, Teflon; Rheodyne, Cotati, CA, USA), an eight-channel peristaltic pump (Ismatec, Glattburg-Zurich, Switzerland) and a spectrofluorimeter (RF-551; Shimadzu, Kyoto, Japan), equipped with a 12 μl flow-through measurement cell.

Data processing and collection were performed by an IBM-compatible personal computer by means of software written in

Fig. 1 Schematic representation of FI manifold employed for the simultaneous determination of Cr(III) and Cr(VI). C1, carrier 1, H2O; C2, carrier 2, a mixture of KIO4 and NaOH; R, reagent, PTQA in H2SO4; S.v., selector valve; P, pump; V, valve; s, sample; D, detector; W, waste; and PC, personal computer.
Microsoft Q Basic. The interface unit was an RTL 800/815 multi-function input/output board. The software allows the automatic injection of the sample, the storage of the FIA curve and the digital presentation of the peak maximum, which is taken as a measure of the concentration of the analyte, CrIII. A Varian (Palo Alto, CA, USA) AA-300 atomic absorption spectrometer using an air-acetylene flame was used for comparison of some results.

Kinetic measurements, for the determination of the most suitable oxidizing agent, were performed with a Biosequential SX17MV stopped-flow apparatus (Applied Photophysics, Leatherhead, UK), equipped with a Varian E-109 spectrometer.

**Reagents**

All chemicals were of analytical-reagent grade or the highest purity available. Distilled water and AnalAr-grade propan-2-ol, which is non-fluorescent under UV radiation, were used throughout.

**CrIII standard solutions**

Volumes of 100 ml of stock standard CrIII solutions (0.52 mg ml⁻¹) were prepared by dissolving 266.5 mg of chromium(III) chloride hexahydrate (99.9%) (Merck, Darmstadt, Germany) in distilled water. The solutions were kept in a calibrated flask and stored in a refrigerator. Working standard solutions were prepared daily by appropriate dilution with distilled water.

**CrVI standard solutions**

Volumes of 100 ml of stock standard CrVI solutions (1 mg ml⁻¹) were prepared by dissolving 294.2 mg of sodium dichromate (99.9%) (Merck) in distilled water. The solutions were kept in a calibrated flask and stored in a refrigerator. Working standard solutions were prepared daily by appropriate dilution with distilled water.

**Periodate solution**

A 100 ml volume of a stock standard solution of periodate (10⁻² M) was prepared by dissolving 218.3 mg of sodium metaperiodate (99.8%) (Merck) in distilled water. The solution was kept in a dark bottle in a refrigerator to avoid photo-degradation.

**Alkaline solution**

A 1 l stock standard alkaline solution (0.1 M) was prepared by dissolving 4.061 g of ACS-grade sodium hydroxide (98.5%) (Riedel-de Haën, Seelze-Hannover, Germany) in distilled water. The solution was kept in a well sealed polyethylene container for preservation.

**H₂SO₄ solution**

A 0.5 l volume of H₂SO₄ stock standard solution (9 M) was prepared by diluting 250 ml of concentrated H₂SO₄ (95–97%) (Merck) with distilled water.

**Carrier solutions**

(1) Distilled water was used as the carrier solution for the determination of CrIII. (2) An alkaline solution containing 10⁻⁴ M periodate + 10⁻² M NaOH was used as carrier for the determination of CrVI. Fresh working standard solutions were prepared daily by appropriate dilution of the stock standard solution with distilled water.

2-(α-Pyridyl)thioquinaldinamidine (PTQA) solution 10⁻³ M

The reagent was synthesized according to the procedure described by Porter. A mixture of 2-aminopyridine (2 mol), quinaldine (1 mol) and sulfur powder (1.5 mol) was refluxed for 6 h in a 250 ml round-bottomed flask fitted with a bulb condenser at a controlled temperature (140–150 °C) at 1 atm pressure over a sand-bath. The reaction mixture was stored overnight. The thiocompound was filtered off and crystallized from light petroleum to give a yellow crystalline solid in the form of small needles. The compound was recrystallized from warm ethanol and was subsequently dried under vacuum (0.1 mmHg) for 24 h.

The melting point of this product (PTQA) was 155 ± 2 °C and the elemental analysis data (C 72.25, N 13.35, H 4.25%) were very close to the expected values. The reagent stock standard solution (10⁻³ M) was prepared by dissolving the requisite amount of PTQA in propan-2-ol (26.6 mg in 100 ml). Working standard solutions of PTQA (2 × 10⁻⁴ M) were prepared by mixing an appropriate volume of the stock standard solution with 9 M H₂SO₄ solution and diluting with distilled water.

**Other solutions**

Solutions of a large number of inorganic ions and complexing agents (Table 2) were prepared from their water-soluble salts. Where insoluble salts were included, special dissolution methods were adopted.

**Preparation of samples**

Tomato juice was obtained from fresh tomatoes, purchased from the local grocery store, using a common household blender.

Lake water samples were taken from a single location at Lake Pamvotis in Ioannina and tap water from a tap in the University of Ioannina.

**Procedure**

Standards (0.1–10 μg ml⁻¹ CrIII or 0.1–1.0 μg ml⁻¹ CrVI) and sample solutions were injected into the carrier stream by means of the peristaltic pump, P (Fig. 1). The solutions were then analyzed by different routes using a double switch. First the switch allowed the flow of distilled water, carrying the sample to the FIA path, where PTQA (2 × 10⁻⁴ M) was added after the sample valve and then the reacting mixture passed directly through the measuring cell where the fluorescence intensity, F1 due to CrIII was measured at 500 nm (excitation at 360 nm). When the carrier switch was turned to position 2 it allowed the flow of the IO₄⁻ – OH⁻ solution, which oxidized CrVI to CrIII. The oxidized sample was then treated with the PTQA solution which merged after the sample switch and the resulting mixture passed through the measuring cell. This time the measured fluorescence, F₂, was due to the total Cr contained initially in the sample (CrIII and CrVI). The concentration of CrIII was then determined from the difference F₂ – F₁ of the peak maxima.

Both reactions are very fast and the fluorescence intensity remains stable for at least 24 h. The PTQA solution shows very low fluorescence compared with that produced by the CrIII – PTQA system.

The concentration of CrIII and/or CrVI was determined from the peak height of the signal which was measured automatically and stored in computer memory. A calibration graph was constructed using standard solutions.

**Results and discussion**

**Selection of oxidizing agent**

The selection of the best oxidizing agent for CrVI was based on its ability to perform the oxidation rapidly and quantitatively,
while not creating interferences in the analytical procedure and problems with the FIA paths. Among several oxidizing agents suggested such as permanganate, peroxodisulfate, hydrogen peroxide and periodate, only the last two met the above criteria. For these oxidizing agents, kinetic studies were carried out using the stopped-flow technique and the results presented in Fig. 2 show a higher efficiency for periodates.

**Optimization of the flow injection system**

Preliminary tests were conducted using different flow assemblies in order to select the optimum manifold configuration. The configuration shown in Fig. 1 was selected since it resulted in the best mixing and reaction conditions and as the optimum peak height and shape.

In order to optimize the selected flow injection manifold, the effect of hydrodynamic and chemical parameters on the peak height, shape and reproducibility were studied.

The univariate method was adopted for optimizing the system. Table 1 shows the results of optimization for Cr(III) and Cr(VI) concentrations of 5 × 10^{-6} and 1 × 10^{-5} M, respectively.

The optimum concentration of the periodate–alkaline solution, along with flow rate and mixing time for the oxidation of the Cr(III) sample, were established by using a 0.5 M KIO_{4} solution. Concentrations of periodate of 10^{-4}–0.1 M were tested, keeping the sample volume at 100 μl and the overall flow rate at 0.5 ml min^{-1}. Different sample volumes (50–500 μl) and flow rates (0.5–1.5 ml min^{-1}) were tested keeping the oxidative reagent concentration at the previously optimized value.

Eventually a compromise had to be established for the analytical conditions in order to achieve efficient reactions in addition to good sampling rates and peak maxima. Concentrations of IO_{4}^{-} and OH^{-} of 10^{-4} and 10^{-2} M, respectively, a sample volume of 150 μl, an overall flow rate of 0.90 ml min^{-1}, reagent and carrier flow rates of 0.45 ml min^{-1}, a mixing time of approximately 40 s and an average temperature of 20–25 °C were selected for subsequent experiments.

Of the various acids (sulfuric, hydrochloric, nitric and phosphoric) studied, sulfuric acid was found to be the most suitable for the system. Different concentrations of sulfuric acid were tested in the range shown in Table 1. The optimum concentration was selected so that the fluorescence of PTQA was negligible in comparison with the Cr(III)–PTQA system, while not being significantly altered by the 0.01 M alkaline solution during the determination of Cr(III). It was found that 1.08 M H_{2}SO_{4} was suitable. Although Fig. 3 shows that 1.08 M H_{2}SO_{4} ensures the maximum responses for both systems, PTQA and PTQA–Cr(III), the values are in favor of the latter. For instance, compared with a 5 × 10^{-4} M concentration of Cr(III) with PTQA, the fluorescence of PTQA alone is about ten times smaller.

Different solvents were examined in order to study their effect on the fluorescence intensity. Chloroform, benzene, carbon tetrachloride and isobutanol showed no fluorescence in the organic phase. Among butan-1-ol, ethanol and propan-2-ol, which gave fluorescence, propan-2-ol performed better as it showed a more intense and constant fluorescence and higher solubility for PTQA. Therefore, 20% propan-2-ol solution was selected for determination in the studied range of Cr concentrations.

The reaction leading to the fluorescent product was very fast. Constant and maximum fluorescence was attained promptly after mixing the reagents and it remained stable for 24 h. Different concentrations of PTQA solutions were tested in the ranges shown in Table 1. A reagent concentration of 2 × 10^{-4} M was the optimum for this manifold.

**Evaluation of the method**

The reproducibility of the procedure and sample throughput were evaluated by repeated injections of 0.5 μg ml^{-1} Cr(III) and 0.5 μg ml^{-1} Cr(VI). The relative standard deviation was found to be 0.1–2.2% (n = 5) for 0.1–1.0 μg ml^{-1} Cr(III) and 0.1–10 μg ml^{-1} Cr(VI), which indicated that the method was precise and reproducible over a wide concentration range. Calibration graphs were obtained using the peak height and were rectilinear for 0.1–1.0 μg ml^{-1} Cr(III) and 0.1–10 μg ml^{-1} Cr(VI). The regression equations of the above calibration graphs are for Cr(III) y = 1261.8x + 1.74 with a correlation coefficient r = 0.9997 and for total Cr y = 24245.5x + 0.17 with r = 0.9982. The detection limit, defined as three times the baseline noise, was 0.05 μg ml^{-1} for Cr(III) and 0.02 μg ml^{-1} for Cr(VI). The sample throughput was 30 measurements per hour, provided that autosampling was available, and the dispersion coefficient was determined with a 0.5 μg ml^{-1} Cr(III) and 0.5 μg ml^{-1} Cr(VI) standard solution as described earlier.

The performance and reproducibility of the proposed method are shown in Tables 2–4. The reliability of the method was achieved in good mixing and reaction conditions and as the optimum peak height and shape.

---

**Table 1** Selected chemical and FIA parameters obtained after the optimization experiments

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Studied range</th>
<th>Selected value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size of sample loop/μl</td>
<td>50–500</td>
<td>150</td>
</tr>
<tr>
<td>Overall flow rate/ml min^{-1}</td>
<td>0.5–1.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Reagent flow rate/ml min^{-1}</td>
<td>0.25–0.75</td>
<td>0.45</td>
</tr>
<tr>
<td>Temperature/°C</td>
<td>20–70</td>
<td>25.0</td>
</tr>
<tr>
<td>H_{2}SO_{4} concentration/M</td>
<td>0.20–1.80</td>
<td>1.08</td>
</tr>
<tr>
<td>PTQA concentration/M</td>
<td>10^{-5}–10^{-3}</td>
<td>2 × 10^{-4}</td>
</tr>
<tr>
<td>KIO_{4} concentration/M</td>
<td>10^{-6}–10^{-2}</td>
<td>10^{-3}</td>
</tr>
<tr>
<td>NaOH concentration/M</td>
<td>10^{-4}–10^{-1}</td>
<td>10^{-2}</td>
</tr>
</tbody>
</table>

**Fig. 2** Reaction curve profiles for the oxidation of Cr(III) to Cr(VI) with IO_{4}^{-} and H_{2}O_{2}, as studied with a stopped-flow spectrophotometer (λ = 373 nm, T = 25 °C).

**Fig. 3** Effect of acidity on the fluorescence of the Cr(VI)–PTQA system (○) compared with the fluorescence of PTQA alone (●).
assessed by analyzing certified reference materials. The results for total Cr were in good agreement with the certified values (Table 2). The method was also tested by analyzing several synthetic mixtures containing standard Cr^vi and Cr^iii (Table 3). Further reliability tests of recovery studies are shown in Table 3. The average recovery obtained by spiking Cr^iv and Cr^iii into water and tomato juice samples was quantitative. The precision and accuracy of the method are satisfactory.

The interference of several ions that may be found in environmental samples in addition to Cr^iii and Cr^vi was studied and accuracy of the method are satisfactory. In order to test the method, all the Cr was first oxidized to Cr^vi, which was determined. Then it was all reduced to Cr^iii by treatment with H_2O_2 in an acidic medium and determined as Cr^iii.

### Table 2 Recoveries of total Cr for certified reference materials

<table>
<thead>
<tr>
<th>Reference material</th>
<th>Cr^iii found ±</th>
<th>Cr^iv found ±</th>
<th>Total Cr certified value/µg g^-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tea</td>
<td>0.78 ± 0.05</td>
<td>0.80 ± 0.05</td>
<td>0.80</td>
</tr>
<tr>
<td>Peach leaves</td>
<td>0.96 ± 0.14</td>
<td>0.93 ± 0.14</td>
<td>0.94 ± 0.14</td>
</tr>
<tr>
<td>Estuarine sediment</td>
<td>190 ± 1</td>
<td>190 ± 1</td>
<td>192</td>
</tr>
</tbody>
</table>

* Average of five runs. The certified values were referred to total Cr. In order to test the method, all the Cr was first oxidized to Cr^ii, which was determined. Then it was all reduced to Cr^iii by treatment with H_2O_2 in an acidic medium and determined as Cr^iii.

### Table 3 Simultaneous determination of Cr^ii and Cr^iv in synthetic mixtures of spiked and standard Cr^ii and Cr^iv in distilled water and real samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cr^ii added/ ng ml^-1</th>
<th>Cr^iv added/ ng ml^-1</th>
<th>Cr^iii found/ ng ml^-1</th>
<th>Cr^ii found/ ng ml^-1</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>0</td>
<td>200</td>
<td>0</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>200</td>
<td>195</td>
<td>200</td>
<td>97.5</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>200</td>
<td>400</td>
<td>404</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>500</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>100</td>
</tr>
<tr>
<td>Tap water</td>
<td>0</td>
<td>200</td>
<td>0</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0</td>
<td>200</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>300</td>
<td>285</td>
<td>310</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>400</td>
<td>425</td>
<td>405</td>
<td>98</td>
</tr>
<tr>
<td>Mineral water</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>300</td>
<td>285</td>
<td>310</td>
<td>95</td>
</tr>
<tr>
<td>Tomato juice</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>310</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>200</td>
<td>195</td>
<td>195</td>
<td>97.5</td>
</tr>
</tbody>
</table>

* Calculated by subtracting Cr^ii from total Cr.

### Table 4 Determination of Cr^ii and Cr^iv in real samples, (n = 5)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cr^ii</th>
<th>Cr^iv/µg g^-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineral water</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Tap water</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Lake water</td>
<td>nd</td>
<td>0.25</td>
</tr>
<tr>
<td>Tomato juice</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

* nd, Not detected.

Determination of total chromium in certified reference materials

In order to mask the co-existing interfering ions, the following procedure was followed. Sediment, tea and peach leaves (1–2 g) were placed in a 50 ml beaker. The sample was digested using the procedure described by Cutter, followed by heating in concentrated HCl to ensure that all chromium was in the form of Cr^ii. The contents of the beaker were filtered through a Whatman No. 40 filter-paper into a 25 ml beaker and then divided into two parts. The first part was conditioned by boiling almost to dryness. It was then collected with a solution containing EDTA and tartrate, filtered through a Whatman No. 40 filter-paper into a 10 ml calibrated flask and diluted to volume.

Having determined the total chromium, as Cr^vi, the second part of the sample was treated with concentrated hydrogen peroxide to reduce Cr^ii to Cr^iv and the excess of H_2O_2 was removed by boiling. The sample was then condensed and treated with a solution containing sodium hydroxide, EDTA and tartrate (0.01 M) and filtered as above. Sodium hydroxide was added to precipitate and remove the cations that would otherwise be precipitated during the on-line FIA procedure, creating significant problems in the FIA paths.

### Table 5 Effect of interfering ions on the determination of 0.5 µg ml^-1 Cr^ii and 0.5 µg ml^-1 Cr^iv

<table>
<thead>
<tr>
<th>Interfering ion</th>
<th>Maximum permissible concentration/ µg ml^-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA, nitrate, tartrate</td>
<td>1000</td>
</tr>
<tr>
<td>Lithium, sodium, potassium, ammonium, chloride, fluoride, phosphate, citrate, bromide, oxalate, azide, perchlorate</td>
<td>500</td>
</tr>
<tr>
<td>Cobalt(II), nickel(II), cadmium(II)</td>
<td>500</td>
</tr>
<tr>
<td>Lead(II), iron(II,III), tin(IV), Zn^2+, antimony(V) ^3+, manganese(II) ^3+</td>
<td>50</td>
</tr>
<tr>
<td>Copper(II), cerium(IV), mercury(II)</td>
<td>10</td>
</tr>
<tr>
<td>Beryllium, calcium, magnesium, arsenic(III), molybdenum(VI)</td>
<td>50</td>
</tr>
<tr>
<td>Silver(I) ^3+</td>
<td>25</td>
</tr>
</tbody>
</table>

* A 5% error criterion is adopted. † These ions have no interfering effect on the determination of Cr^iv because they are precipitated during the process. ‡ Removable interference with EDTA. § Removable interference with sodium tartrate.
For tea, interference from permanganate was easily removed by using sodium azide and boiling the solution before measurement. The results of total chromium determined either as Cr$^{VI}$ or as Cr$^{III}$ were in good agreement with the certified values. The results are given in Table 2.

**Simultaneous determination of Cr$^{III}$ and Cr$^{VI}$ in synthetic mixtures**

Mixtures of standard Cr$^{III}$ and Cr$^{VI}$ solutions of different concentrations were prepared in distilled water. The Cr$^{III}$ and Cr$^{VI}$ contents were determined fluorimetrically as described above. The precision for the determination of Cr$^{III}$ and Cr$^{VI}$ was measured by analyzing the samples listed in Table 3 (n = 5). The relative error for all samples was < 2%.

**Analysis of spiked environmental water and tomato juice samples**

The proposed method was applied to the determination of Cr$^{III}$ and Cr$^{VI}$ added to environmental water and tomato juice samples from the Ioannina area. A preliminary study showed Cr$^{III}$ and Cr$^{VI}$ to be below the limits of detection in the samples with the exception of lake water (Table 4), where chromium was determined following the procedure recommended for the spiked samples. Detection of Cr$^{III}$ in lake water was expected, owing to its presence in lake sediment, whereas Cr$^{VI}$ was not expected because of the abundance of organic reducing substances. The samples were spiked with different concentrations of Cr$^{III}$ and Cr$^{VI}$ with distilled water and the recovery was determined following the procedure recommended for the spiked samples. Detection of Cr$^{III}$ in lake water was expected, owing to its presence in lake sediment, whereas Cr$^{VI}$ was not expected because of the abundance of organic reducing substances. The samples were spiked with different concentrations of Cr$^{III}$ and Cr$^{VI}$ with distilled water and the recovery was determined. Prior to that samples were treated with a solution containing OH$^-$. EDTA and tartrate at concentrations of 1 M and filtered through a Whatman No. 40 filter-paper to eliminate interferences. In the case of tomato juice, plant tissues and other insoluble parts were removed by centrifugation and filtration through a 0.45 μm membrane filter and the remainder was decolorized with polyamide. The recoveries in all cases were high (between 95.0 and 104%), as shown in Table 3.

**Conclusions**

The application of on-line oxidation of Cr$^{III}$ by periodate and fluorimetric detection of Cr$^{VI}$ with PTQA, combined with FIA, has been shown to be effective for the simultaneous determination of Cr$^{III}$ and Cr$^{VI}$ and therefore for the speciation of chromium. Automation of the system resulted in much shorter analysis times, with the optimum possible oxidation efficiency, 23 than when using conventional, time-consuming, oxidation methods that require boiling or extreme conditions. The proposed FIA procedure using PTQA is not only a sensitive method for the simultaneous determination of Cr$^{III}$ and Cr$^{VI}$ but is also very simple and, with suitable masking, very selective.

This work was partly supported by the General Secretariat of Research and Technology (Greece), Grant PENED 95, and partly by the Research Committee of the University of Ioannina, Project No. 589. We thank Dr. C. Stalikas and the European Environmental Research Institute (EERI) in Ioannina for performing the AAS measurements and for supplying the certified reference material samples.

**References**