Sequential Spectrophotometric Determination of Chromium(III) and Chromium(VI) Using Flow Injection Analysis

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A flow injection analysis (FIA) procedure for the speciation of Cr(III) and Cr(VI) using the 1,5-diphenylcarbazide (DPC) method is presented. As Cr(III) does not interfere in the Cr(VI) - DPC reaction, both Cr(II) and total chromium (after the on-line oxidation of Cr(III) by Ce(IV)) are sequentially determined. Cr(III) is obtained by difference. Under the experimental conditions described, the calibration graphs are linear up to 2 μg ml⁻¹ of Cr(VI) and 4 μg ml⁻¹ of Cr(III). The detection limits found were 18 ng ml⁻¹ for Cr(VI) and 55 ng ml⁻¹ for Cr(III), at a signal to noise ratio of 3. The common interfering elements in the Cr(VI) - DPC reaction were investigated under dynamic FIA conditions. The FIA method was also compared with the conventional spectrophotometric procedure.

Keywords: Chromium speciation; flow injection analysis; spectrophotometry; chromium determination

Chromium may exist in many chemical forms¹ and in nature it is usually encountered in the oxidation states III and VI. It is well known that each of these oxidation states has very different biological, medical and toxicological properties.² Chromium(III) is an essential element to mammals, whereas chromium(VI) is considered to be a moderate to severe toxic agent.¹²

A number of papers have described specific analytical procedures for the speciation of chromium ions using atomic-absorption and atomic-emission spectroscopy.³⁻⁴ However, it is not always necessary to use expensive and/or sophisticated apparatus and methods for the trace analysis of chromium (and/or speciation studies), as demonstrated by several recent papers,⁵⁻¹¹ which are based on the highly sensitive and selective 1,5-diphenylcarbazide (DPC) spectrophotometric procedure.¹²,¹³

Considering the analytical interest in speciation studies involving chromium, we report here a rapid, precise and inexpensive on-line procedure for the sequential spectrophotometric determination of Cr(VI) and Cr(III), using the DPC technique.¹⁴,¹⁵ As the DPC reagent is selective for Cr(VI), Cr(III) was oxidised to Cr(VI), using the on-line procedure described previously,¹⁰ and the concentration of Cr(III) was obtained by difference.

Experimental

Reagents

Analytical-reagent grade chemicals and de-ionised water were used to prepare all solutions, which were stored in high-density polyethylene bottles.

Stock standard solutions of Cr(III) and Cr(VI), and nitric acid, sulphuric acid, 1,5-diphenylcarbazide and stock Ce(IV) solutions, were prepared as previously described.¹⁵,¹⁶ Synthetic mixtures of Cr(III) and Cr(VI) were prepared by mixing appropriate volumes of the stock standard solutions.

Further tests were performed in a sample of effluent from a leather treatment plant located near the metropolitan region of Campinas, collected before reaching the waste water recovery plant. This sample was filtered in situ to remove coarse grains and particles, then stored in a high-density polyethylene bottle at pH ca. 1.5 (H₂SO₄). Aliquots of 10 ml of this sample were then treated with 0.25 g of trichloroacetic acid to coagulate the leather proteins.² After a settling period of 30 min, the supernatant was filtered with a 0.45-μm cellulose membrane filter (Millipore, Type HA), according to the EPA procedure for water analysis,¹⁷ into a 100-ml calibrated flask. The solution was diluted to volume with water, keeping the final pH at ca. 2 (H₂SO₄).

Stock solutions (1000 μg ml⁻¹) of Fe(III), Mo(VI), Hg(II), Mn(II), V(V), Cu(II) and Cl⁻ were prepared for on-line interference studies. These tests were performed by adding, separately, appropriate aliquots of each of these solutions to Cr(III) (2 μg ml⁻¹) and Cr(VI) (1 μg ml⁻¹) solutions.

Conditions

The FIA arrangement used for the sequential determination of Cr(VI) and Cr(III) is shown in Fig. 1. This arrangement is similar to that previously reported for the on-line oxidation of Cr(III),¹⁰ except for a switching device (a modified proportional injector¹⁸) that is placed between the thermostated bath and the main FIA proportional injector. The temperature of the oxidation step was controlled to within ± 0.1 °C. The reagents and the sample were pumped at a flow-rate of 1.2 ml min⁻¹, using an Ismatec Mini-S 840 peristaltic pump and Tygon peristaltic pump tubing (Technicon). Polyethylene tubing (i.d. 0.8 mm) was used for the oxidation, mixing and reaction coils. The absorbance measurements were made using a Zeiss PM 2A spectrophotometer at 540 nm, using an 80-μl Zeiss flow cell (optical path 10 mm). A sample injection volume of 164 μl was used throughout.

Results and Discussion

Total chromium is measured setting the switching device in the position shown in Fig. 1(a). Using this configuration the sample [Cr(III) + Cr(VI)] is mixed with the Ce(IV) solution at the confluence point G prior to the injection of the sample volume to be analysed by reaction with DPC. Cr(VI) is directly measured setting the switching device as shown in Fig. 1(b). In both instances the sample is pumped through the feedline IV.

The absorbance due to the Cr(III) species is obtained through the equation Aₗ = (AVₗRₗ) + AₗH, where Aₗ is the absorbance observed for total chromium [Fig. 1(a)], AVₗ is the absorbance due to Cr(VI) species [Fig. 1(b)], AₗH is the absorbance due to only the Cr(III) species and Rₗ is the ratio of flow-rates (as defined previously¹⁹), used to correct the Aₗ value for dilution. The actual concentrations of both Cr(III) and Cr(VI) are obtained from calibration graphs constructed from FIA calibration runs for Cr(III) and Cr(VI).
higher sensitivity conditions, the determination of Cr(VI) is more precise using the FIA technique, although the conventional procedure has procedure,12.13 is linear up to 1.2 pg ml\(^{-1}\) (seven points) for Cr(VI), using the conventional static ml\(^{-1}\) level). The detection limits (signal to noise ratio of 0.5% at the RSD values ranged from 2% at the 0.2 yg ml\(^{-1}\) level to 0.7% at the 2 yg ml\(^{-1}\) level (minimum points) is hear up to 4 pg ml\(^{-1}\) (pg ml\(^{-1}\))

\[ R_f = \frac{A \times V}{m} \]

Interference factor

\[ \text{Interference factor}^* = \frac{[\text{interfering species}]_0}{[\text{Cr}]_0} \]

Table 1. Interferences in the direct determination of chromium with DPC by the FIA procedure. Cr(VI), 1 µg ml\(^{-1}\); volume, 100 ml

<table>
<thead>
<tr>
<th>Interfering species</th>
<th>Added as</th>
<th>Amount/ mg</th>
<th>Interference factor*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr(III)</td>
<td>Sulphate</td>
<td>20</td>
<td>1.00 1.00</td>
</tr>
<tr>
<td>Fe(III)</td>
<td>Chloride</td>
<td>5</td>
<td>1.04 1.08</td>
</tr>
<tr>
<td>Mo(VI)</td>
<td>Molybdate</td>
<td>2</td>
<td>0.96 0.95</td>
</tr>
<tr>
<td>Hg(II)</td>
<td>Chloride</td>
<td>1</td>
<td>0.96 1.05</td>
</tr>
<tr>
<td>Mn(II)</td>
<td>Chloride</td>
<td>20</td>
<td>1.00 1.00</td>
</tr>
<tr>
<td>V(V)</td>
<td>Vanadate</td>
<td>2</td>
<td>1.14 1.00</td>
</tr>
<tr>
<td>Cu(II)</td>
<td>Sulphate</td>
<td>2</td>
<td>1.14 1.00</td>
</tr>
<tr>
<td>Cl(^-)</td>
<td>Sodium</td>
<td>50</td>
<td>1.00 1.00</td>
</tr>
</tbody>
</table>

\[ A = 3.70 \times 10^{-3} + 0.282 \text{[Cr] (µg ml}^{-1}\); where A = absorbance; correlation coefficient, \(r = 0.999\). Under the same experimental conditions and using an \(R_f\) value\(^*\) of 0.452, the calibration graph for Cr(III) (eight points) is linear up to 4 µg ml\(^{-1}\) \(A = 3.70 \times 10^{-3} + 0.127 \text{[Cr] (µg ml}^{-1}\); \(r = 0.998\). The relative standard deviation (RSD), determined with ten replicates, for Cr(VI) ranged from 3% at the 0.1 µg ml\(^{-1}\) level to 0.7% at both 0.2 µg ml\(^{-1}\) level (minimum RSD = 0.4% at 1.5 µg ml\(^{-1}\)). For the Cr(III) determinations the RSD values ranged from 2% at the 0.2 µg ml\(^{-1}\) level to 0.5% at the 4 µg ml\(^{-1}\) level (minimum RSD = 0.3% at the 1 µg ml\(^{-1}\) level). The detection limits (signal to noise ratio of 3) were calculated as recommended\(^19\) and are 18 ng ml\(^{-1}\) for Cr(VI) and 55 ng ml\(^{-1}\) for Cr(III). The calibration graph (seven points) for Cr(VI), using the conventional static procedure,\(^12,13\) is linear up to 1.2 µg ml\(^{-1}\) \(A = -1.21 \times 10^{-2} + 0.740 \text{[Cr] (µg ml}^{-1}\); \(r = 0.998\). Under these experimental conditions, the determination of Cr(VI) is more precise using the FIA technique, although the conventional procedure has higher sensitivity.

Table 2. Interferences in the determination of chromium by the DPC-FIA procedure, observed when the interfering species are added to a Cr(III) standard solution, prior to the oxidation step. Cr(III), 2 µg ml\(^{-1}\); volume 100 ml

<table>
<thead>
<tr>
<th>Interfering species</th>
<th>Added as</th>
<th>Amount/ mg</th>
<th>Interference factor*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr(VI)</td>
<td>Dichromate</td>
<td>5</td>
<td>1.00 1.00</td>
</tr>
<tr>
<td>Fe(III)</td>
<td>Chloride</td>
<td>10</td>
<td>1.00 1.15</td>
</tr>
<tr>
<td>Mo(VI)</td>
<td>Molybdate</td>
<td>0.3</td>
<td>0.93 0.93</td>
</tr>
<tr>
<td>Hg(II)</td>
<td>Chloride</td>
<td>1</td>
<td>0.96 1.05</td>
</tr>
<tr>
<td>Mn(II)</td>
<td>Chloride</td>
<td>0.5</td>
<td>0.93 0.95</td>
</tr>
<tr>
<td>V(V)</td>
<td>Vanadate</td>
<td>1</td>
<td>1.03 1.03</td>
</tr>
<tr>
<td>Cu(II)</td>
<td>Sulphate</td>
<td>1</td>
<td>0.93 0.92</td>
</tr>
<tr>
<td>Cl(^-)</td>
<td>Sodium</td>
<td>50</td>
<td>1.00 1.00</td>
</tr>
</tbody>
</table>

\[ * \text{See footnote to Table 1.} \]

In theory, as the \(R_f\) value reflects the dilution to which the sample is submitted in the oxidation line, the sensitivity for the total chromium determinations [and by implication the Cr(III) determinations] could be increased by decreasing the extent of the sample dilution by the oxidant solution (i.e., increasing the \(R_f\) value), using a more concentrated solution of Ce(IV). However, care must be taken with this procedure because a lack of repeatability may be observed and double peaks may be recorded, depending on the concentration of the Ce(IV) solution. In addition, the sampling rate will decrease as a lower flow-rate is required when a higher concentration of Ce(IV) is used. Although not relevant for the determination of Cr(VI), the amount of sulphuric acid added to the sample may greatly affect the determination of total chromium, owing to the possible formation of sulphato complexes of Cr(III), which may affect the oxidation yield. Therefore, it is recommended\(^18\) that the concentration of sulphuric acid should not be higher than 1 \(\times 10^{-2}\) M.

According to the literature, the static conventional DPC spectrophotometric procedure is almost specific for Cr(VI),\(^12,13\) and interferences in the determination of Cr(VI) are only observed from Fe, V, Mo, Cu and Hg.\(^12\) However, considering that the dynamic conditions of FIA may not...
respond to the static conditions, a re-investigation of interferences was carried out, both for the Cr(VI) - DPC reaction (Table 1) and for total chromium, by adding the interfering element to a Cr(III) standard solution, prior to the oxidation step (Table 2). These studies were carried out using both HNO$_3$ and H$_2$SO$_4$ (each at 0.80 M) in confluence with the DPC solution. 5, 16 Tables 1 and 2 indicate the minimum amount of interfering species for which the relative error in absorbance is higher than ±3% or the maximum amount of these species tested when the relative error in absorbance is lower than ±3%. The absorbance values were corrected against the blank.

The behaviour of the interfering species for the direct Cr(VI) - DPC reaction in FIA (Table 1) indicates a more pronounced interference than when using the conventional procedure. 12 Perhaps this is because the FIA data are obtained 30 s after each sample injection (i.e., the FIA residence time), whereas those for the static procedure relate to a minimum period of 2 min.

One cannot assign precisely the role of the interfering species in the Cr(VI) - DPC reaction because the reaction mechanism involving these two reagents is still under discussion, 20, 21 even though DPC has long been used for Cr(VI) determination. A Cr to DPC ratio of 2:3 for the reaction between Cr(VI) and DPC is the only well established parameter, obtained under rigorously controlled experimental conditions, 21 a ratio which is consistent with earlier data. 20

An even more pronounced interference can be observed if the interfering species is added to the Cr(III) solution, prior to the oxidation step (Table 2). This behaviour is even more difficult to explain because unstable intermediates, such as Cr(IV) and Cr(V), may be involved in the oxidation of Cr(III) by Ce(IV). 22 Certainly those species that may react with Ce(IV) appear to give more interference than those for which a reaction is not likely.

The proposed FIA methodology for the speciation of Cr(III) and Cr(VI) was tested against the conventional static spectrophotometric procedure. 12, 13 Synthetic mixtures of Cr(III) and Cr(VI), and also a real sample, were prepared as described under Experimental. In one set of samples, chromium(VI) was determined by the conventional (static) method without previous oxidation. 13 Using another set of samples, total chromium was determined by the conventional method, after oxidation with potassium peroxydisulphate (potassium persulphate). 13 The last set of samples were analysed for Cr(III) and Cr(VI) using the FIA procedure described in this paper. In all instances the determinations of chromium were carried out in triplicate and the values were corrected against the blank. Table 3 reports the mean values.

The maximum absolute error found is 6 μg of Cr (in 100 ml) for the FIA method, which is very close to the calculated detection limit for Cr(III). In the same concentration range, the static conventional procedure has a maximum absolute error of 8 μg with respect to the same total volume of 100 ml.

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References