Research article

Determination of chromium species in water using diphenylcarbazide with a sequential spectrophotometric discrete robotic analyser

Jerry Dikobe [a,b], Funzani Asnath Melato [a,*], Carel Johannes Lombard Adlem [b], Khathutshelo Netshiongolwe [a]

[a] *Tshwane University of Technology, Department of Chemistry, Private Bag X680, Pretoria, 0001, South Africa*
[b] *Council for Scientific and Industrial Research, Meiring Naude Road, Brummeria, Pretoria, 0001, South Africa*

ARTICLE INFO

ABSTRACT

There is an increasing need for fast and reliable analytical methods for the determination of chemical forms of elements in environmental samples. The interest in chromium is driven by the fact that its toxicity depends on its oxidation state. Although chromium (III) is essential for mammals to maintain their metabolism of proteins, fats, and carbohydrates, chromium (VI) is toxic to humans. For chromium speciation, several costly analytical methods coupling separation methods with atomic absorption and emission spectroscopy have been developed. This article presents the online robotic discrete analyser procedure with the 1,5 diphenylcarbazide (DPC) method for the speciation of Cr (III) and Cr (VI). Cr (III) was determined by difference since it does not interfere with the reaction of Cr (VI)-DPC. Chromium (VI) and total chromium were characterised sequentially (after online oxidation of Cr (III) by Cerium (Ce (IV)). The calibration graphs were linear under experimental conditions up to 1 mg/L Cr (VI) and 2 mg/L total Cr with correlation coefficient R^2, 0.9997 and 0.9999 respectively. At a signal-to-noise ratio of three, the detection limits were 0.004 mg/L Cr (VI) and 0.015 mg/L total Cr. Good agreement between the real values of certified reference materials and the chromium species content was obtained in this study. The method was precise with a percentage relative standard deviation of less than 2 for hexavalent chromium and total chromium. The t-stat demonstrates that there was no significant difference between the developed robotic discrete analyser method and the ICP-MS method. Except for effluent water, which had recoveries between 65 and 75 % in the assessment of the devised method's selectivity, the overall percentage of recoveries fell between 90 and 110 %, which was generally satisfactory. This method proved to be appropriate for its intended use.

1. Introduction

The primary source of the metallic element chromium is the mineral chromite, which is found in gasses, soil, water, rocks, animals, and plants. Wastewater from anthropogenic activities such as tanning, metallurgy, electroplating, metal smelting, and the dyestuff industry is a significant source of chromium, which is mostly present in the water [1,2]. Metallic chromium (oxidation state 0), is

---

mainly found in alloys such as stainless steel and chrome-plated objects. It is the supreme additive, endowing alloys or materials with new properties, such as resistance to corrosion, wear, temperature and decay, as well as strength, hardness, permanence, hygiene, and colour [3].

Chromium (III) is considered a trace element essential for living organisms to function properly [4]. It regulates glucose and lipid metabolism in mammals [5,6]. The discovery of the so-called glucose tolerance factor (GTF), which is composed of Cr(III), glutamic acid, glycine, cysteine, and nicotinic acid, provided support to this theory [7,8].

Cr (VI) exerts toxic effects on biological systems and workplace exposure to hexavalent chromium compounds leads to several kinds of clinical conditions [9]. Inhalation and retention of Cr(VI)-containing material can cause nasal septum perforation, pneumonitis, asthma, bronchitis, inflammation of the liver and larynx, and an increased chance of developing bronchogenic cancer [10]. When the Cr(VI) compounds come into contact with the skin, they can induce dermatitis, dermal necrosis, dermal corrosion, and skin allergies [11] Chromate's harmful properties are from their strong oxidative potential and their ability to freely diffuse across cell membranes [12]. The toxicological effects of Cr(VI) are caused by its oxidising capabilities and the generation of free radicals during the intracellular reduction of Cr(VI) to Cr(III) [13]. Because of its strong ability to coordinate organic molecules, chromium (III), which is produced during reduction, may also be detrimental at high doses, inhibiting several metalloenzyme systems [14].

The total chromium measurement cannot be used to determine the actual environmental impact of chromium (III and VI) speciation in the environmental samples, as it is necessary to accurately assess the pollution levels [15]. Characterising elemental species provides information on the exact amount of an element in environmental samples [16].

Several specific analytical methods have been developed for the speciation of chromium ions using atomic absorption and atomic emission spectroscopy coupled with separation techniques [17]. Numerous advances based on the extremely selective and sensitive 1, 5-diphenylcarbazide spectrophotometric method, on the other hand, revealed that expensive and advanced technologies are not always required for speciation investigations and trace analysis of chromium. Considering the analytical interest in chromium speciation investigations, this study aimed to develop a rapid, accurate, precise, and cost-effective online procedure for chromium species characterisation utilising the 1,5-diphenylcarbazide method with a discrete robotic analyser.

The discrete analyser uses a real discrete direct read-measuring device and is a dual-beam, high-resolution digital detector that is reusable and fully automated. It uses a sturdy robotic sampling arm with a syringe powered by a stepper motor to aspirate, dispense, and mix precise amounts of sample and reagent in tiny test tubes known as cuvettes. In the reaction well cuvettes, the sample and reagent are incubated for a specified amount of time. The sample is analysed at a temperature of 37 °C, which reduces the time needed to reach a stable colourimetric endpoint absorbance and accelerates the rate of reaction. Then, a single aliquot is put into an optical cuvette made of glass. For the best possible signal-to-noise ratio, the absorbance is then measured on the stationary reactant.

Other techniques that have been used for chromium speciation analysis are complex and non-economical compared to the discrete analysis method for routine analysis of chromium species in environmental samples.

In recent years, electromembrane microextraction (EME) and gel-electromembrane extraction (G-EME) techniques have been used widely for the analysis of chromium species (trivalent and hexavalent chromium) in different environmental samples [18]. This method is mainly based on the migration of the charged analytes from the aqueous donor phase (DP) to the acceptor phase (AP) solution across a membrane by applying an external electrical field [19]. Pre-analytical treatment by G-EME offers the benefit of reducing matrix effects and enhancing the concentration of the target analytes, thus enabling modern analytical instruments to achieve improved sensitivity [18]. However, the cost of membranes used in these methods is high. The EME technique uses organic solvents and additives to improve extraction efficiency and the selection of a compatible solvent and the additive remains a challenge. On the other hand, G-EME is a green extraction technique and does not require the addition of additives but it is less selective than EME, and electroendosmosis (EEO) can occur during the extraction process which can affect the sensitivity. The applicability of the gel with high viscosity as a membrane can also influence the mass transfer and both the EME and G-EME are hardly automated [20,21].

2. Experimental

2.1. Reagents

All the chemicals used were pure analytical-grade reagents that were acquired from various suppliers. Total chromium standard solutions were prepared from 1000 mg/L stock solutions of Cr(III) and Cr(VI) respectively. The chromium (III) nitrate reagent (Sigma-Aldrich, Darmstadt, Germany) was used to prepare the Cr (III) stock solution, whereas the potassium dichromate reagent (Minema, Johannesburg, South Africa) was used to prepare the Cr(VI) stock solution. A 0.5 mg/L total chromium verification standard solution was prepared from a 1000 mg/L Cr(VI) standard solution. Potassium chromate reagent (Sigma-Aldrich, Darmstadt, Germany) was used to prepare a 0.3 mg/L Cr(VI) verification standard solution. Cerium (IV) Sulphate (Sigma-Aldrich, Darmstadt, Germany) reagent was used to prepare the oxidant, 250 mg/L in 0.25 M sulfuric acid (Sigma-Aldrich, Darmstadt, Germany). The colour reagent 1,5-diphenylcarbazide was purchased from Anatech (Johannesburg South Africa). For all processes requiring deionized water, ultra-pure water (electrical conductivity, 0.05 S cm^{-1}), produced by a Milli-Q water purification system (GIC Scientific, Johannesburg, South Africa), was used. Chromium standards for ICP-MS were prepared using 1000 mg/L ICP grade stock standard solutions (Inorganic venture, Christiansburg, USA) and used for instrument calibration in the range of (0.001–0.5) mg/L. The sample matrices were matched by adding 1 % (v/v) prepared from ultrapure (65 %) HNO$_3$ (Merck, Johannesburg, South Africa) to the working standards. Instrument calibration was verified by analysis of 0.010 mg/L Cr standard (LGC, Teddington, UK.

2.2. Sample collection and preparation

Different types of water samples were collected from various locations in South Africa, including the tap water from the Environmental Labs Pretoria campus of the Council for Science and Industrial Research (CSIR), a borehole at the Newcastle Landfill Site, the Ncandu River in Newcastle's Industrial Center, and the Musina Tiger Brand Tomato Unit's effluent facility. Grab samples were collected in plastic bottles and kept chilled in a cooler box before being taken to the lab. Before analysis, samples were kept at 4 °C in the lab, and turbid samples were filtered through a 0.45 μm pores filter before use. Thereafter, all the samples were spiked with chromium concentrations covering the working range of 0.1–2 mg/L Cr.

2.3. Method optimisation

The predominant parameters in the optimisation procedure were chromium wavelength selection and online oxidation of Cr (III) to Cr (VI) in the reaction chamber at a constant temperature for the determination of total chromium. These parameters influence the intensity of the absorption line used to determine the analyte and the extent of the recovery of total chromium from water samples. The wavelength scan was performed using a spectrophotometer (HACH DR 6000, South Africa) on a reddish-violet chromium diphenylcarbazide complex after reaction of chromium (VI) standard with 1,5 diphenylcarbazide reagent in a conical flask.

By varying the oxidation reaction time at intervals of 60 s, 120 s, 240 s, 360 s, 480 s, 600 s, 720 s, 840 s, 960 s, 1080 s, 1200 s, 1320 s, and 1500 s, the absorbances determined with the length of oxidation were varied at chromium (III) concentrations (1, 2 and 3) mg/L covering the operating range of the method. By varying the oxidant (Ce^{4+}) concentrations (50, 100, 150, 200, 250, and 300 mg/L in 0.25 M H_2SO_4), it was possible to determine the effect of the oxidant Ce(IV) concentration on the measured absorbance with the length of oxidation at chromium(III) concentrations (1, 2 and 3 mg/L) covering the working range of the method with temperature constant at 37 °C and a reaction time of 360 s.

2.4. Operating condition and instrumentation

Samples and colour reagents were combined using specialised robotics and automated syringes, and the resulting colour formation was examined spectrophotometrically as shown in Table 1. Neither a reaction cartridge nor a chemical manifold is used by discrete analysers. Samples and reagents are mixed and blended in a cuvette. To ensure reproducible results, discrete analyser manufacturers adhere to Environmental Protection Agency Procedures and Standard Methods.

There are two different kinds of discrete analysers available: (1) systems that mix their samples and reagents in a reaction cuvette and then analyse the sample in a separate optical well; and (2) systems that mix their sample and reagents in the same cuvette that the system uses to analyse the sample, like the Gallery PLUS discrete analyser used in this study.

A completely automated discrete photometric analyser designed primarily for food, beverage, water, and soil testing is a high-capacity bench-top Gallery Plus (Thermo Scientific, Pretoria, South Africa). The high-capacity bench-top Gallery Plus analyser delivers time and cost benefits by allowing for the simultaneous analysis of many parameters from a single sample, real-time reagent monitoring, and automatic dilutions.

2.5. Characterisation of hexavalent chromium

The intensity of reddish-violet colour resulting from the reaction of 1,5-diphenylcarbazide and hexavalent chromium in a moderately acidic solution was measured spectrophotometrically at 540 nm [22]. The standards, samples and reagents were mixed by a robotic analyser automatically in a reaction incubator, as indicated in Fig. 1 and Table 1.

2.6. Characterisation of total chromium

Chromium (III) in the sample was quantitatively oxidised to chromium (VI), by reaction with the oxidant Ce (IV) [23]. The intensity of the reddish-violet colour produced by the reaction of chromium (VI) (oxidised chromium (III) plus original chromium (VI) in the sample) with 1,5-diphenylcarbazide was measured spectrophotometrically at a wavelength of 540 nm [24]. The standards, samples

Table 1
Operating parameters of the Galley plus discrete analyser (hexavalent chromium).

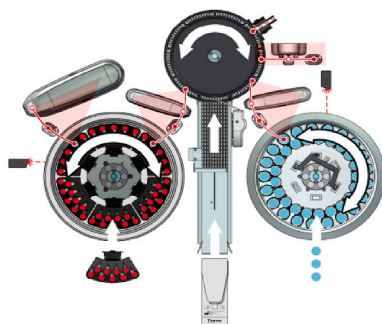| Operational Condition	Parameters
Sample volume	120 μl
Sample incubation	18 s
Reagent 1 (1,5 Diphenylcabazide) volume	40 μl
Reaction time	1500 s
Main wavelength	540 nm
Side wavelength	880 nm
Residual net Absorbance	0

Fig. 1. Direct read Gallery discrete analyser (Thermo Fisher Scientific, Pretoria, South Africa).

and reagents were mixed by robotics automatically in a reaction incubator, as indicated in Table 2 and Fig. 1.

2.7. Characterisation of trivalent chromium (by calculation)

The amount of chromium (III) was calculated by subtracting the amount of chromium (VI) from the amount of total chromium overall which was done by the instrument software.

2.8. Validation of the characterisation technique

The method's operational range was determined through the measurement of chromium species standards in the range of 0.05–2 mg/L. The precision, accuracy, and linearity of 10 samples at each concentration (0.05, 0.1, 0.5, 1, and 2) mg/L were determined after the method's operating range was specified.

To assess the method's precision, the tap water sample was spiked with the analytes at varied concentrations over the working range. The homogeneous sample was tested ten times at concentrations of (0.3, 0.5, 0.7, 1, and 2) mg/L. Analysis of the chromium in the certified reference material (seawater LRAA4152) was used to determine the total chromium accuracy. Chromium (VI) analysis in the certified reference sample (seawater LRAA7118) was used to evaluate the accuracy of the hexavalent chromium level. The same methods utilised in the analysis of water samples were used in the CRMs.

The tap water, groundwater, surface water, and effluent were spiked with the analyte and the percentage recoveries of chromium species concentrations were calculated to evaluate the matrix effect. The selectivity of total chromium was assessed by adding 0.1, 0.5, and 1.5 mg/L of Cr(III) to various types of water (tap water, groundwater, surface water, and the effluent), covering the method dynamic range, and then analysing samples following the pre-established procedure to determine total chromium in water. After the spiking of several types of water (tap water, groundwater, surface water, and effluent), with 0.3, 0.5, and 0.7 mg/L of Cr(VI), samples were analysed using the pre-set procedure to quantify hexavalent chromium in water. This enabled the chromium (VI) selectivity to be evaluated.

Total chromium in water samples was quantitatively measured using the Inductively Coupled Plasma- Mass Spectroscopy (ICP-MS) (Agilent 7900, California, USA) instrument and the operational parameters are shown in Table 3. Compared to other ionization methods, such as flame ionization, plasma ionization has several benefits, such as the ability to occur in a chemically inert environment, which prevents oxide formation, and its greater thoroughness. To further reduce the effects of self-absorption, the torch's temperature profile is quite uniform.

There was a dynamic reaction cell (DRC) in the ICP-MS. Between the lens and the quadruple, in the vacuum chamber. When using the DRC in DRC mode, the ion beam underwent chemical treatment to remove interferences. The computer software was used to set the pressure and kind of reaction gas. Interrupting the series of reactions that would otherwise result in interferences helped to avoid them. The ions were transported to the MS analyser chamber through the multipole DRC.

Table 2
Operating parameters of the Galley Plus discrete analyser (total chromium).

Operational Condition	Parameters
Sample volume	120 μl
Sample incubation	18 s
Reagent 1 (Cerium Sulphate) volume	30 μl
Reaction Time	360 s
Reagent 1 (1,5 Diphenylcabizide) volume	40 μl
Reaction time	1500 s
Main wavelength	540 nm
Side wavelength	880 nm
Residual net Absorbance.	0

Table 3
ICP-MS operational parameters.

Operational Conditions	ICP-MS parameters
Rate of nebulizer gas flow (mL/Min)	1.1
Auxiliary gas flow rate (L/Min) = 1.0	1.0
Flow rate of plasma gas (20 L/min)	20
Power (W) of radio frequency (RF)	1500
Rate of coolant gas flow (L/Min)	12
Temperature of cooling water (^0C)	15–20
Exhaust duct (M^3/Min)	5–7
Peristaltic pumping rate (rps)	0.3

The ions were distributed according to their mass-to-charge ratio (m/z) in the mass analyser. Except for the signal processor and readout, all instrument parts for mass spectrometers needed to operate at low pressures.

Results from the developed online robotic discrete analyser method were compared with the approved ICP-MS approach to assess the ruggedness of the method.

3. Results and discussion

3.1. Optimisation

A maximum absorbance at 540 nm was selected for the analysis of chromium species given the pick saturations of a curve as shown in Fig. 2.

At a fixed temperature of 37 °C, equilibrium was established within 360 s, as shown in Fig. 3, even though the oxidation yield increased with longer reaction times in the reaction chamber. The graphs demonstrated that at 1, 2 and 3 mg/L Cr^{3+}, the time vs absorbance curves stabilise at 360s. Trivalent chromium was completely oxidised to hexavalent chromium at 360s with cerium (IV) kept constant at 250 mg/L in an acidic medium.

As shown in Fig. 4, the concentration of the Ce(IV) solution used in confluence with the chromium solution also influenced the overall online oxidation process. This observation seems to be consistent with the rate law d[Cr(VI)]/dt = k[Ce(IV)]^2[Cr(III)]/[Ce (III)], reported by Tong and King [25] for the oxidation of Cr(III) by Ce(IV) in aqueous acidic sulphate medium. Considering these data, 250 mg/L was taken as the most appropriate concentration of Ce(IV) to be used in the online oxidation of Cr(III) to Cr(VI). The graph of Cr concentration versus absorbance at 50 mg/L Ce(IV) in Fig. 4 was linear up to 1 mg/L showing that Ce(IV) has little impact on the online oxidation of Cr(III) to Cr(VI) since there wasn't sufficient Ce(IV) concentration to complete the oxidation reaction beyond 1 mg/L of Cr(III). The graph of Cr concentration versus absorbance at 100 mg/L Ce(IV) in Fig. 4 was linear up to 2 mg/L indicating the partial effect of an online oxidation of Cr(III) to Cr(VI) because there was no sufficient Ce(IV) concentration to complete the oxidation reaction beyond 2 mg/L of Cr(III). The graph of Cr concentration vs absorbance at 150, 200, 250 and 300 mg/L Ce(IV) in Fig. 4 was linear up to 3 mg/L indicating the efficacy of online oxidation of Cr(III) to Cr(VI) by Ce(IV). At the concentration of 250 mg/L and more of the Ce(IV) concentration, more acid was required to dissolve Cerium (IV) sulphate and the introduction of more acid increased the effect of the cerium sulphate complex. This effect of sulfuric acid concentration may be primarily related to the creation of the anionic species, $Ce(SO_4)_3^{2-}$, which resulted in a modest alteration in the formal potential of the Ce(IV)–Ce(III) couple. To dissolve the cerium (IV) salt and avoid its hydrolysis, working solutions of cerium (IV) were prepared using the least quantity of sulfuric acid necessary.
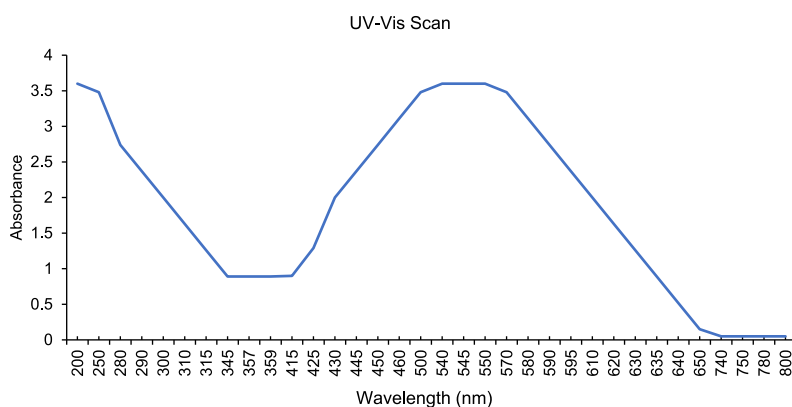
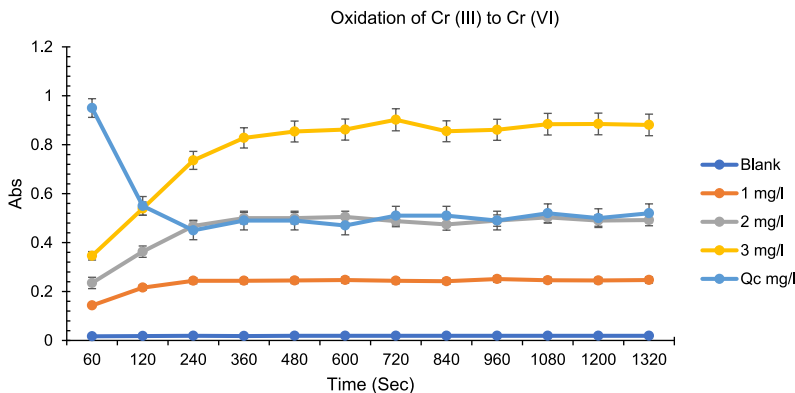**Fig. 2.** Chromium diphenylcarbazide complex UV/Vis Wavelength scan.

Fig. 3. Oxidation of Cr (III) with Ce (IV): variation of the measured absorbance with length of time in the reaction incubator at 37 °C.
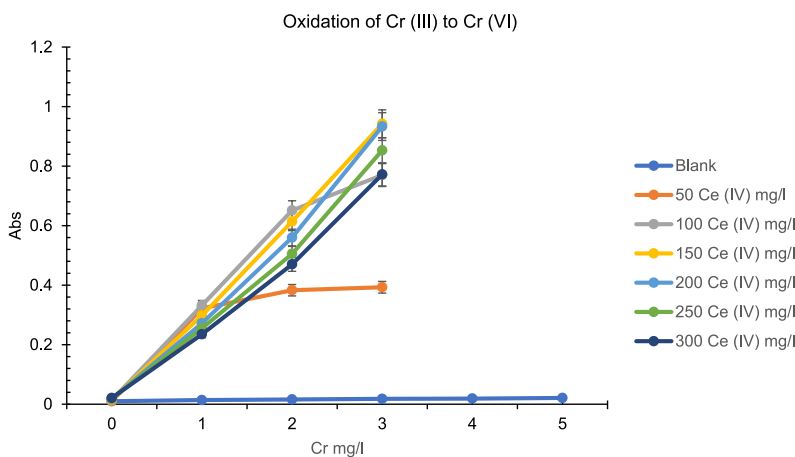

Fig. 4. Oxidation of Cr (III) to Cr (VI) with Ce (IV).

3.2. Characterisation technique evaluation

In the application of analytical sciences, method validation is a crucial prerequisite. It's not always obvious why it ought to be done, when it should be done, or what exactly must be done. It was the purpose of method validation to show that the test results were appropriate for their intended application. An extensive amount of data was generated during a validation investigation [26]. The technique has been evaluated for working range, linearity, accuracy, precision, robustness/ruggedness, selectivity/specificity, limit of quantification and limit of detection. The entire analytical process, including sample preparations before analysis, was validated. It
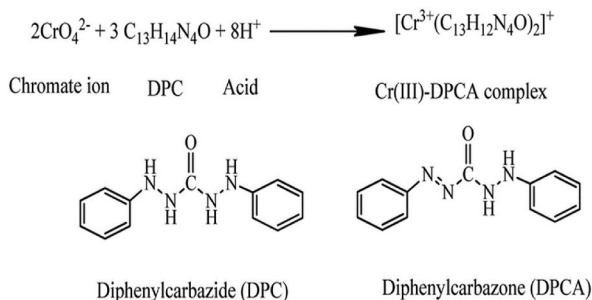

Fig. 5. Chromium Diphenylcarbarzone complex formation.

was validated for each type of matrix where it would be used, as well as for the entire range of analyte concentrations indicated in the method scope [27].

3.3. Characterization of hexavalent chromium

The intensity of chromium diphenylcarbazide reddish-violet complex formed after a reaction of chromate ions and diphenylcarbazide in an acidic medium as per the equation in Fig. 5 was quantified spectrophotometrically at 540 nm.

3.4. Characterization of total chromium

The total chromium was determined sequentially by two-step reactions before quantified spectrophotometrically at 540 nm as shown in the flow chart in Fig. 6. Firstly, trivalent chromium in the water sample was oxidised to hexavalent chromium by cerium (IV) sulphate and secondly, hexavalent chromium present in the sample combined with oxidised trivalent chromium was quantified as total chromium by reaction of chromate ion with diphenylcarbazide to form a reddish-violet complex which was measured at 540 nm. Reactions in the two steps are carried in an acidic medium.

3.5. Working range

An analytical technique's range is the range between higher and lower levels of an analyte, encompassing values generated with an acceptable and established degree of precision, accuracy, and linearity. The units used in the standard expression of the working range are the same as those used in the analytical method test findings. At high concentrations, a negative deviation from linearity is anticipated. A minimum of the prescribed recovery range should fall between 90 and 110 percent of the analytical test concentration for the assay test. An examination of percentage recovery is done to see how well the approach works and to determine the correctness and precision of the test result. The maximum concentration level in this investigation was 2 mg/L, and the lower concentration level began at 0.05 mg/L.

The parameters to demonstrate the range of the analytical method were within specifications, the percentage recoveries were between 90 % and 110 % at all concentration levels covering the entire range. All levels' relative standard deviations were less than 2 % as shown in Table 4, which is an indication of high precision. Hexavalent chromium had a working dynamic range of 0.05–1 mg/L and 0.1–2 mg/L for total Chromium.

3.6. Linearity

According to the analytical method's response function or calibration curve, the correlation between the analytical signal and the analyte concentration was monotonic. There are non-linear models as well as linear models for the response function. In this study, there was a highly significant linear relationship between instrument signals and concentrations. Up to seven standards, including a blank, were selected, ranging from the concentration of 50 %–150 % of the target analyte.

Using calibration standards without a matrix sample, the response functions for total chromium and hexavalent chromium were determined [28]. The relationship between the responses and concentrations is illustrated in Fig. 7. The calibration graph's exceptional linearity was demonstrated by the correlation coefficient (R^2) of 0.9997 for hexavalent and 0.9999 for total chromium closer to 1.0000 (ideal correlation coefficient).

Oxidation of Cr³⁺
- $Cr^{3+} + Ce^{4+} \rightarrow Cr^{6+} + Ce^{3+}$
- In Acidic medium

Total content of Chromium
- Cr^{6+} in water sample + Cr^{6+} after oxidation

Detection of Total Chromium
- $2CrO_4^{2-} + 3C_{13}H_{14}N_4O + 8H^+ \rightarrow [Cr^{3+}(C_{13}H_{12}N_4O)_2]^+$ (reddish- Violet)
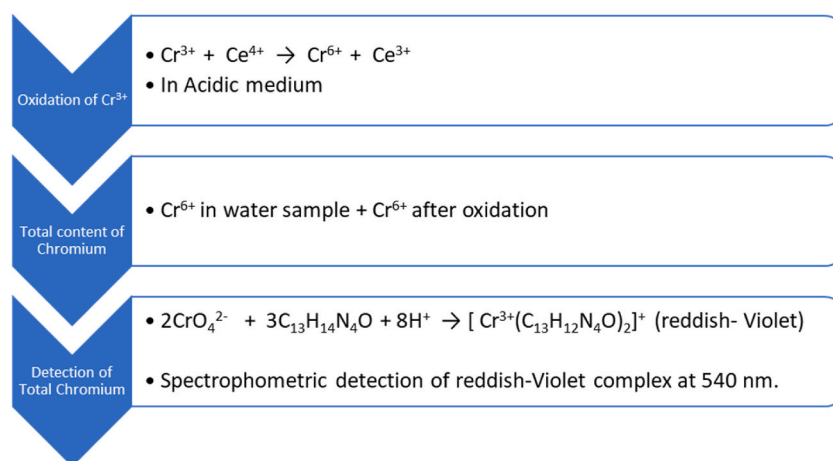- Spectrophometric detection of reddish-Violet complex at 540 nm.

Fig. 6. Flow chart of sequential spectrophotometric determination of total chromium.

Table 4
Range of the method for total chromium and hexavalent chromium.

Total chromium					hexavalent chromium				
Spiked Cr^{3+} (mg/L)	Average results (mg/L)	Recovery (%)	SD	% RSD	Spiked Cr^{6+} (mg/L)	Average results mg/L	Recovery (%)	SD	% RSD
0.1	0.10	100.1	0.0015	1.5	0.05	0.05	103.0	0.0005	1.0
0.5	0.49	97.9	0.0066	1.4	0.5	0.51	103.0	0.0036	0.71
1	1.00	100.1	0.0114	1.1	1	1.02	101.6	0.0019	0.19
2	1.94	96.9	0.0276	1.4	0.05	0.05	103.0	0.0005	1.0
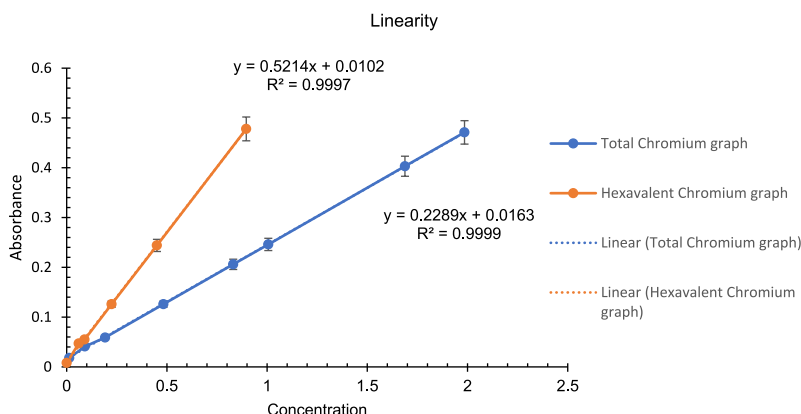
Fig. 7. Linearity graphs for total chromium and hexavalent chromium.

3.7. Precision

The amount of scatter in the findings from several tests of a homogenous sample determines how precise an analytical process is.

Spiking drinking water with concentrations covering the operating range allowed for the demonstration of precision over the calibration range. Table 5 shows the homogenous sample examined ten times at 0.5, 1 and 2 mg/L, and the findings of the hexavalent chromium analysis at 0.3, 0.5, and 0.7 mg/L were also shown in Table 5. The chromium species characterisation showed good precision, with all levels of the percentage relative standard deviation (%RSD) being less than 2 %.

3.8. Accuracy

The degree to which the measured value resembles the true value is a measure of a method's accuracy. A certified reference material Seawater LRAA4152 (Sigma-Aldrich) was used to verify the accuracy as shown in Table 6. There was significant agreement between the total chromium content of 0.571 mg/L achieved by the robotic discrete analyser approach and the true value of 0.535 mg/L at a 95 % confidence interval (0.375–0.696 mg/L). According to these findings, the method recommended for chromium trace analysis in complex matrices was accurate and precise.

The hexavalent chromium content of the certified reference material Sea water LRAA7118 (Sigma-Aldrich, Darmstadt, Germany) was determined precisely. The hexavalent chromium content of 0.432 mg/L produced by the robotic discrete analyser approach and the true value of 0.419 mg/L at a 95 % confidence interval range (0.377–0.461 mg/L) as shown in Table 6 had a satisfactory agreement. These findings revealed that the approach was accurate for hexavalent chromium trace analysis in complex matrices [29].

Table 5
The precision of the method for total chromium and hexavalent chromium.

Total chromium				Hexavalent chromium			
Spiked Cr^{3+} (mg/L)	Average results (mg/L)	SD	%RSD	Spiked Cr^{6+} (mg/L)	Average results (mg/L)	SD	%RSD
0.5	0.49	0.0066	1.4	0.3	0.30	0.0017	0.57
1	1.00	0.0114	1.1	0.5	0.51	0.0076	1.1
2	1.94	0.0276	1.4	0.7	0.73	0.0144	1.9

Table 6
Accuracy of the method for Total Chromium and Hexavalent Chromium.

Total Chromium					Hexavalent Chromium				
CRM	True value mg/L	Result (mg/L)	Limits	Recovery (%)	CRM	True value mg/L	Results (mg/L)	Limits	Recovery (%)
LRAA4152	0.535	0.571	0.375 to 0.696	106.7	LRAA7118	0.419	0.432	0.377 to 0.461	103.1

3.9. Selectivity and specificity

The selectivity of an analytical procedure is the extent to which it remains unaffected by other species present in the sample matrix. Analyte performance in a test combination with every possible matrix component is compared with analyte solution performance. To investigate the matrix effect, tap water, groundwater, surface water, and effluent were spiked with the analyte. The percentage recoveries of chromium species concentrations were then determined.

To investigate the matrix effect of the total chromium, a variety of water types, including tap water, groundwater, surface water, and effluent, were spiked with 0.1, 0.5, and 1.5 mg/L of Cr(III). The recoveries ranged between 90 and 110 % as shown in Table 7, which is generally regarded as satisfactory, except for effluent, which ranged between 65 and 75 % which is below the acceptable range of between 75 % and 125 % [30]. Inadequate recovery rates from the wastewater sample could have been caused by other chemical species in the wastewater that could have reacted with Cr(III) and interfered with its measurements.

To investigate the matrix effect of chromium (VI), tap water, surface water, groundwater, and effluent were spiked with 0.3, 0.5, and 0.7 mg/L of Cr(VI), which covered the method's dynamic range. As shown in Table 7, all recoveries fell between 90 and 110 %, which is generally regarded as acceptable. The range of acceptable values is typically 75 %–125 %.

3.10. Ruggedness

The analytical results of the robotic discrete analyser technique and the ICP-MS technique were subjected to the student t-test. At the 95 % confidence level, the t-Stat (0.192) value was lower than the t-critical value (1.75), hence the results showed no significant differences between the two approaches. Detailed results are provided in Table 8. Less than 15 % of the results for all samples in Table 8 varied between Gallery Plus and ICP-MS, indicating satisfactory robustness, precision, and accuracy.

3.11. Limit of detection (LOD) and limit of quantification (LOQ)

The detection limit of a technique is the lowest analyte concentration that causes a detectable response above the system noise level, which is typically three times the noise level. The limit of quantification of a technique is the lowest analyte level that can be precisely and correctly quantified; this level is typically ten times the noise level. Ten blank samples were analysed to establish the limit of detection and quantification. The LOD and LOQ values as shown in Table 9 were determined using the student's test at a 95 % confidence level in the following manner:

LOD = t xs

t = 3.14 x s [for 10 replicates]

LOD: 3.14 x s.

LOQ: 10 x s.

4. Conclusion

The major objective of this study was to develop an inexpensive, rapid, precise and accurate online technique to determine chromium species in water using a 1,5-diphenylcarbazide and a robotic discrete analyser. To achieve this, a robotic spectrophotometric discrete analyser was used, along with chromium species standard solutions, oxidising reagent, colour (complexing) reagent, and certified reference material, in sequential order to set up, refine, and validate a procedure to measure total chromium, hexavalent chromium, and trivalent chromium by difference.

The operating range, linearity, calibration verification, precision, accuracy, selectivity, robustness, ruggedness, limit of detection, and limit of quantification were all investigated to confirm that the test method was appropriate for its intended application. Total chromium had a working dynamic range of 0.1–2 mg/L, while hexavalent chromium had a working dynamic range of 0.05–1 mg/L. The Cr and Cr(VI) calibration graphs had excellent linearity as indicated by the correlation coefficients, R^2, which were both closer to 1.000 (ideal correlation coefficient). The instrument operating parameters were also optimized by a procedure which included chromium wavelength selection and online oxidation of Cr(III) to Cr(VI) in the reaction chamber at a constant temperature for the determination of total chromium.

To determine the method's consistency during calibration, drinking water was spiked with concentrations that covered the operational range. Then, ten examinations were conducted on a homogeneous sample. The percentage relative standard deviation (% RSD) for total and hexavalent chromium readings was less than 2 % at all levels, showing remarkable instrument precision. The analysis of chromium species in certified reference materials was conducted to evaluate the technique's reliability for total and

Table 7
Matrix effect of the method for Total chromium and Hexavalent chromium).

Total chromium						
Sample Type	Conc. (mg/L)	Recovery (%)	Conc. (mg/L)	Recovery (%)	Conc. (mg/L)	Recovery (%)
Drinking Water	0.1	107.0	0.5	100.0	1.5	104.1
Surface Water	0.1	103.7	0.5	93.0	1.5	93.0
Effluent Water	0.1	68.8	0.5	72.2	1.5	65.1
Hexavalent chromium						
Drinking Water	0.3	101.1	0.5	101.4	0.7	103.2
Surface Water	0.3	102.0	0.5	102.8	0.7	105.4
Effluent Water	0.3	104.2	0.5	102.4	0.7	103.0

Table 8
Comparison of the Cr results obtained using a Robotic Discrete Analyser and ICP-MS.

Sample	Discrete analyser mg/L	ICP-MS (mg/L)	% Difference
Tap Water (spiked)	0,101	0,0883	12,6
Tap Water (Spiked)	0,253	0,226	10,7
Tap Water (Spiked)	0,324	0,299	7,7
Surface Water (Spiked)	0,165	0,151	8,5
Surface Water (Spiked)	0,335	0,307	8,4
Surface Water (Spiked)	0,659	0,617	6,4
Ground Water (Spiked)	0,157	0,140	10,8
Ground Water (Spiked)	0,393	0,394	−0,3
Ground Water (Spiked)	0,642	0,643	−0,2
% Difference (Average)	7.2		
Two-Sample Assuming Equal Variances (*t*-Test)			
	Variable 1		*Variable 2*
Mean	0,33656		0,31837
Variance	0,04059		0,04026
Observations	9		9
Pooled Variance	0,04043		
Hypothesized Mean Difference	0		
df	16		
t Stat	0,19190		
P(T ≤ t) one-tail	0,42512		
t Critical one-tail	1,74589		
P(T ≤ t) two-tail	0,85023		
t Critical two-tail	2,11991		

Table 9
Limit of detection and limit of quantification.

Parameters	Total Chromium	Chromium (VI)
Average	0.0112	0.02113
STDEV	0.004984	0.0003536
LOD	0.0157	0.0011
LOQ	0.04980	0.0035

hexavalent chromium. The chromium species contents were measured using the developed robotic discrete analyser technique and the suggested real values showed good agreement.

The method's selectivity was assessed by adding chromium species that fell within the method's dynamic range to several types of water including tap water, groundwater, surface water, and effluent and measuring the proportion of chromium species recovered. Most percentage recoveries were between 90 and 110 %, which was satisfactory. By comparing the proposed approach to intriguing methodologies, such as ICP-MS, different water samples were analysed to determine the techniques' robustness. Since the t-stat value at the 95 % confidence level was less than the t-critical value, there was no significant difference between the ICP-MS method and the proposed robotic discrete analyser approach. Except for effluent water, which had recoveries ranging from 65 to 75 %, in the investigation of the selectivity of the developed method, most recoveries ranged from 90 to 110 %, which was considered average. Chemical species present in wastewater could have reacted with Cr(III) which might led to poor effluent sample recoveries.

Data availability statement

The data discussed in this study may be obtained on request from the corresponding author.

CRediT authorship contribution statement

Jerry Dikobe: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Funzani Asnath Melato:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Conceptualization. **Carel Johannes Lombard Adlem:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **Khathutshelo Netshiongolwe:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

References

[1] V. Gómez, M.P. Callao, Chromium determination and speciation since 2000, TrAC, Trends Anal. Chem. 25 (10) (2006) 1006–1015, https://doi.org/10.1016/j.trac.2006.06.010.

[2] L.S. McNeill, J.E. McLean, J.L. Parks, M.A. Edwards, Hexavalent chromium review, Part 2: Chemistry, occurrence, and treatment, Journal AWWA 104 (7) (2012), https://doi.org/10.5942/jawwa.2012.104.0092.

[3] J. Guertin, Toxicity and health effects of chromium (all oxidation states), in: J. Guertin, C. Avakian, J. Jacobs (Eds.), Chromium(VI) Handbook, CRC Press, 2004, pp. 215–234, https://doi.org/10.1201/9780203487969.ch6.

[4] M. García, E, C. Cabrera, J. Sánchez, M.L. Lorenzo, M.C. López, Chromium levels in potable water, fruit juices and soft drinks: influence on dietary intake, Sci. Total Environ. 241 (1–3) (1999) 143–150, https://doi.org/10.1016/S0048-9697(99)00340-X.

[5] W.-Y. Chen, C.-J. Chen, J.-W. Liao, F.C. Mao, Chromium attenuates hepatic damage in a rat model of chronic cholestasis, Life Sci. 84 (17–18) (2009) 606–614, https://doi.org/10.1016/j.lfs.2009.02.003.

[6] S. Şahan, Ş. Saçmacı, Ş. Kartal, M. Saçmacı, U. Şahin, A. Ülgen, Development of a new on-line system for the sequential speciation and determination of chromium species in various samples using a combination of chelating and ion exchange resins, Talanta 120 (2014) 391–397, https://doi.org/10.1016/j.talanta.2013.12.030.

[7] E.M. Horvath, L. Tackett, A.M. McCarthy, P. Raman, J.T. Brozinick, S. Jeffrey, J. S. Antidiabetogenic effects of chromium mitigate hyperinsulinemia-induced cellular insulin resistance via correction of plasma membrane cholesterol imbalance, Mol. Endocrinol. 22 (4) (2008) 937–950, https://doi.org/10.1210/me.2007-0410.

[8] Z. Krejpcio, Essentiality of chromium for human nutrition and health, Pol. J. Environ. Stud. 10 (2001).

[9] T. Pavesi, J.C. Moreira, Mechanisms and individuality in chromium toxicity in humans, J. Appl. Toxicol. 40 (9) (2020) 1183–1197, https://doi.org/10.1002/jat.3965.

[10] C. Zhang, J. Lin, X. Jia, B. Peng, A salt-free and chromium discharge minimizing tanning Technology: the novel cleaner integrated chrome tanning process, J. Clean. Prod. 112 (2016) 1055–1063, https://doi.org/10.1016/j.jclepro.2015.07.155.

[11] H.H. Abbas, F.K. Ali, Study the effect of hexavalent chromium on some biochemical, citotoxicological and histopathological aspects of the orechromis spp. Fish, Pakistan J. Biol. Sci. 10 (22) (2007) 3973–3982, https://doi.org/10.3923/pjbs.2007.3973.3982.

[12] Z. DeLoughery, M.W. Luczak, A. Zhitkovich, Monitoring Cr intermediates and reactive oxygen species with fluorescent probes during chromate reduction, Chem. Res. Toxicol. 27 (5) (2014) 843–851, https://doi.org/10.1021/tx500028x.

[13] T.L. DesMarias, M. Costa, Mechanisms of chromium-induced toxicity, Current Opinion in Toxicology 14 (2019) 1–7, https://doi.org/10.1016/j.cotox.2019.05.003.

[14] M. Shadreck, T. Mugadza, Chromium, an essential nutrient and pollutant: a review, Afr. J. Pure Appl. Chem. 7 (2013) 310–317.

[15] M.S. El-Shahawi, H.M. Al-Saidi, A.S. Bashammakh, A.A. Al-Sibaai, M.A. Abdelfadeel, Spectrofluorometric determination and chemical speciation of trace concentrations of chromium (III & VI) species in water using the ion pairing reagent tetraphenyl-phosphonium bromide, Talanta 84 (1) (2011) 175–179, https://doi.org/10.1016/j.talanta.2010.12.039.

[16] S. Testa, J. Jacobs, Overview of chromium(VI) in the environment: background and history, in: J. Guertin, C. Avakian, J. Jacobs (Eds.), Chromium(VI) Handbook, CRC Press, 2004, pp. 1–21, https://doi.org/10.1201/9780203487969.ch1.

[17] W. Van Der Merwe, J. Beukes, P. Van Zyl, Cr(VI) formation during ozonation of Cr-containing materials in aqueous suspension – implications for water treatment, WSA 38 (4) (2012) 505–510, https://doi.org/10.4314/wsa.v38i4.4.

[18] W. Alahmad, S.A. Mohamed, A. Halabi, Recent elaborations in electromembrane microextraction technique for preconcentration of chromium species: a mini-review, Advances in Sample Preparation 8 (2023) 100097, https://doi.org/10.1016/j.sampre.2023.100097.

[19] H. Tabani, T. Samkumpim, W. Alahmad, F. Dorabadizare, P. Varanusupakul, In-tube gel electro-membrane combined with microfluidic paper-based device: a green and miniaturized extraction mode for the chromium speciation, Advances in Sample Preparation 3 (2022) 100036, https://doi.org/10.1016/j.sampre.2022.100036.

[20] R.O. Martins, G.L. De Araújo, R.C. Simas, A.R. Chaves, Electromembrane extraction (EME): fundamentals and applications, Talanta Open 7 (2023) 100200, https://doi.org/10.1016/j.talo.2023.100200.

[21] J. Li, R. Zhu, X. Shen, C. Huang, Functional materials and chemicals in electromembrane extraction, TrAC, Trends Anal. Chem. 150 (2022) 116574, https://doi.org/10.1016/j.trac.2022.116574.

[22] M. Quack, F. Merkt (Eds.), Handbook of High-resolution Spectroscopy, first ed., Wiley, 2011 https://doi.org/10.1002/9780470749593.

[23] T.H. Randle, A.T. Kuhn, Kinetics and mechanism of the cerium(IV)/Cerium(III) redox reaction on a platinum electrode, J. Chem. Soc., Faraday Trans. 79 (8) (1983) 1741, https://doi.org/10.1039/f19837901741, 1.

[24] J.E.T. Andersen, Introduction of hydrogen peroxide as an oxidant in flow injection analysis: speciation of Cr(III) and Cr(VI), Anal. Chim. Acta 361 (1998) 125–131.

[25] J.Y.-P. Tong, E.L. King, The kinetics and mechanism of the reaction of cerium (IV) and chromium (III) [1,2], J. Am. Chem. Soc. 82 (15) (1960) 3805–3809, https://doi.org/10.1021/ja01500a001.

[26] V.J. Barwick, P.P.M. Bravo, S.L.R. Ellison, J. Engman, E.L.F. Gjengedal, U. Lund, B. Magnusson, H. Müller, M. Patriarca, B. Pohl, P. Robouch, L.P. Sibbesen, E. Theodorsson, F.J.L.A. Vanstapel, I. Vercruysse, A.T. Yılmaz, P.Y. Ömeroğlu, U. Örnemark, The Fitness for Purpose of Analytical Methods A Laboratory Guide to Method Validation and Related Topics, second ed., 2014.

[27] A. Gustavo González, M. Ángeles Herrador, A practical guide to analytical method validation, including measurement uncertainty and accuracy profiles, TrAC, Trends Anal. Chem. 26 (3) (2007) 227–238, https://doi.org/10.1016/j.trac.2007.01.009.

[28] A.M. Stoyanova, Catalytic spectrophotometric determination of chromium, Turk. J. Chem. 29 (2005) 367–375.

[29] L.V. Mulaudzi, J.F. Van Staden, R.I. Stefan, Determination of chromium(III) and chromium(VI) by use of a spectrophotometric sequential injection system, Anal. Chim. Acta 467 (1–2) (2002) 51–60, https://doi.org/10.1016/S0003-2670(02)00188-5.

[30] B. Markiewicz, I. Komorowicz, A. Sajnóg, M. Belter, D. Barałkiewicz, Chromium and its speciation in water samples by HPLC/ICP-MS – technique establishing metrological traceability: a review since 2000, Talanta 132 (2015) 814–828, https://doi.org/10.1016/j.talanta.2014.10.002.