Nitrite interference and elimination in diphenylcarbazide (DPCI) spectrophotometric determination of hexavalent chromium

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ABSTRACT

Cr(VI) is highly noted as a carcinogenic, mutagenic, and teratogenic pollutant. However, accurate determination of Cr(VI) in aqueous samples is difficult using the conventional diphenylcarbazide (DPCI) spectrophotometric method upon being interfered by co-existed nitrite. This paper illustrates how to eliminate the nitrite influence in a simple but efficient method based on a detailed analysis of interference mechanism. High-performance liquid chromatography analysis revealed that under acidic condition, DPCI was oxidized by nitrite to other substrates, which could not react with Cr(VI). The final oxidation product of DPCI was further purified by thin-layer chromatography and identified as diaryl carbodiazone by Fourier Transform Ion Cyclotron Resonance-Mass Spectrometry (FTICR-MS) and nuclear magnetic resonance. Consequently, an improved method was proposed by simply adding sulfamic acid for eliminating the nitrite interference in Cr(VI) determination. The proposed method was successfully confirmed by the accurate recovery of Cr(VI) from spiked water samples and further proven with inductively coupled plasma-atomic emission spectroscopy, which demonstrated a great potential for determining Cr(VI) concentration in aqueous samples containing nitrite.

Key words | diphenylcarbazide, elimination, hexavalent chromium, nitrite interference, sulfamic acid

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INTRODUCTION

Chromium in aqueous solution mainly exists in forms of trivalent chromium and hexavalent chromium. Cr(III) is an essential element playing an important role in glucose, lipid, and protein metabolism for the human body (Mertz 1993), while Cr(VI) is a toxic substance with intense carcinogenicity, mutagenicity, and teratogenicity (Cieslak-Golonka 1996). As an important industrial material, chromium is widely used in electroplating, dye and pigment manufacturing, leather tanning, and alloy production, which could be inevitably released to the surrounding environment. Therefore, strict water quality standards for Cr(VI) were executed to reduce environment pollution and health risks, for instance, the maximum contaminate level of Cr(VI) in domestic water supplies is 0.05 ppm (USEPA 1980).

The measurement methods of Cr(VI) concentration in aqueous solutions include spectrophotometric, chromatographic, atomic absorption methods. Among them the diphenylcarbazide (DPCI) spectrophotometric method has been most frequently used due to the highly sensitive and selective reaction of DPCI and Cr(VI) (Gomez & Callao

2006). Note that existence of some ions such as V(V), Fe(III), Cu(II), Mo(VI), and permanganate could interfere with the reaction of DPCI and Cr(VI), some methods have been proposed to solve these problems, e.g. removal of V(V), Fe(III), Cu(II), and Mo(VI) by chloroform extraction of the metals formed cupferrates and masking permanganate by agent azide (APHA 1998).

Recently, combined pollution of heavy metals and other contaminants in water attracts more attentions (Zhang et al. 2009; Tao et al. 2011), especially co-occurrence of chromium and nitrite. On one hand, nitrite contamination became more serious in some regions with industrial and agricultural development (Rawat et al. 2012). As a precursor of carcinogenic N-nitrosamines (Bassir & Maduagwu 1978), nitrite would induce cancer in human and animals. Conversely, chromate-reducing activity in bacteria was found to have inseparable relationships with denitrification, a biological process reducing nitrate stepwise to nitrite, nitric oxide, nitrous oxide, and finally nitrogen gas, in which nitrite could be accumulated to a considerable concentration. Co-reduction

of Cr(VI) and nitrate by *Staphylococcus epidermidis* L-02 accompanied with nitrite accumulation has been reported under anaerobic condition (Vatsouria *et al.* 2005). Nitroreductase was confirmed to be able to act as a chromate reductase (Kwak *et al.* 2003). Moreover, the nitrite reduction pathway is related to Cr(VI) reduction in *Shewanella oneidensis* MR-1 (Viamajala *et al.* 2002).

Unfortunately, it was observed in previous studies that nitrite at 5 mM concentration made DPCI unworkable to Cr(VI) and completely hindered the DPCI spectrophotometric method, therefore a sophisticated method liquid chromatography-inductively coupled plasma mass spectrometry (LC-ICP-MS) was carried out to quantify Cr(VI) concentrations (Han *et al.* 2010). Moreover, five drops of 5% sodium nitrite would cause a 33% negative error at 10 mg L^{-1} Cr(VI) (Burns & Dunford 1996). However, the mechanism of nitrite interference in DPCI spectrophotometric process had rarely been investigated previously. Thus, how nitrite interferes in determining Cr(VI) concentration and how to eliminate the interference become the primary concern for accurate determination of Cr(VI) in the presence of nitrite, and this study would propose a simple and cost-effective solution based on the insights into mechanisms of nitrite interference.

MATERIALS AND METHODS

Apparatus

A UV–vis spectrophotometer (UV-4802, Shanghai Unico Instrument Co. Ltd, China) with 10-mm quartz cells was used for absorption measurements. A high-performance liquid chromatography (HPLC) system (Agilent 1200, USA) equipped with a ZORBAX SB-Aq column (250 mm × 4.6 mm, 5.0 μm) and an ultraviolet visible detector was used for detection of the intermediate and final products of reactions. Nuclear magnetic resonance (NMR) spectra were analyzed on a Bruker AVANCE NMR spectrometer (AV400, Bruker, Switzerland) at a resonance frequency of 400 MHz for ^1H, and 400 MHz for ^{13}C. Mass spectrometric experiment was carried out on a Bruker APEX IV FTICR mass spectrometer (APEX IV, Bruker, USA) equipped with a 7.0 T superconducting magnet and EI, ESI source. Ultrasonic apparatus (KH-100B, Kunshan Hechuang Instrument Co. Ltd, China) was used for accelerating the reaction of sulfamic acid and nitrite. Inductively coupled plasma-atomic emission spectroscopy (ICP-AES, Prodigy, Leeman, USA) was employed for testing accuracy

of the improved method in determining different Cr(VI) concentrations.

Reagents and solutions

Dichloromethane and methanol used in this study were chromatographically pure. Diphenylcarbazone (DPCO) was analytical reagent grade (purity $\geq 40.0\%$, mixture with DPCI). Other solutions were prepared by analytical reagent grade chemical and double-distilled water. A stock solution of 1,000 mg L^{-1} Cr(VI) was prepared by dissolving 2.829 g potassium dichromate, previously dried at 120 °C for 2 h, in 1,000 mL water. A standard solution of 10 mg L^{-1} Cr(VI) was prepared by diluting 1 mL stock chromium solution to 100 mL. DPCI solution was prepared by dissolving 200 mg 1,5-DPCI in 50 mL acetone and diluting to a final volume of 100 mL. Sulfuric acid solution (1:1) and phosphoric acid solution (1:1) were prepared by diluting 50 mL acid to 50 mL water. Sodium nitrite solution (10 g L^{-1}) and sulfamic acid solution (20 g L^{-1}) were prepared by dissolving 1 g sodium nitrite in and 2 g sulfamic acid in 100 mL water, respectively. A solution of 500 mg L^{-1} Cr(III) was prepared by dissolving 0.0256 g chromium(III) chloride hexahydrate in 10 mL water.

Reaction and product identification of nitrite and DPCI

The interference of nitrite in determining Cr(VI) concentrations was investigated by measuring absorbance of solutions containing 1 mg L^{-1} Cr(VI) and increasing nitrite with DPCI spectrometric method. To further explore the interference mechanism, reaction process of DPCI and nitrite at acidic condition was confirmed by analyzing the intermediates and final products with HPLC. Briefly, 40 μL DPCI solution was diluted with 1 mL water, after adding 10 μL sulfuric acid solution and 10 μL phosphoric acid solution, 0, 0.4, 1, 1.5, 2, 3, 40 μL nitrite solution were added, respectively. Thereafter the reaction system were supplemented with water to a final volume of 1.1 mL for HPLC analysis, in which separation was accomplished with elution of a mobile phase comprising water and methanol (40:60 v/v) at a flow rate of 1 mL min^{-1} and column temperature of 30 °C and signal detection was performed at 234 nm.

The final reaction product was purified with thin-layer chromatography (TLC) silica gel plate (GF254, Shanghai Yucheng Chemical Co. Ltd, China). The developing solvent comprised dichloromethane and methanol (12:1 v/v). Products on silica-coated plates, visible under UV light, were eluted by dichloromethane and methanol (9:1 v/v). After

being dried by nitrogen gas, a portion of the samples were resuspended in 500 μL methanol for Fourier Transform Ion Cyclotron Resonance-Mass Spectrometry (FTICR-MS) analysis and the other in 500 μL CD$_3$OD (Cambridge Isotope Laboratories, Inc., USA) for ^{1}H NMR and ^{13}C NMR analysis.

General and improved procedure for Cr(VI) determination

General procedure for determination of Cr(VI) concentrations was as follows. Place a known volume of sample containing no more than 5 μg Cr(VI) in a 10 mL colorimetric tube, make up the volume to 5 mL with water, add 50 μL sulfuric acid and 50 μL phosphoric acid, mix well, add 200 μL DPCI solution, mix well again, and keep it still for 10 min for full color development. The absorbance was determined at 540 nm with a 10 mm cell. A blank solution (only water) was conducted in parallel and taken as reference. The improved procedure was carried out for completely eliminating nitrite interference in determining Cr(VI) concentration. The major steps were similar as the general procedure except adding 200 μL sulfamic acid solution into the mixture and placing the colorimetric tube in ultrasonic apparatus (100 W) for 5 min before adding sulfuric acid. The ultrasonic process aimed to promote the reaction of sulfamic acid and nitrite and release the gaseous product from the reaction system. Nitrite concentration was determined by N-(1-naphthalene)-diaminoethane spectrophotometric method (APHA 1998).

RESULTS AND DISCUSSION

Nitrite interference in determining Cr(VI) concentrations

The results of nitrite interference in determining Cr(VI) concentrations were shown in Figure 1. When solutions containing 1 mg L^{-1} Cr(VI) was supplemented with increasing sodium nitrite, the absorbance decreased significantly. The apparent Cr(VI) concentration decreased to 90.13% of the real value in the presence of only 0.61 mg L^{-1} nitrite (NO$_2^-$-N). It also decreased to 15.22% when nitrite concentration reached 7.6 mg L^{-1}. When nitrite reached 60.87 mg L^{-1}, the chromogenic reaction was completely invalidated. All concentrations were calculated in a volume of 5 mL.

The same phenomenon was observed in previous studies (Burns & Dunford 1996; Han *et al.* 2010). The
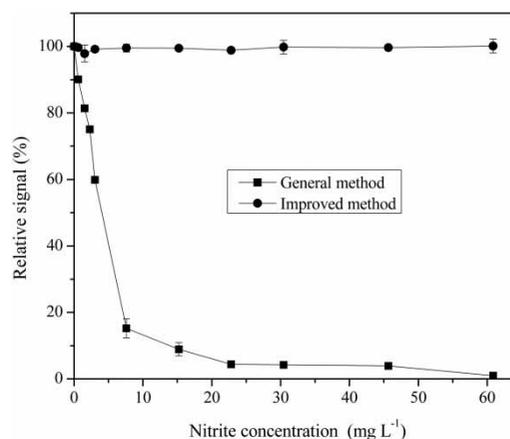

Figure 1 | Absorbance variations of 1 mg L^{-1} Cr(VI) supplemented with different nitrite concentrations. Improved method was treated with sulfamic acid.

presence of nitrite indeed hindered the color reaction of Cr(VI) and DPCI, leading to a fake disappearance of Cr(VI) in the typical DPCI spectrophotometric method. The classical color reaction between Cr(VI) and DPCI has been investigated a lot previously (Bose 1952; Pflaum & Howick 1956; Lichtenstein & Allen 1961). It is postulated that under the acidic condition Cr(VI) oxidized DPCI to DPCO and was reduced to Cr(III) itself, thereafter, the newly produced DPCO and Cr(III) formed a complex which was responsible for the intense magenta color (Pflaum & Howick 1956). It was initially suspected that the interference was ascribed to the redox reaction of nitrite and Cr(VI) at acidic condition due to the strong oxidability of Cr(VI), however, it turned out that this chemical reaction did not occur, which was checked by the insignificant variations of nitrite concentration within 30 min in the mixture of HNO$_2$ (acidified nitrite) and Cr(VI) solution. Thus, further tests were needed to explore the mechanism of nitrite interference in determining Cr(VI) concentration by DPCI spectrophotometric method.

Reaction mechanism of nitrite and DPCI

After ruling out the possibilities of reaction between nitrite and Cr(VI), the reaction process of nitrite and DPCI in the acidic condition was investigated and confirmed by analyzing the intermediates and final products with HPLC (Figure 2). With injection of solution of DPCI, DPCO (mixture with DPCI) and HNO$_2$, retention times of 4.24, 5.03, and 3.59 min, respectively, were obtained (Figures 2(a)–2(c)). With the volume of nitrite dosage increased from 0.4 to 40 μL, the peak of DPCI reduced gradually and finally
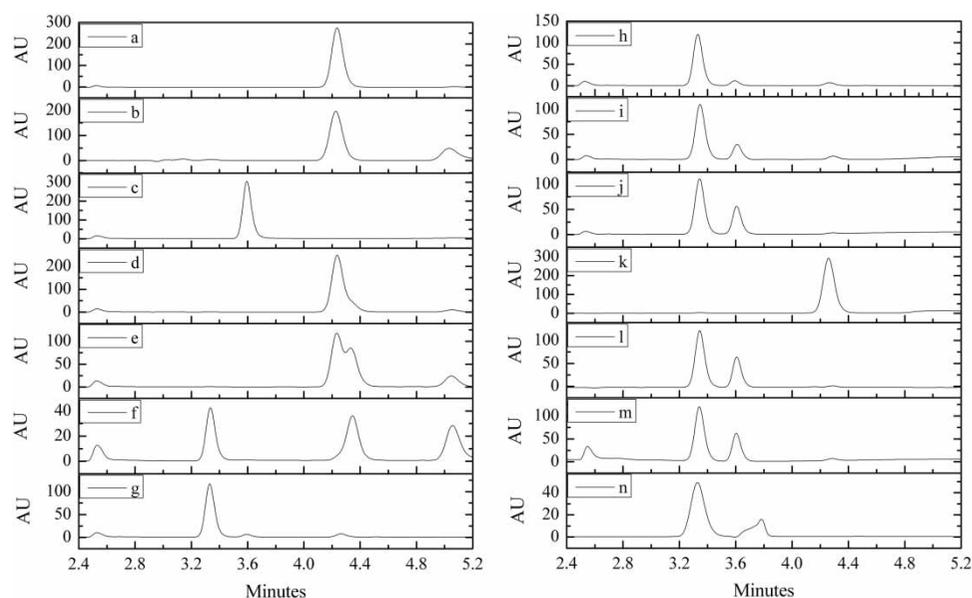
Figure 2 | High-performance liquid chromatographic elution profile of: (a) DPCI; (b) DPCO; (c) $NO_2^- + H^+$; (d) DPCI + 0.4 μL nitrite; (e) DPCI + 1 μL nitrite; (f) DPCI + 1.5 μL nitrite; (g) DPCI + 2 μL nitrite; (h) DPCI + 3 μL nitrite; (i) DPCI + 40 μL nitrite; (j) DPCO + 40 μL nitrite; (k) DPCI + 40 μL nitrite, without H^+; (l) DPCI + 40 μL nitrite, with H_3PO_4; (m) DPCI + 40 μL nitrite, with H_2SO_4; (n) production purified by TLC.

disappeared (Figures 2(d)–2(i)), and simultaneously several new obvious peaks formed at retention times of 4.35 min (Figures 2(e) and 2(f)), 5.06 min (Figures 2(e) and 2(f)), 3.33 min (Figures 2(f)–2(i)), and 3.61 min (Figure 2(i)), indicating formation of new compounds. The peak at retention time of 5.06 min (Figures 2(e) and 2(f)) formed and disappeared again, which matched DPCO well (Figure 2(b)) and could be inferred as an immediate. The substrate at 3.61 min (Figure 2(i)) matched HNO_2 well, indicating nitrite was excess compared to DPCI. As the last appeared and stable existed peak, it could be inferred that the substrate peaked at 3.33 min was the final reaction product of nitrite and DPCI. It was also produced in the reaction between DPCO and HNO_2 (Figure 2(j)), which further verified that DPCO was an intermediate and the substrate at 3.33 min was the final product of reaction between DPCI and HNO_2.

Moreover, H^+ was proved to be necessary in the reaction of nitrite and DPCI, since the reaction did not occur without acid addition (Figure 2(k)) and occurred when either H_3PO_4 or H_2SO_4 was supplemented (Figures 2(l) and 2(m)). In addition, it was notable that a new substrate, peaking at 4.33–4.35 min in Figures 2(e) and 2(f) (overlapping DPCI peak in Figure 2(e)) appeared and disappeared later, demonstrating that another unknown intermediate was formed during the reaction process of nitrite and DPCI. Unfortunately, this unknown intermediate was not separated efficiently and needs to be further studied in the future. The final product was successfully purified by TLC

silica gel plate and peaked at 3.33 min (Figure 2(n)), which matched the peaks obtained in former tests. The purified substrate was further identified by FTICR-MS and NMR.

The peak of $[M + H]^+$ by FTICR-MS analysis under the positive ion mode was at m/z 239.09274 $[C_{13}H_{11}N_4O]^+$ (0.82 ppm), indicating the molecular mass of the final product was 238.0848. Thereby the formula $C_{13}H_{10}N_4$ (theoretical mass 238.2447) was presumed, which coincided with diaryl carbodiazone (DPCDO), a dehydrogenation product of DPCO. The structure of the final product was further explored by ^{1}HNMR and ^{13}CNMR spectrum. The data (Table S1 in the Supplementary Material, available online at http://www.iwaponline.com/wst/072/203.pdf) were consistent with those reported for diaryl carbodiazone (Wang *et al.* 1998), which further proved DPCDO was the final product in the reaction of nitrite and DPCI. From the HPLC, FTICR-MS, ^{1}H NMR, and ^{13}C NMR analysis, it could be inferred that nitrite interference in determining Cr(VI) concentration by DPCI spectrophotometric method was ascribed to the reaction of DPCI and nitrite, i.e. DPCI was oxidized by nitrite stepwise to DPCO and DPCDO under acid conditions, as shown in Figure 3.

DPCO and DPCDO could not react with Cr(VI) ion except for DPCI (Pflaum & Howick 1956). If DPCI was consumed by exited nitrite, reduction of Cr(VI) to Cr(III) and the following chromogenic reaction of Cr(III) and DPCO were both hindered, and thereby a negative result was shown. The phenomenon of nitrite interference also indicated that
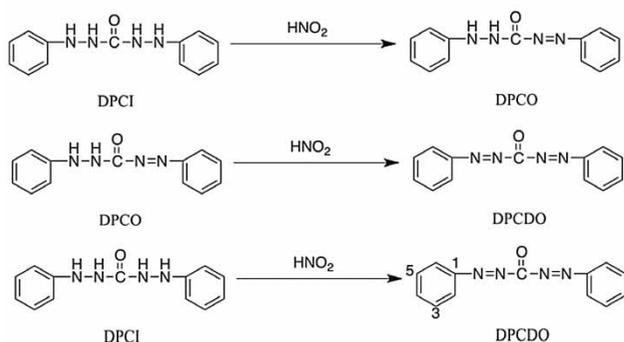
Figure 3 | Reaction mechanism of DPCI and nitrite revealed by HPLC, FTICR-MS, ^{1}H NMR and ^{13}C NMR analysis.

nitrite was more competitive than Cr(VI) during the oxidation of DPCI to DPCO. In previous research (Li et al. 2002), a reagent system $NaNO_2/NaHSO_4 \cdot H_2O/SiO_2$ had been developed to oxidize DPCI to DPCO, which could not be further oxidized to DPCDO even in presence of excess oxidant $NaNO_2$ and efficient catalyst SiO_2. While other investigators (Nagashima et al. 1984) indicated that DPCI could be oxidized stepwise to DPCO and further to DPCDO by H_2O_2, in the present study, it was proved possible to oxidize DPCI to DPCDO by nitrite under acidic conditions.

Elimination of nitrite interference with sulfamic acid

As it was a big concern to accurately determine Cr(VI) concentration when nitrite was present, it was necessary to explore an effective method to eliminate the interference. In this study, an improved method was proposed where sulfamic acid was used as a masking agent to eliminate the existed nitrite.

Sulfamic acid had been used to enhance the efficiency of high-concentration nitrite-containing wastewater by microwave treatment process (Li et al. 2010), and remove nitrite for accurate determination of nitrate or chemical oxygen demand (COD) (Marouf-Khelifa et al. 2006). However, the performance of sulfamic acid in eliminating nitrite for accurately determining Cr(VI) by DPCI spectrophotometric method had never been investigated. The reaction of sulfamic acid and nitrite was shown as the following reaction equation (Yu 2010):

$$NH_2SO_3H + NaNO_2 \rightarrow N_2 \uparrow + H_2O + NaHSO_4 \qquad (1)$$
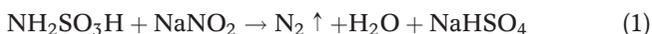
In this study, the sulfamic acid added into the reaction system was 4 mg, enough to eliminate 115 mg L^{-1} NO_2^--N

calculated in a volume of 5 mL according to equation (1). As can be seen from Figure 1, in the improved method the absorbance recovered 96.1–101.6% of the real value, indicating the interference of nitrite was eliminated effectively and efficiently. In the eliminating process, the produced nitrogen gas should be released sufficiently to ensure that nitrite had been fully consumed by sulfamic acid. It was also notable that if nitrite concentration in water samples was more than 115 mg L^{-1}, the concentration of sulfamic acid could correspondingly increase to meet the requirement of eliminating nitrite. Carbamide was reported to be able to reduce nitrite to nitrogen gas (Eszterle 2004), nevertheless, only a proportion of real absorbance was recovered when carbamide was used to eliminate nitrite in our study.

When 60 mg L^{-1} NO_2^--N was present, the slope of standard curve of Cr(VI) (0–1 mg L^{-1}) determined by general method was nearly zero, whereas in the improved method the calibration curve $y = 0.3603x + 0.0022$ ($R^2 = 0.9998$) was almost the same with $y = 0.3750x + 0.0017$ ($R^2 = 0.9997$) produced in the nitrite absence system (y refers to the absorbance and x implies the Cr(VI) concentration, mg L^{-1}), revealing a total elimination of nitrite interference by sulfamic acid. The limit of detection was 0.0040 mg L^{-1} using the general procedure and 0.0039 mg L^{-1} using the improved procedure calculated by tripling the standard deviation of blank sample.

The relative standard deviation of five repeated measurements of two Cr(VI) samples with unknown concentrations below 1 mg L^{-1} (both supplemented with 60 mg L^{-1} NO_2^--N) were 0.88% (average 0.522 mg L^{-1}) and 1.04% (average 0.647 mg L^{-1}), respectively, lower than 5%, demonstrating the high repeatability of the improved method. Since sulfamic acid did not react with Cr(VI) or DPCI, the excess added sulfamic acid was not needed to be decomposed, indicating this method was selective and feasible in eliminating nitrite interference in DPCI spectrophotometric method.

Applicability of the improved method

For further test application of the improved method, water samples spiked with known amounts of Cr(VI) and different amounts of nitrite were investigated. As shown in Table 1, the obtained recoveries of Cr(VI) were in the range of 98.20–102.61%, demonstrating the high accuracy and application capability of the improved method for determining Cr(VI) in nitrite contaminate water samples.

Table 1 | Recovery of Cr(VI) determined by the improved method

NO$_2^-$-N added (mg L^{-1})	Cr(VI) added (mg L^{-1})	Cr(VI) measured (mg L^{-1})[a]	Recovery (%)
3	0.2	0.201 ± 0.007	100.43
3	0.5	0.493 ± 0.013	98.59
30	0.2	0.205 ± 0.003	102.61
30	0.5	0.492 ± 0.007	98.40
60	0.2	0.205 ± 0.006	102.61
60	0.5	0.491 ± 0.002	98.20

[a]Samples were measured in triplicate and represented by mean ± standard deviation.

The accuracy of the improved method was also checked by ICP-AES. Five water samples containing 0–5 mg L^{-1} Cr(VI) were diluted five times (added nitrite concentration ranging from 1 to 60 mg L^{-1}) and analyzed by the general method, the improved method and ICP-AES, as presented in Table 2. Statistical differences between the improved method and ICP-AES were analyzed by paired-samples *T*-test using SPSS 20.0 software. The improved method demonstrated a good agreement with ICP-AES ($P = 0.501 > 0.05$), indicating the simple present method could be employed instead of expensive ICP-AES method for determining Cr(VI) in nitrite present samples. Even though the plasma-based analytical techniques are currently prevailing in analysis of heavy metals in aqueous samples, conventional colorimetric methods with UV-vis instruments still provide a valuable tool when the large and expensive analytical equipment is not available, especially during *in situ* field sampling measurements.

Samples containing 0.5 mg L^{-1} Cr(VI), 60 mg L^{-1} nitrite and different amounts of Cr(III) were also measured by the improved method, as shown in Table 3. It can be seen that only when the concentration of Cr(III) reached 50 mg L^{-1}, an interference (deviation above 5%) was observed.

Table 2 | Determination of Cr(VI) concentrations in water samples by general method, improved method and ICP-AES

Water sample[a]	General method[b]	Improved method[b]	ICP-AES[b]
1	0.070 ± 0.009	0.613 ± 0.041	0.607 ± 0.010
2	0.059 ± 0.009	1.162 ± 0.009	1.154 ± 0.018
3	0.334 ± 0.009	2.454 ± 0.038	2.522 ± 0.022
4	0.054 ± 0.009	3.160 ± 0.071	3.199 ± 0.013
5	0.134 ± 0.009	4.365 ± 0.049	4.337 ± 0.022

[a]All water samples did not contain Cr(III) and were added a random nitrite amount from 1 to 60 mg L^{-1}.
[b]Samples were measured in triplicate and represented by mean ± standard deviation, mg L^{-1}.

Table 3 | Determination of Cr(VI) concentrations in water samples containing Cr(III) by the improved method

Cr(III) added (mg L^{-1})	Cr(VI) measured (mg L^{-1})[a]	Recovery (%)
0	0.513 ± 0.001	102.7
0.5	0.504 ± 0.000	100.8
1	0.524 ± 0.000	104.8
10	0.506 ± 0.005	100.7
25	0.521 ± 0.000	104.3
50	0.525 ± 0.001	105.2
100	0.537 ± 0.000	107.4

[a]Samples were measured in duplicate and represented by mean ± standard deviation.

However, it was concluded that the interference was due to the original color of Cr(III) in such a high concentration which has a certain absorbance at 540 nm, since the reaction between Cr(VI) and DPCI was extremely specific. Moreover, there was no significant interference when Cr(III) concentrations were at lower levels, even though these were already dozens of times higher than Cr(VI). This result demonstrated that the proposed method still works for determining Cr(VI) concentrations when Cr(III) and nitrite co-exist in water samples. Moreover, under this situation, the ICP-AES method could only determine the concentration of total Cr rather than Cr(VI), but the improved method proposed in this study still works when determining Cr(VI) concentration regardless of Cr(III) and nitrite interference, indicating its great potential application for Cr(VI) determination.

CONCLUSIONS

Nitrite could severely interfere in the accurate determination of Cr(VI) concentration in terms of DPCI spectrophotometric method. The interference mechanism was explored that nitrite would oxidize DPCI stepwise to DPCO and DPCDO under acidic condition and thereby hinder the color reaction of Cr(VI) and DPCI. Based on the mechanism a simple and efficient method was proposed for eliminating nitrite by sulfamic acid addition. The improved method was verified by high recovery of Cr(VI) from spiked water samples compared with ICP-AES and exhibited excellent characteristics of feasibility, repeatability and accuracy, which demonstrated a great application prospect in determining Cr(VI) concentrations in nitrite and Cr(III) co-occurred aqueous samples.

ACKNOWLEDGEMENTS

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