Annals of Agrarian Science

Journal homepage: http://journals.org.ge/index.php

Determination of Cr (III) by Means of Atom Absorption Spectrometry

A. Rcheulishvili[a], O. Rcheulishvili[a, b], L. Tugushi[a], E. Ginturi, L. Gheonjian[a, c], V. Mayer[a], D. Qaadze[a]

[a] Elefter Andronikasvili Institute of Physics, Ivane Javakhishvili Tbilisi State University; 6, Tamarashvili street, Tbilisi, 0172, Georgia

[b] Ilia State University; 3/5, Kakutsa Cholokashvili Ave., Tbilisi, 0162, Georgia

[c] University named after St. Queen Tamara under the Patriarchate of Georgia 68, Uznadze street, Tbilisi 0102, Georgia

Received: 21 January, 2022; Accepted: 30 August, 2022

A B S T R A C T

The atomic absorption (AA) method is proposed for determination of trivalent chromium [Cr(III)] concentration in the samples containing the mixture of Cr (III) and Cr (VI). It was found that addition of chloric acid ($HClO_4$) to Cr (III) solution substantially increases AA spectrometric signal. However, with the addition of $HClO_4$ to Cr (VI) solution the signal remains unchanged. The addition of chloric acid to the mixture of chromium ions causes the increase of the signal of absorption that is related to the presence of Cr (III) ions. This fact allows quick and selective determination of Cr (III) concentration in the samples without prior separation of chromium compounds with different valences. The proposed method can be used for both atomic absorption spectrometry and atomic fluorescence spectrometry. Possibility of experimental application of the method is shown on the example of examination of interaction between Cr (VI) ions and chromium bacteria *Arthrobacter oxydans*.

***Key words*:** Atomic absorption spectrometry, Chromium, Cr (III), Chloric Acid

* Corresponding author: Alexandre Rcheulishvili, e-mail address: archeuli@gmail.com

Introduction

Chromium is one of the most problematic metals with both toxic and vitally important properties [1, 2]. Of several possible oxidation states from Cr (II) to Cr (VI), two states such as trivalent Cr (III) and hexavalent Cr (VI) are characterized by stability in natural and biological systems. Cr (VI) compounds are readily soluble, highly toxic and carcinogenic, while Cr (III) compounds are less soluble and less toxic. Moreover, Cr (III) is a trace element vital for functioning of living organisms. Through various processes (chemical and / or biological), the toxic form of Cr (VI) can be transformed into non-toxic form of Cr (III). Therefore, the express information on the chromium content in the environment is essential for assessing and prevention its harmful effects.

Currently, various methods are used to determine the chromium content, such as electron spin resonance spectrometry, ion chromatography, high-pressure liquid chromatography, inductively coupled plasma mass spectrometry, neutron activation analysis, etc. Atomic absorption spectrometry (AAS) is the simplest and cheapest method used as the main technique for determining the chromium content [3, 4].

However, for a quantitative determination of Cr (VI) or Cr (III) concentrations, the preliminary separation and concentration procedures are necessary, which require additional time and costs. Besides, the process of chemical separation can cause errors in determining the concentration [3].

In recent years, Gaspar et al. carried out researches to improve the efficiency of Cr (VI) and Cr (III) concentration determination by means of

preliminary separation of Cr ions in biological and environmental samples using high-pressure liquid chromatography and AAS [5-8].

In our previous studies, we had an attempt to simplify the determination of Cr (VI) and Cr (III) concentrations by means of AAS method without their preliminary separation in the test solutions. Two well-known facts, which are essential in the atomic absorption analysis of chromium, were taken as the basis. First, in some test conditions (using C_2H_2-air flame in atomic absorption or atomic emission spectrometry) Cr (III) detection sensitivity is somewhat better (up to 10%) than that of Cr (VI) [9, 10]. Similar results were obtained in the case of atomic fluorescence spectrometry (AFS) [11]. We found that Cr (VI) detection sensitivity becomes twice greater than that of Cr (III) when the mixed propane-butane-air flame is used in AAS and AFS [11, 12]. Second, it is known that the presence of some special "additives" in the sample can change the signal of the element during AAS or AFS [3]. Based on this, we examined the effect of various chemical reagents (H_2SO_4, H_2O_2, HCl, H3PO4, KOH, FeSO4) on the magnitude of the atomic absorption and atomic fluorescence signals of chromium upon spraying Cr (VI) and Cr (III) solutions [13]. Experiments showed that hydrogen peroxide (H_2O_2) is most suitable for determination of Cr (VI) concentration in all test compounds. The addition of H_2O_2 to the sample solutions had practically no effect on the atomic absorption (atomic fluorescence) signal of Cr (III), while the signal of Cr (VI) was halved.

However, due to the fact that in atomic absorption analysis the samples are often preashed (wet ashing) with nitric acid (HNO_3), the use of H_2O_2 does not always work in practice, since the presence of HNO_3 in the sample reduces the effect of hydrogen peroxide and complicates the selective determination of Cr (III).

The paper presents the results of further investigations of effective reagents for detection of Cr (III) by means of AAS or AFS. When chloric acid was used as a reagent in the experiment, the presence of HNO_3 had no effect on the results.

Objectives and Methods

Equipment used. In the experiments, we used a double-beam atomic absorption spectrometer Beckman-495 with a double-slit burner for H_2O_2-air flame. To obtain the flame of the mixed propane-butane-air a forced mode of burning gas and reduced air flow (P≈18 psig) (1psig ≈ 1/15 atm) were used. The distance between the transmission beam and the burner nozzle was 10 mm. A spectral lamp with a hollow cathode of "ЛСП-1" type (Russia) was used as a source of primary radiation. The lamp current for Cr was 25 mA (continuous). Chromium resonance line of 357.9 nm wavelength and 0.5 nm slit width was used.

In the experiments the atomic fluorescence spectrometer described in [14] was also used. For excitation of the chromium atoms the "ЛСП-1" hollow cathode lamp was used. The lamp was powered by "ППСЛ-2" device (Russia) in the external modulation mode with the supply current of 23 mA, on average. Modulation was carried out at frequency of 350 hertz using "3Г-10" generator (Russia). The photomultiplier "ФЭУ-71" (Russia) was used as a photodetector. From the photomultiplier the electronic signal was transmitted to a narrow-band amplifier tuned to the frequency of 350 hertz, and then to a synchronous detector "СД-9" (Russia). The reference signal was transmitted from the generator "3Г-10". The outgoing signal from "СД-9" was recorded by means of a potentiometric recorder "ЛКС-3" (Russia).

Sample Preparation

Initial solutions of Cr (VI) and Cr (III) were prepared with concentrations of C = 4 mg / ml each. For preparation of Cr (VI) solution we placed m=1.1315 g of compound $K_2Cr_2O_4$ in 100 ml flask and filled it with bidistilled water. For preparation of Cr (III) solution, we placed m = 1.9207 g of compound CrK (SO_4), $12H_2O$ in 50 ml flask and filled it with bidistilled water. Then, 0.1 ml of initial solution of Cr (VI) was transferred into eight test tubes. The test tubes were added by solution of $HClO_4$ (d = 1.25 kg / L) in the following amounts: 0 ml, 0.03 ml, 0.06 ml, 0.1ml, 0.2 ml, 0.3 ml, 0.4 ml, 0.6 ml, respectively. All tubes were filled with bidistilled water up to 8 ml, so that the concentration of Cr (VI) in each tube was 50 μg/ml (first series). Another eight tubes prepared in the same way were added by extra 0.2 ml of HNO_3 (73%) per tube (second series). In the third series each tube prepared in the same way was added by 0.4 ml of HNO_3.

The mixtures were prepared from the initial

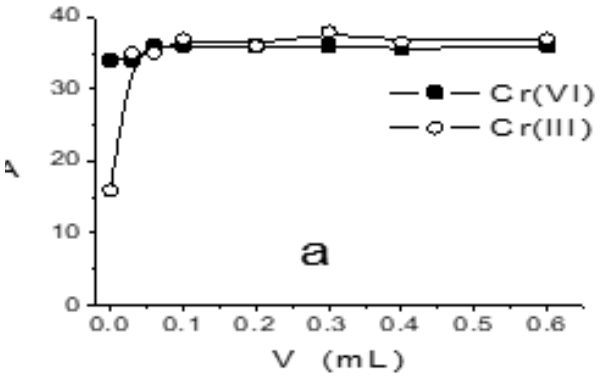
solution of Cr (III) in the same way. Concentration of Cr (III) in each tube was also 50 μg / ml. The eight test tubes of the first series were added by $HClO_4$ in the amount of 0 ml, 0.03 ml, 0.06 ml, 0.1ml, 0.2ml, 0.3ml, 0.4 ml, 0.6 ml, respectively. The test tubes of the second series prepared in the same way as those in the first series were added by an extra 0.2 ml of HNO_3 per test tube, and those of the third series were added by 0.4 ml.

The signals of atomic absorption (AA) and atomic fluorescence (AF) of chromium were measured by spraying the obtained solutions. As far as the results obtained in AA and AF coincided, only the data of AA measurements are presented below.

Results and Discussion

It turned out that the absorption signal remained practically unchanged when about 0.6 ml of $HClO_4$ was added to 8 ml of Cr (VI) solutions (Fig. 1a). Addition of a small amount of $HClO_4$ to Cr (III) solutions (0.03 ml per 8 ml) increases the atomic absorption signal approximately twice. Further increase of the amount of added $HClO_4$ practically does not cause any more changes in the absorption signal. The dependences will be similar if extra amount 0.2 ml of HNO_3 (Fig. 1b) or 0.4 ml of HNO_3 (Fig. 1c) is added to Cr (VI) and Cr (III) solutions.

The results obtained show that if a certain amount of $HClO_4$ is added to a mixture of Cr (VI) and Cr (III) solutions, the atomic absorption signal increases by ΔA (absorption jump) that is caused exclusively by the presence of trivalent chromium in the test solution. The magnitude of the atomic absorption signal ΔA depends only on the concentration of Cr (III) in the test solution.



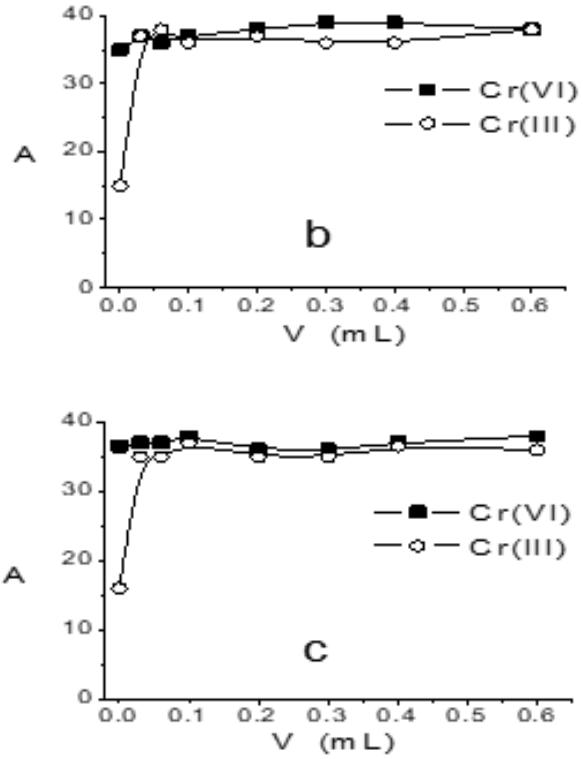


Fig. 1.

Dependence of the atomic absorption signal (A) of chromium (C = 50 μg / ml) on the volume V (ml) of $HClO_4$ added to the test solutions. The volume of the test solutions is 8 ml. Flame: propane + butane - air.
■ - Cr (VI) solutions, ○ - Cr (III) solutions
a - without adding HNO_3 to the test solutions.
b - added by 0.2 ml of HNO_3 (70%) to all tubes.
c - added by 0.4 ml of HNO_3.

To establish the dependence of the absorption jump ΔA on the concentration of Cr (III), we prepared standard aqueous solutions of trivalent chromium with concentrations of 3.2 μg / ml, 6.4 μg / ml, 12.8 μg / ml and 25.6 μg / ml in 25 ml flasks. The content of each flask was divided into two halves. One of them was added by 200 μL of $HClO_4$. Thus, by adding $HClO_4$ and without its adding we obtained two types of solutions with different concentrations of Cr (III). After measuring the atomic absorption signals, calibration curves were plotted (Fig. 2). As Fig. 2 shows, the addition of $HClO_4$ to the standard solutions of Cr (III) sharply increases the slope of the calibration curve.

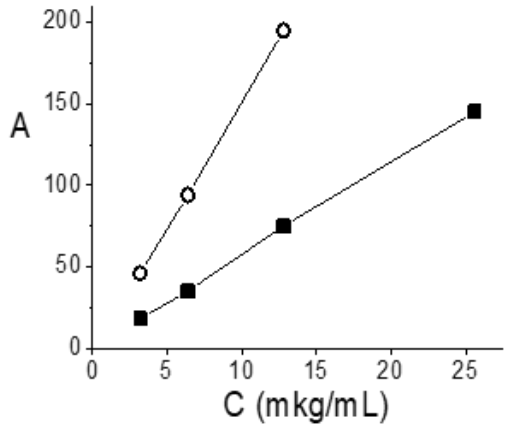


Fig. 2.

Dependence of the atomic absorption signal A on the concentration of Cr (III).
■ - without $HClO_4$
○ - with 200 µl of $HClO_4$ (d = 1.25g / ml) per 12 ml solution

For the same concentration of Cr (III), we calculated the difference ΔA between the absorption signals obtained by $HClO_4$ addition (A ‘) and without its addition (A):

ΔA = A′-A. The dependence of the absorption jump ΔA of Cr (III) concentration is shown in Fig. 3. As Fig. 3 shows, the dependence of the absorption jump ΔA of Cr (III) concentration is linear. The dependence does not change when extra 0.2 ml or 0.4 ml of HNO_3 is added to the sprays.

Taking into consideration that addition of 0.4 ml of HNO_3 to the solutions of Cr (III) and Cr (VI) does not change the effect of $HClO_4$ on the atomic absorption signal, the proposed method can be used for the analysis of such samples where HNO_3 is present, such as the biological samples ashed by means of concentrated HNO_3.

The scheme for determining the concentration of Cr (III) is as follows: After ashing the sample, its volume is increased up to 6 ml with bidistilled water (the volume should be sufficient for two atomic absorption measurements). The sample is divided into two approximately equal halves (~ 3 ml), one part of which is added by 30 µl of $HClO_4$. The same amount of bidistilled water is added to the second part of the sample. The solutions obtained are sprayed, the corresponding signals A’ and A of atomic absorption of chromium are measured, and the difference between them ΔA is calculated. The same procedures are carried out for the standard samples with known concentrations of Cr (III). The desired concentration of Cr (III) is calculated by means of the obtained value ΔA and the calibration graph obtained by means of the above method (Fig. 3) [15].

The minimum effective amount of chloric acid used in the experiments was 0.1% of the total volume of the sample. In our opinion, within the investigated range of 0.1 - 10% the most effective amount of $HClO_4$ is within the range of 0.1 - 1%. The concentration of the main (added) solution of chloric acid is 35%.

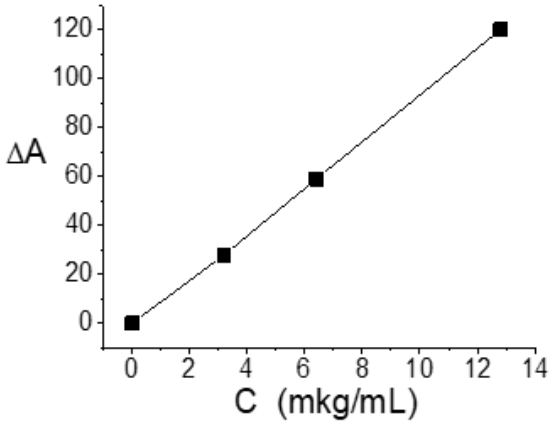


Fig.3.

Dependence of the absorption signal change ΔA (absorption jump) on Cr (III) concentration. ΔA = A'-A, where A - atomic absorption signal obtained by spraying Cr (III) solutions without $HClO_4$.
A ‘ - atomic absorption signal in case of adding 200 µl of $HClO_4$ per 12 ml of Cr (III) solution.

Examples of Application of The Method

The proposed method was used in our research to determine Cr (III) content. From this point of view, our focus was on the chromium-tolerant bacteria, which has the ability to reduce Cr (VI) to Cr (III) [16]. In our experiments, we used *Arthrobacter oxydans (A. oxydans)* as model bacteria.

The experiments carried out using Fourier transform synchrotron radiation and infrared spectroscopy showed that the Gram-positive bacteria *A. oxydans* isolated from the basalt samples collected in Columbia (USA) are Cr (VI)-tolerant, which has the ability to reduce Cr (VI) to Cr (III) [17]. The

mechanisms of Cr (VI) reduction by means of *A. oxydans* bacteria were considered in our previous studies [18-21].

Within the present work, general content of chromium as well as Cr (III) was determined in *A. oxydans* cells after their exposure to 35 mg /l Cr (VI) (in the form of K_2CrO_4) for one and seven days, respectively.

Bacterial cells were grown under the conditions described in [18]. The cells were separated by centrifugation (10,000 rpm 15 min, 40℃), washed three times with NaCl solution (0.15M, pH7), and analyzed by means of AAS.

To determine the concentration of Cr (III), dry bacterial samples were weighed, placed in test tubes and added by 0.4 ml of HNO_3 (70%). After ashing the cells, the test tubes were filled by 6 ml of distilled water. Then, each sample was divided into two equal parts, one was added by 30 µl of distilled water, and the other by 30 µl of chloric acid (0.1%).

The solutions were sprayed. Atomic absorption signals A_1 and A_2 of corresponding chromium were measured and the difference ΔA between them was calculated. Using this value, the concentration of Cr (III) was determined according to the calibration curve.

It was found that after the exposure to Cr (VI) for several days, the content of Cr (III) in *A. oxydans* cells reaches ~ 15% of the total amount of chromium. After seven days, Cr (III) fraction in the total amount of chromium accumulated by bacterial cells significantly increases (up to 70%), which coincides with the results obtained by us earlier [18, 21].

Thus, the proposed method of Cr (III) detection by means of AAS and APS significantly increases the possibility of their use in practice.

Conclusion

The possibility of Cr (III) concentration determination without its preliminary separation from the test solution is shown. For this purpose, by spraying Cr (III) and Cr (VI) in a propane + butane - air flame the effect of H_2SO_4, H_2O_2, $HClO_4$, HNO_3, HCl, H_3PO_4, KOH, and $FeSO_4$ on the signal of atomic absorption of Cr was studied. It was found that the effect of $HClO_4$ is convenient for determination of Cr (III) concentration. Addition of a small amount of $HClO_4$ to Cr (VI) solution does not change the atomic absorption signal, while addition of the same amount of $HClO_4$ to Cr (III) solution approximately twice increases the absorption signal. An increase in the atomic absorption signal upon addition of $HClO_4$ to the test solutions is caused only by the presence of Cr (III) in the solution, and the magnitude of the absorption signal determines Cr (III) concentration. The effect of $HClO_4$ on the atomic absorption signal does not change when 0.4 ml of HNO_3 is added to Cr (III) and Cr (VI) solutions. This fact makes allows us to ash the preparations tested for Cr (III) content in HNO_3.

Acknowledgement

This work was supported by Project AR-18-629 from the Shota Rustaveli National Science Foundation of Georgia (SRNSF).

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