ANALYTICA CHIMICA ACTA 630 (2008) 181–185

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/aca

# Selective, peroxidase substrate based "signal-on" colorimetric assay for the detection of chromium (VI)

*Jing Li, Hui Wei, Shaojun Guo, Erkang Wang**

*State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Graduate School of the Chinese Academy of Sciences, Changchun, Jilin 130022, China*

ARTICLE INFO

*Article history:*
Received 10 July 2008
Received in revised form
29 September 2008
Accepted 1 October 2008
Published on line 14 October 2008

*Keywords:*
Colorimetric assay
Signal-on
Chromium (VI)
Peroxidase substrate

ABSTRACT

Due to the potentially adverse effects of the chromium (VI) on the human health and also on the environment, the quantitative determination of Cr(VI) is of particular interest. This work herein reports a facile, selective and rapid colorimetric determination of Cr(VI) based on the peroxidase substrate-2,2′-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS) as the color developing agent. ABTS, which was usually acted as peroxidase substrate for the enzyme linked immunosorbent assay, is used here for the first time to fabricate the "signal-on" colorimetric Assay for Cr(VI). The ABTS was chosen instead of the commonly used 1,5-diphenylcarbazide (DPC) due to its good solubility, stability, sensitivity and low background. This method provided a convenient colorimetric detection of Cr(VI) with a wider linear range from 8.33 $\mu g L^{-1}$ to 1.25 $mg L^{-1}$ by recording the absorption spectra at the wavelength of 419 nm and a low detection limit of 7.87 $\mu g L^{-1}$. In addition, the entire detection takes less than 10 min.



## 1. Introduction

Chromium (Cr) exists in two main environmentally relevant valence states as Cr(III) and Cr(VI) in natural samples. However, they have a contrasting impact on environment and the human health. Cr(III) compounds are relatively harmless and act as a bioessential species for the maintenance of glucose, lipid and protein metabolism [1]. In contrast to the Cr(III) compounds, Cr(VI) compounds can cross cell membranes and are known as toxic pollutant and potentially carcinogenic agents for a variety of living organisms [2]. Due to the different toxicities of these two valence states of chromium compounds, it is essential to determine Cr(VI) rather than the total concentration of Cr for the human health and environmental monitoring.

Up to date, many techniques have been developed for the quantification of Cr(VI) in environment samples, which can be achieved by atomic spectroscopy (e.g., classics absorption/emission spectrometry and fluorescence spectrometry) [3–5], mass spectrometry (e.g., inductively coupled plasma-mass spectrometry) (ICP-MS) [6], UV–vis spectrophotometry [7] and electrochemical techniques (e.g. adsorptive striping voltammetry and the direct reduction of Cr(VI) on the electrode) [8–14]. Although these techniques possess a various attractive analytical "figures of merit", each of them has one or several undesirable limitations [15]. For example, ICP-MS, as the most sensitive and multielement detection technique [16], is a rather bulky (i.e. unsuitable for the in situ field monitoring) [17], expensive and not selective to the different charge states and chemical forms of an element. While electrochemical techniques are simple, inexpensive and portable, they are subjected to the interference resulting from the sample matrixes or analyte reaction product and it is necessary to clean up the electrode surface prior to the next cycle detec-

* *Corresponding author*. Tel.: +86 431 85262003; fax: +86 431 568 9711.
E-mail address: ekwang@ciac.jl.cn (E. Wang).
0003-2670/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.aca.2008.10.004

tion, which is time-consuming. Thus, the development of new, practical and rapid assays for the determination of Cr(VI) still remains a challenge.

Facile colorimetric assays could potentially eliminate the use of analytical instruments and are paid more and more attention. Colorimetric method for Cr(VI) determination has been depicted in the literature [18–22]. In an acid medium, 1, 5-diphenylcarbazide (DPC) reacts with Cr(VI) and a red–violet colored complex produced. The molar absorption spectra based on Cr(VI) at the wavelength of 540 nm are recorded. However, the DPC is insoluble in water and the solution prepared with acetone cannot conserve for a long time, just for one week. Furthermore, the color reaction between DPC and Cr(VI) needs the participation of sulfuric acid and phosphatic acid to avoid the interference, which makes the process complicated and inconvenient. Therefore, developing a new, simple, selective and rapid colorimetric assay for the determination of trace Cr(VI) in aqueous solution is important to monitor the environment.

2,2′-Azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS), which is usually acted as the peroxidase substrate for the enzyme linked immunosorbent assay (ELISA), has been used as the probe to determine $H_2O_2$ based on the catalytic activity and mimetic activity of the $Fe_3O_4$ magnetic nanoparticles in our group [23]. However, ABTS as the color developing agent for the detection of metal ions has not been reported. In this work, we make use of the ABTS as the probe for the colorimetric detection of Cr(VI). The sensing mechanism of this colorimetry is based on the oxidation of ABTS by Cr(VI) in acid medium (Eq. (1)), which produces a soluble end product that is green in color and can be read spectrophotometrically at 419 nm. This approach shows its superiority due to its good solubility in water, stability (to be stored at room temperature for six months), compared with DPC.

$$\mathrm{Cr(VI)} + \mathrm{ABTS} \xrightarrow{\mathrm{H^+}} \mathrm{Cr(III)} + \mathrm{Oxidized\ \ ABTS} \quad (1)$$

## 2. Experimental

### 2.1. Chemical and reagents

2,2′-Azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS) was purchased from Sigma–Aldrich (Milwaukee, WI). $K^+$, $Ag^+$, $Pb^{2+}$, $Hg^{2+}$, $Cu^{2+}$, $Cd^{2+}$, $Zn^{2+}$, $Ba^{2+}$, $Ni^{2+}$, $Al^{3+}$, $Cr^{3+}$, $CrO_4^{2-}$ and $MnO_4^{2-}$ were prepared by diluting the corresponding standard stock solutions prepared with $KNO_3$, $AgNO_3$, $CdCl_2$, $Pb(NO_3)_2$, $HgCl_2$, $CuCl_2{\cdot}2H_2O$, $ZnCl_2$, $Ba(NO_3)_2$, $NiCl_2{\cdot}6H_2O$, $CrCl_3{\cdot}6H_2O$, $AlCl_3$, $K_2CrO_4$ and $K_2MnO_4$ respectively. The water used throughout all experiments was purified by a Milli-Q system (Millipore, Bedford, MA). 0.2 M acetate buffer, prepared by mixing appropriate amounts of $CH_3COOH$ and $CH_3COONa$, and was served as the supporting electrolyte. All solutions were prepared with double-distilled water. All chemicals employed in this work were of analytical reagent grade and used as received.

### 2.2. Instrumentation

Absorption spectra were recorded on a Cary 500 scan UV–vis-NIR spectrophotometer (Varian, Harbor City, CA).

### 2.3. Procedures

Determination of Cr(VI) using ABTS as the Probe. 30 μL of 60 mM solution ABTS, 170 μL of 0.2 M HAc-NaAc buffer and 40 μL certain concentration of Cr(VI) stock solution were mixed together. A portion of the above reaction mixture (100 μL) was diluted to 1 mL with water and used for the absorption spectroscopy measurement. The absorption spectrum at 419 nm without Cr(VI) were recorded for the control experiment.

To examine the influence of reaction buffer pH on the determination of Cr(VI) based on the oxidation of ABTS, 0.2 M acetate buffer solutions from pH 2.0 to 7.0 were investigated.

To investigate the influence of co-ions on the change of UV–vis absorption spectra based on the oxidation of ABTS by Cr(VI). The interference study was performed by adding various foreign ions into a solution containing Cr(VI) ($C_{\text{co-ions}}$:$C_{\text{Cr(VI)}}$ = 2:1).

The preparation and determination of real sample: 0.1 g polypropylene was acidified in the 8 mL concentrated nitric acid for 5 h, and then the sample was solubilized by the microwave-accelerated reaction system. Finally, the sample was transferred into the flasks and diluted to 25 mL as the stock solution. For the analysis, 20 μL of polypropylene, 140 μL of 0.2 M acetate buffer and 30 μL of 60 mM solution ABTS were prepared. The lake water was used for analysis without any treatment.

## 3. Results and discussion

### 3.1. Study of the catalytic oxidation of ABTS by Cr(VI)

To investigate the behavior of ABTS as the color developing agent, the colorimetric assay with ABTS for the determination of Cr(VI) was tested. As shown in Figs. 1 and 2, the addition of the 0.833 mg $L^{-1}$ Cr(VI) to the ABTS solution could oxidize the peroxidase substrate-ABTS to produce the oxidized colored product and gave a noticeably enhanced response at 419 nm compared with the solution without Cr(VI) in 0.2 M acetate buffer (pH 2.0). Therefore, we can fabricate a new and facile colorimetry to determine the Cr(VI) using ABTS as the probe by monitoring the absorption of oxidized ABTS at the wavelength of 419 nm.

### 3.2. Effect of the reaction pH

The influence of the pH on this system was studied in the range of pH 2.0–7.0 and the corresponding pH-dependent response curves were recorded in Fig. 3. The results found that the tested buffer accelerated the oxidation of ABTS compared with that in the aqueous solution, especially, in acid solution. In the pH 2.0 buffer, the response at 419 nm enhanced obviously in the presence of Cr(VI), on the contrary, there is hardly any change in the absorption value at 419 nm in the presence of Cr(VI) from pH 6.0 to 7.0 NaAc-HAc buffer. This

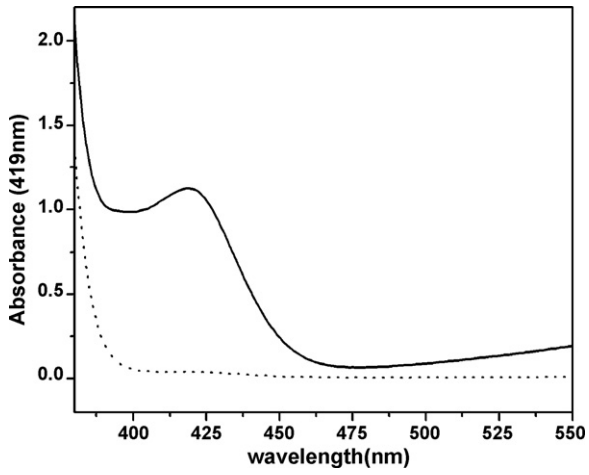



**Fig. 1 – Typical absorption curves of ABTS reaction solutions in the absence of Cr(VI) (dotted line) and in the presence of 0.833 mg $L^{-1}$ Cr(VI) (solid line) in 0.2 M HAc-NaAc buffer (pH 2.0).**

indicated the oxidation of ABTS by Cr(VI) was pH-relevant and the Cr(VI) exhibited stronger catalytic activity for the oxidation of ABTS in acid medium compared with that in neutral or basic medium. Therefore, 0.2 M pH 2.0 acetate buffer solution was taken as the optimal reaction buffer for the subsequent determination.

### 3.3. *Effect of the reaction time*

The reaction time was optimized by monitoring the curve of absorbance response at 419 nm vs time. As illustrated in the Fig. 4, the absorbance response increased initially with the increasing reaction time and then reached plateau when the reaction time was ca 10 min at room temperature. Thus the reaction time of 10 min was selected for the determinations of Cr(VI).

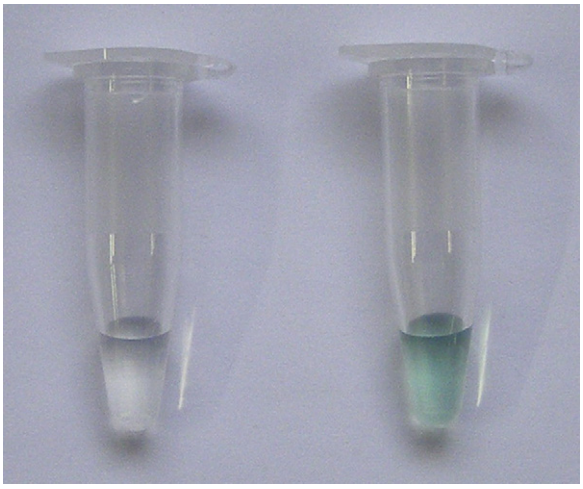


**Fig. 2 – Typical photographs of diluted reaction mixture in the absence of Cr(VI) (left) and in the presence of Cr(VI) (right) in 0.2 M HAc-NaAc buffer (pH 2.0).**

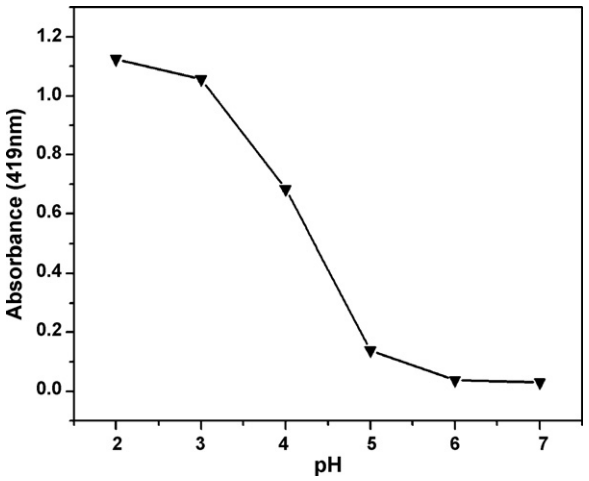



**Fig. 3 – The pH-dependent response curves of ABTS reaction solutions oxidized by 0.833 mg $L^{-1}$ Cr(VI) in 0.2 M HAc-NaAc buffer.**

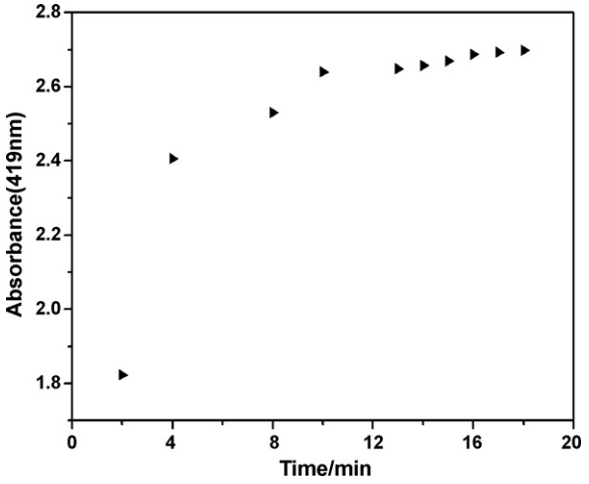



**Fig. 4 – The absorbance–time curves of the 30 μL 60 mM ABTS reaction solutions catalytically oxidized in the presence of 2.5 mg $L^{-1}$ Cr(VI) in the 170 μL 0.2 M HAc-NaAc buffer and 600 μL water.**

### 3.4. *Interference study*

In order to verify the selectivity of this approach for the detection of Cr(VI) in practical applications, the interference of some common metal ions on the determination of Cr(VI) was investigated. We recorded the data of the absorbance at 419 nm with Cr(VI) before ($A_0$) and after adding the interferents ($A$) into solutions with Cr(VI). As shown in the Fig. 5, the absorbance ratio ($A/A_0$) exhibited no variation by the addition of the following metal ions (a 2-fold mass concentration over Cr(VI)): $K^+$, $Ag^+$, $Pb^{2+}$, $Hg^{2+}$, $Cu^{2+}$, $Cd^{2+}$, $Zn^{2+}$, $Ba^{2+}$, $Ni^{2+}$ or $Al^{3+}$, $Cr^{3+}$), and the mutual interference ($A - A_0/A_0$) did not exceed the accepted error level of 4.4%. However, the presence of Mn(VII) led to the obviously increased signal change (ca. 40%) with the identical concentration as Cr(VI) and had serious interference on the determination Cr(VI). In order to overcome the influ-

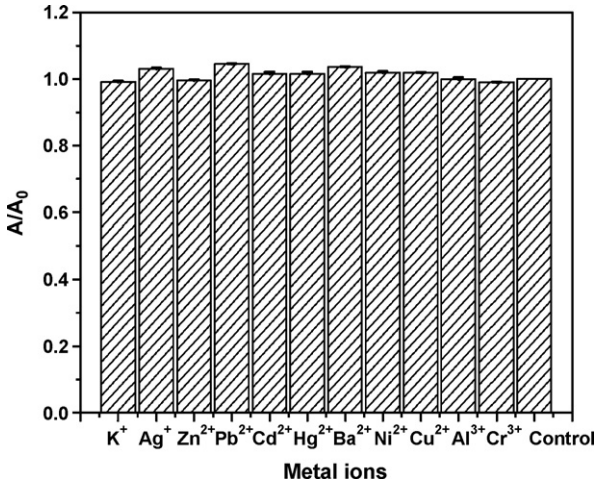
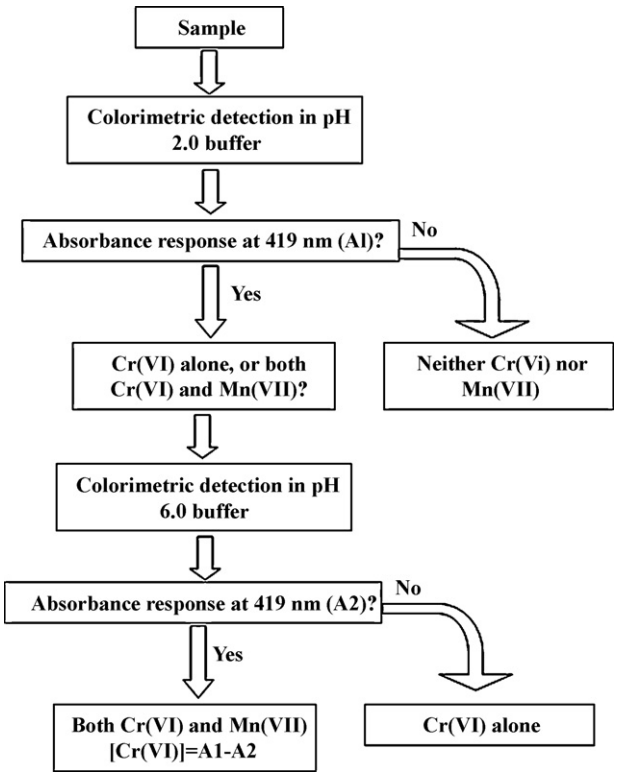



**Fig. 5 – The effects of the coexistence metal cations on the UV–vis absorption spectra in 0.2 M HAc-NaAc buffer (pH 2.0) with Cr(VI) ($C_{co\text{-}ions}$:$C_{Cr(VI)}$ = 2:1).**




**Scheme 1 – The experimental protocol to check and eliminate the interference of Mn(VII).**

ence of Mn(VII), the effects of pH on the responses of these two ions were investigated. As mentioned above, the oxidation of ABTS by Cr(VI) was pH-relevant and Cr(VI) exhibited stronger catalytic activity in the pH 2.0 buffer. However, in the range of pH 6.0–7.0, taking the response of pH 6.0 for example (Fig. 6A), the UV–vis absorption spectra with 0.833 mg $L^{-1}$ Cr(VI) is similar to the original solution without Cr(VI). On the contrary, the pH has little effect on the oxidation of ABTS by Mn(VII) compared with Cr(VI) from pH 2.0 to 6.0 (Fig. 6B). It may be attributed to the different oxidized abilities of these two ions. Mn(VII) showed stronger oxidized ability than Cr(VI), especially, in the range of pH 6.0–7.0. Therefore, the interference of Mn(VII) could be easily checked and eliminated by controlling the pH of the reaction solution from pH 2.0 to 6.0. (See Scheme 1.) pH 6.0 was chosen to detect the concentration of Mn(VII) in the presence of Cr(VI) due to the good sensitivity of Mn(VII) and noninterference of Cr(VI) in this condition (Fig. 6A).

### 3.5. *Linearity and limit of detection*

Since the oxidation activity of the ABTS is Cr(VI) concentration dependent (Eq. (1)), the system discussed above could be used to determine Cr(VI). Under the optimal conditions, we measured the linearity and limit of detection of Cr(VI). The resulting calibration plot is linear over the range from 8.33 μg $L^{-1}$ to 1.25 mg $L^{-1}$. The linear regression equation of Cr(VI) was $A = 0.0013\ C + 0.02447$ (A: absorbance at 419 nm; C: μg $L^{-1}$) with a correlation coefficient of 0.999. Based on three times the background noise (S/N = 3), the limits of detection

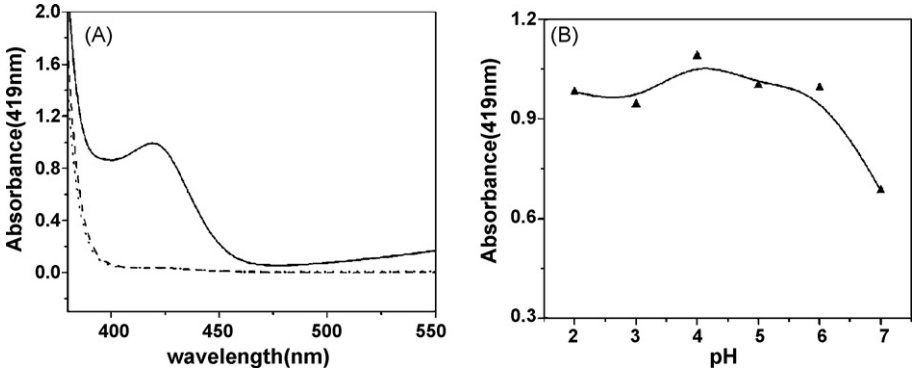



**Fig. 6 – (A) Typical absorption curves of ABTS reaction solutions catalytically oxidized in the absence of Cr(VI) (dashed line), in the presence of 0.833 mg $L^{-1}$ Cr(VI) (dotted line), and in the presence of 0.833 mg $L^{-1}$ Mn(VII) in 0.2 M HAc-NaAc buffer (pH 6.0). (B) The pH-dependent response curves of ABTS reaction solutions oxidized by 0.833 mg $L^{-1}$ Mn(VII) in 0.2 M HAc-NaAc buffer.**

was 7.87 $\mu g L^{-1}$ for Cr(VI), which is slightly lower than that reported by DPC as the color reagent [24] ($\geq$10 $\mu g L^{-1}$).

### 3.6. *Application*

The selective detection of Cr(VI) in the polypropylene was presented. Before the determination of Cr(VI), pH 6.0 was chose to eliminate the interference of Mn(VII). In pH 6.0 buffer, the absorbance at 419 nm showed no variation by adding the sample into the ABTS solution. It was indicated the noninterference of Mn(VII). To determine the Cr(VI), pH 2.0 was used. The detected value of 0.28 $\mu g mL^{-1}$ measured by standard addition method is close to the value determined by inductively coupled plasma-optical emission spectroscopy of 0.23 $\mu g mL^{-1}$. Furthermore, the amount of Cr(VI) added (0.24 $\mu g mL^{-1}$) also correlated well the value measured (0.238 $\mu g mL^{-1}$) with a recovery of ~99.4%. Determination of Cr(VI) in the lake water was also demonstrated by the proposed set-up method. We could not observe any variation of the absorbance by the addition of the lake water. However, the recoveries for the samples after spiking with various concentrations of Cr(VI) falling in the window of 86.9–96.6% proved that the proposed method could be applied for the determination of Cr(VI) in aqueous solutions.

## 4. Conclusions

In summary, we have demonstrated a new colorimetric assay for the detection of Cr(VI) and it was based on the oxidation of peroxidase substrate-ABTS by Cr(VI) in the acid medium. The signal-on absorption response enhancement resulted from the colored oxidized ABTS. Herein, the ABTS as the color developing agent for the determination of Cr(VI) not for the ELISA provided a convenient colorimetric assay for Cr(VI) and this colorimetric assay showed good response towards the determination of Cr(VI) with a wider linear range from 8.33 $\mu g L^{-1}$ to 1.25 $mg L^{-1}$. Furthermore, this approach provided advantages of rapidity (within 10 min), simplicity, good sensitivity and low cost.

## Acknowledgements

This work is supported by the National Natural Science Foundation of China with the Grants 20575063 and the 863 project No. 2006AA020701 as well as 973 project 2007CB714500, and the Chinese Academy of Sciences KJCX2-YW-H11.

## REFERENCES

[1] W. Yang, Z. Zhang, W. Deng, Anal. Chim. Acta 485 (2003) 169.
[2] J. Wang, K. Ashley, E.R. Kennedy, C. Neumeister, Analyst 122 (1997) 1307.
[3] S. Balasubramanian, V. Bugalenthi, Talanta 50 (1999) 457.
[4] A.C. Sahayam, Anal. Bioanal. Chem. 372 (2002) 840.
[5] S. Rubio, A. Gómez-Hens, M. Valcárcel, Anal. Chem. 56 (1984) 1417.
[6] R. Nusk, K.G. Heumann, Fresenius J. Anal. Chem. 357 (1997) 1050.
[7] F. Shemirani, M. Rajabi, Fresenius J. Anal. Chem. 371 (2001) 1037.
[8] N.A. Malakhova, A.V. Chernysheva, Kh.Z. Brainia, Electroanalysis 3 (1991) 803.
[9] M. Korulczuk, M. Grabarczyk, Talanta 49 (1999) 703.
[10] J. Golimowski, P. Valenta, H.W. Nurnberg, Fresenius J. Anal. Chem. 322 (1990) 315.
[11] J. Wang, J. Lu, C. Olsen, Analyst 117 (1992) 1913.
[12] E. Chatzitheodorou, A. Economou, A. Voulgaropoulos, Electroanalysis 16 (2004) 1745.
[13] N. Carrington, L.A. Yong, Z.L. Xue, Anal. Chim. Acta 572 (2006) 17.
[14] C.M. Welch, O. Nekrassova, R.G. Compton, Talanta 65 (2005) 74.
[15] D.A. Skong, F.J. Holler, T.A. Nieman, Principles of Instrumental Analysis, 5th ed., Saunders college publishing, Philadelphia, 1992, p. 206.
[16] Y. Zhang, M. Xu, Y. Wang, F. Toledo, F. Zhou, Sensors Actuat. B 123 (2007) 784.
[17] H.E. Taylor, Inductively Coupled Plasma Mass Spectrometry: Practices and techniques, Academic Press, San Diego, 2001.
[18] L.S. Clesceri, A.E. Greenberg, A.D. Eaton (Eds.), Standard Methods for the Examination of Water and Wastewater, 20th ed., APHA; AWWA and WEF, Washington, DC, 1998.
[19] X.B. Long, M. Miro, E.H. Hansen, Anal. Chem. 77 (2005) 6032.
[20] M.A.S. Pressman, J.H. Aldstadt, J. Environ. Monitor. 7 (2005) 809.
[21] N. Bardiya, Y.-W. Hwang, J.-H. Bae, Anaerobe 10 (2004) 7.
[22] N. Rajesh, V. Agarwal, S. Aarthy, Can. J. Chem. Eng. 86 (2008) 72.
[23] H. Wei, E.K. Wang, Anal. Chem. 80 (2008) 2250.
[24] Hach Company, Chromium, hexavalent, for water and wastewater, In: Hach Co., Method 8023, DR/2000 Spectrophotometer Procedures Manual, seventh ed., Hach Co, Loveland, 1992, p. 113.