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A rapid, eco-friendly, and reliable microplate method for determination of Cr(VI)

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Abstract: In the present work, the reaction of Cr(VI) with 1,5-diphenylcarbazide in microplates (enzyme-linked immunosorbent assay microtiter plate) and a subsequent determination in a plate reader at 540 nm were performed. The final volume utilized for each determination was 200 μL, and the cost and waste were reduced for hexavalent chromium [Cr(VI)] quantification. The use of microplates allowed for the analysis of a large number of samples, reducing analysis time. No statistically significant differences were observed between the results obtained in the analysis of Cr(VI) in real samples by the conventional procedure and by the proposed methodology in this work (analysis of variance, $P < 0.05$). Employing the proposed method, the limit of detection (5.95 μg L^{-1}) of Cr(VI) was lower than that obtained with the traditional method. Thus, the analytical results obtained suggest that this methodology is faster, more economical, and more environmentally friendly than the traditional method.

Key words: Cr(VI) determination, 1,5-diphenylcarbazide, environmental samples, enzyme-linked immunosorbent assay

1. Introduction

Chromium compounds are widely used in the electroplating industry, for the development of dyes and pigments, in leather tanning, and in wood preservation, among others. Some of these industries discharge their wastes directly into surface waters and soils. The most stable oxidation states in nature of chromium are trivalent chromium (Cr(III)) and hexavalent chromium (Cr(VI)). Depending on its oxidation state, chromium exerts different effects on living beings. It has been shown that the compounds of Cr(VI) are 500–1000 times more toxic than Cr(III); it has also been reported that Cr(VI) is mutagenic and carcinogenic.[1,2] For these reasons, there have been a large number of methods established for the determination of Cr(VI), including spectrophotometric, electrochemical, and competitive immunoassay, and recently those involving the use of the so-called hybrid techniques for the determination of Cr(VI) in different types of samples, which can quantify trace amounts (μg L^{-1}) and allow for chromium speciation. However, for implementation of these complicated procedures and equipment, a high cost is involved. The advantages and disadvantages of some techniques used for Cr(VI) determination in several matrices are depicted in Table 1.

Because of their versatility and accessibility to the majority of laboratories, spectrophotometric techniques have been utilized for direct and indirect determination of Cr(VI).[3–8] Within this context, the most widely used method for direct determination of Cr(VI) is that which employs 1,5-diphenylcarbazide (DPC).[9] This method is based on the oxidation of DPC in a strongly acidic medium due to the reduction of Cr(VI) and

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Table 1. Advantages and disadvantages of some techniques used for Cr(VI) determination in several matrices.

Technique	Advantages	Disadvantages	Ref
Spectrophotometric UV-Vis	Fast, simple, sensitive, and low cost for implementation in the laboratory	Relativity high LOD (mg L^{-1})	3
Electrochemical	High selectivity and low LOD (μg L^{-1})	Mechanical renewal of electrode surface prior to the analysis	4
Competitive immunoassay	High selectivity and low LOD (μg L^{-1})	Expensive	5
Hybrid techniques	Very low LOD (ng L^{-1}) and analytical speciation of Cr(III) and Cr(VI)	High cost and complicated procedures for implementation	6,7

the generation of a purple-red complex (Cr(III)-1,5-diphenylcarbazone), whose absorbance can be registered in the visible region of the electromagnetic spectrum (540 nm). This method has a limit of detection (LOD) of approximately 10 μg L^{-1},[10] which is below the permissible value of the World Health Organization (WHO), which established the acceptable content of Cr(VI) in groundwater as 50 μg L^{-1}.[11] Additionally, this method has demonstrated great versatility and robustness. Consequently, the DPC method has been used in a large number of studies.[12−14]

The concept of green analytical chemistry (GAC) is defined as a trend to develop, modify, and employ procedures to minimize the requirements of raw materials, products, solvents, reagents, etc.; these products may be hazardous to human health and even for the environment.[15,16] From an economic point of view, GAC has also exerted a positive influence on reducing operating costs and the treatment of waste generated by analytical procedures. In addition, the use of environmentally friendly procedures is required for International Organization for Standardization (ISO) 14000 certification.[17] Thus, the study of analytes of environmental importance, such as herbicides,[18] cadmium,[19,20] mercury,[21,22] chromium,[5] and copper,[23] using techniques such as the enzyme-linked immunosorbent assay (ELISA), is becoming increasingly important due to the advantages that they present, such as speed, sensitivity, specificity, the small volumes of sample required, simultaneous analysis of multiple samples, and the use of low-cost equipment for implementation.[24,25]

This work presents the results of applying analytical methodology for the determination of Cr(VI) based on the reaction with DPC in microplates (ELISA microtiter plate) followed by quantification in a reader plate device at 540 nm. This micromethod was assessed using the standard method (macroapproach) and was applied to the analysis of Cr(VI) in water samples from an effluent and soil samples. The purpose of the proposed method is to reduce the amount of reagents, minimize the cost, and decrease the amount of waste in the determination of Cr(VI).

2. Results and discussion

The aim of this work was to conduct a study on the effect of the volume employed for colorimetric determination of Cr(VI) by the DPC method. Thus, it is proposed to employ the minimal amount of reactants and samples, according to the principles stated by GAC. Therefore, in this work, microplates (ELISA microtiter plate) and an ELISA plate reader were used. Quantification of Cr(VI) was determined as established by the standard method at 540 nm,[26] together with the additional advantage of the ELISA plate-reader system, which permits the analysis of a great amount of samples in a short time.

2.1. Determination of analytical parameters

For the standard method (macroapproach), a final volume of 2.5 mL was used (see Section 3.3.1). The effect of the sample's volume on the ELISA plate (length path) was studied in this investigation, considering the analytical parameters obtained from the calibration curves for Cr(VI) determination, through the use of the methodologies previously described.[8–10] For the micromethod (see Section 3.3.2), the volumes evaluated on the ELISA microplate were selected based on the maximal amount that the well could hold without spills after the shaking process. Moreover, the minimal volume was studied to obtain a reliable analytical signal (absorbance); thus, the volumes examined on the ELISA microplate were 50, 100, 150, and 200 μL. The results obtained from the calibration curves for Cr(VI) determination employing these volumes are depicted in Table 2. As can be seen, the results obtained from the micromethod with final volumes of 150 μL (E150) and 200 μL (E200) are similar to those obtained with the standard method. Additionally, for the LOD of Cr(VI) obtained for the three volumes mentioned previously, the ranges are below the permissible value that the WHO requires for the content of Cr(VI) in groundwater (50 μg L^{-1}).[11] On the other hand, the methodology for employing volumes of 50 μL (E50) and 100 μL (E100) was discarded due to the poor value of LOD, and the error (%) obtained was overly high. There are two possible explanations for these observed effects: (a) the high LOD could be related to the low volumes used in the microplates, which are correlated with the optical length path, affecting the absorbance and the LOD, according to the Lambert–Beer law, and (b) the overly high % error is produced because of the manipulation of low volumes of aliquots, introducing an intrinsic error. Therefore, the following studies were undertaken utilizing the analytical parameters obtained for the E150 and E200 micromethods.

Table 2. Parameters of the analytical curves evaluated for the standard method (macromethod) and the proposed micromethod for the determination of Cr(VI).

Parameter	Macromethod	Micromethod			
		E50	E100	E150	E200
Cr(VI), mg L^{-1}	0.2–1.0	0.2–1.0	0.2–1.0	0.2–1.0	0.2–1.0
Equation	$A = 0.833_{Cr(VI)} - 0.009$	$A = 0.078_{Cr(VI)} - 0.001$	$A = 0.215_{Cr(VI)} - 0.005$	$A = 0.330_{Cr(VI)} - 0.007$	$A = 0.453_{Cr(VI)} - 0.005$
R^2	0.9993	0.9970	0.9986	0.9995	0.9991
LOD, μg L^{-1}	15.8	149	8.70	7.09	5.95
LOQ, μg L^{-1}	52.7	498	29.0	23.6	19.8
Error (%) 0.2 mg L^{-1} Cr(VI)	2.11	12.5	10.5	2.91	2.24
Error (%) 0.9 mg L^{-1} Cr(VI)	0.44	4.84	2.11	0.78	0.64

A: Absorbance. LOD: Limit of detection. LOQ: Limit of quantification. Error: $\alpha = 0.05$ and n = 10.

There is evidence that molybdenum, vanadium, mercury, and iron ions may interfere in the DPC–Cr(VI) reaction, especially when the Cr(VI) concentration in the medium is relatively low. However, these interferences could be avoided by strict pH control in the medium and the use of the appropriate wavelength.[26] Based on these facts and the experimental conditions employed in this investigation, the interference study was not conducted.

2.2. Determination of Cr(VI) in environmental water and industrial effluent samples

The standard method (macroapproach) and micromethods E150 and E200 were used to determine Cr(VI) in samples from an electroplating industry (A), leachates (B), and solid wastes (C) (see Table 3). Statistical analysis demonstrated that there is not a significant difference among concentrations obtained by micromethod E200 and the standard method. However, the concentration determined by micromethod E150 is significantly different from that of the standard method ($P < 0.05$). This indicates that micromethod E200 is a reliable method for Cr(VI) determination in real samples.

Table 3. Comparison of the analytical results obtained in the determination of Cr(VI) in the samples analyzed by the standard method (macromethod) and the micromethod proposed in this work.

Sample	Cr(VI) ± SD		
	Macromethod	E150	E200
A, g L^{-1}	10.99 ± 0.05	9.80 ± 0.18*	11.08 ± 0.07
B, g L^{-1}	11.90 ± 0.14	10.88 ± 0.11*	12.14 ± 0.17
C, g kg^{-1}	0.15 ± 0.01	0.14 ± 0.02*	0.15 ± 0.01

SD: Standard deviation, n = 3. *: Statistically significant difference compared to the macromethod ($P < 0.05$).

In conclusion, micromethod E200, which was proposed for Cr(VI) determination, revealed that it is possible to use lesser amounts of the reagents and samples; therefore, waste generation is reduced according to GAC principles. In addition, reading time could be decreased from hours to minutes, depending on the number of samples to be analyzed, as seen from application of the ELISA plate-reader system. The results demonstrated that the proposed micromethod E200 has better analytical parameters than the standard method (macroapproach), as the LOD and limit of quantification in micromethod E200 were lower than those obtained from the standard method. Moreover, the amount of Cr(VI) determined in water and solid wastes with the proposed methodology does not differ statistically from the values obtained with the standard method. Therefore, micromethod E200 for Cr(VI) determination could be a reliable method for Cr(VI) determination in water and solid wastes. Finally, this method complies with GAC principles in that it reduces waste, time, and costs.

3. Experimental

3.1. Experimental setup

For the standard method (macroapproach), an Ultrospec 4300 Pro UV/Visible Spectrophotometer (Amersham Biosciences, USA) was utilized with a spectral bandwidth of <1.8 nm. Spectral data acquisition, storage, and manipulation were performed employing SWIFT II applications software. Absorption spectra were registered using plastic cuvettes of 3 mL and path length of 10 mm (BRAND, USA).

In the micromethods, an ELISA plate reader (Labsystem Multiskan MS, Finland) was employed. Absorption spectra were registered using the ELISA microtiter plate with a 96-well plate.

High-purity water of 18 MΩ cm was obtained with reverse osmosis equipment (model Q842-210, Quimis, Brazil), followed by purification using equipment model Simplicity UV, from Millipore.

3.2. Standards, reagents, and samples

All chemicals were of analytical reagent grade. Acetone (Fisher Scientific, USA) was used throughout. DPC, sulfuric acid, and Cr(VI) were purchased from Aldrich (USA). The DPC solution was prepared as follows: 0.125 g of DPC was weighed and dissolved in 100 mL of acetone : 0.5 mol L^{-1} H_2SO_4 solution (1:1). The Cr(VI) standard (1000 mg L^{-1}) was purchased from PerkinElmer (USA).

The samples utilized in this research were obtained from an electroplating industry effluent located in Celaya City, Guanajuato, Mexico. Leachates and solid wastes with Cr(VI) were provided by a local company located in San Francisco del Rincón, Guanajuato, Mexico. Solid samples were treated in a similar way to that reported by Villalobos-Aragón et al.:[27] approximately 2.5 g from the samples was weighed and added to 25 mL of deionized water. This mixture was then shaken for 1 min by a vortex and was left to separate into two phases. Later, the liquid phase was filtered (0.45-μm pore filter paper) and the extraction process was repeated twice. The leachates obtained were collected and gauged at 250 mL with distilled water. Liquid samples were also filtered and subsequently diluted with deionized water.

3.3. Analytical procedure

3.3.1. Procedure for standard method (macroapproach)

A total of 1.0 mL of DPC solution was added to 1.5 mL of a Cr(VI) solution in order to obtain final concentrations of 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 mg L^{-1} of Cr(VI). The samples were vortex-mixed for 10 s and then left to rest at room temperature for 30 min. Afterward, 2.0 mL of the sample was poured into a plastic cell.[7] The absorbance for a blank and for each solution was measured at 540 nm by triplicate.

3.3.2. Procedure for micromethod

For easy handling of the reagents, the methodology previously described in the literature was utilized.[7] The solutions employed to obtain the calibration curves were prepared according to the final volumes for testing (50, 100, 150, and 200 μL), maintaining the DPC solution : sample ratio (3:2) to obtain the final concentrations of 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 mg L^{-1} of Cr(VI). The microplates were shaken on a rocker platform (Bellco Glass, Inc., USA) at 100 rocks min^{-1} at room temperature for 30 min. The colorimetric change was measured with a UV plate reader at 540 nm. Absorbance for a blank and for each solution was measured at 540 nm in triplicate.

4. Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) post hoc test performed by Minitab ver. 17.1.0 (Minitab, USA). When noted, a Student t-test was also used to compare significant differences between two population means ($P < 0.05$).

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