NU Science Journal 2009; 6(S1): 64 -72

Hexavalent Chromium Determination Using Diphenylcarbazide Coated Cellulose Technique

Supamas Danwittayakul[*] and Aree Thanaboonsombut

National Metal and Materials Technology Center, 114 Thailand Science Park,
Klong 1, Klong Luang, Pathumthani, Thailand
*Corresponding author.E-mail: supamasd@mtec.or.th,
Tel: +66-2-5646500 ext 4214

ABSTRACT

Diphenylcarbazide coated cellulose materials combined with UV-VIS spectrophotometer were introduced as the simple detection method for quantitative determination of hexavalent chromium (Cr(VI)). The preparation of diphenylcarbazide coated cellulose materials was, first, cut cellulose materials into small pieces sized 1 cm x 1 cm, then, dip them into the 1,5-diphenylcarbazide solution (acetone) at the concentration of 0.04 M and, finally, evaporate the coated materials in air. For the calibration curve preparation, diphenylcarbazide coated cellulose materials were immersed in 25 mL of Cr(VI) solutions at pH 2 with concentrations ranging from 0 μg/L to 100 μg/L. The calibration curve obtained shows the relationship between the absorbance of Cr(VI)-diphenylcarbazide complexes and their concentrations. Additionally, the complex solutions developed different color shades starting from colorless to pink-violet color according to their concentrations. The detection limit could be obtained at the concentration of 1.43 μg/L with sufficient precision and accuracy. With this technique, color interferences from the presence of some salts, i.e., NO_3^-, SO_4^{2-}, PO_4^{3-}, Na^+, K^+, Mg^{2+}, Cl^-, Al^{3+}, Cu^{2+}, Zn^{2+}, Pb^{2+} and Fe^{3+}, did not occur at some particular salt concentrations with the Cr(VI) concentration at 100 μg/L. It could be concluded that the diphenylcarbazide coated cellulose technique is the simple detection method for Cr(VI) determination and could be applied to quantify the concentration of Cr(VI) in real samples, e.g., the extraction solution from plated-metallic sheet.

Keywords: simple detection, heavy metal, hexavalent chromium, on-site measurement

INTRODUCTION

The determination and monitoring of hexavalent chromium (Cr(VI)) in real samples, such as waste water, water sample extracted from metal, are of importance due to its high toxicity. Several studies have shown that Cr(VI) compounds can enhance the risk of lung cancer. The World Health Organization (WHO) has determined the carcinogenity of Cr(VI). The Department of Health and Human Services (DHHS) has also determined that certain Cr(VI) compounds are known to cause cancer in humans (U.S Environmental Protection Agency, 1992; Meissam and Mozhgan, 2003). The available techniques for Cr determination, e.g., ion chromatography (IC), atomic absorption spectrometry (AAS), inductively coupled plasma-optical emission spectrometry (ICP-OES), inductively coupled plasma-mass spectrometry (ICP-MS) and etc, are expensive and require technical skills. Simple detection method for heavy metal contaminants available for in situ analysis has gained much interest (Andrade *et al.*,1983; Andrade *et al.*,1996). These simple detection methods are rapid, cheap and user-friendly techniques. In 1980s, the simple detections, which are the combination between solid state analytical devices and dry chemical analysis, were used as the analytical methods especially in clinical diagnosis (Capitan-Vallvey *et al.*, 2002). One of the most popular simple detection methods is a test strip method for ion analysis, both cations and anions, in water sample solution. Test strip methods are usually prepared by immobilizing the chemical substances, necessary for analysis, on the carriers, i.e., polymer films, membranes, papers and glasses (Yukiko *et al.*, 2006; Yukiko *et al.*, 2007). One of these simple detections was the test strip for Pb(II) determination. It was prepared by dissolving an immobilizer, e.g., *O*-donor macrocycles and an anionic dye, together with a carrier such as a polymer film, and, then, coating the solution onto a substrate (Capitan-Vallvey *et al.*, 2002; Yukiko *et al.*, 2007). For all simple detection method, the reaction between target ions, or analytes, and detection chemical substances depends on the rate of reaction, the period of contact and the opportunity of contact. The analytical results are presented in various ways, e.g., a change in color of test strip, a change in fluorescence properties (Prestel *et al.*, 200), etc. 1, 5-diphenylcarbazide (DPC) is one of the most chemical substances used for Cr(VI) determination in water sample. The Cr(VI) concentration in the solution is evaluated by its reaction with DPC under acidic conditions resulting in a pink-violet-colored complex compound. The complex solution is then quantified by a UV-VIS spectrometer at 540 nm (Qingjun *et al.*, 2007; Zhiqiang, *et al.*, 1998; Hua *et al.*, 2008).

In this study, diphenylcarbazide coated cellulose materials (DPC-CC materials) was prepared as test strips. The test strip method equipped with a UV-VIS spectrophotometer was selected as the simple detection method for quantitative determination of Cr(VI). In the calibration curve method, the curve showing the relationship between the absorbance of the analytes, Cr(VI), and concentration was developed. The effects of cellulose support size, sample volume and ions interference on the Cr(VI) determination were then studied. The detection limit and repeatability were also evaluated. Moreover, this method was applied to determine Cr(VI) in chrome plated metallic samples.

MATERIALS AND METHODS

All chemical substances in analytical grade were used for this work. All standard solutions were prepared using deionized water. To prepare support materials, a cellulose material (alpha cellulose cotton linter) with 1 mm of thickness was cut to small pieces sized 1 cm x 1 cm. Two steps of DPC-CC preparation were dipping and evaporation. First, a cellulose support material sized 1 cm x 1 cm was dipped into DPC solution (acetone) at the concentration of 0.04 M for 60 seconds. Then, the dipped cellulose was evaporated in air. The microstructures of DPC coated and uncoated cellulose specimens were investigated using SEM (JSM: 6301F, JEOL) technique.

For detection method, the DPC coated specimen was immersed in 25 mL of Cr(VI) solution under controlled acidic condition at pH 2. The color of complex solution was gradually developed into pink-violet until it was stable within 30 minutes. Then, the absorbance of complex solution was measured using UV-VIS spectrophotometer (UV Mini 1240 UV-VIS, Shimadzu). The calibration curve was prepared using the relationship between the absorbance of Cr(VI)-DPC complex compounds and their concentrations, ranging from 0 μg/L to 100 μg/L. In addition, effects of the size of cellulose support materials and the volume of Cr(VI) solutions were studied. The boundary of study was limited to three sets of two experiments, one at 0.5 cm x 0.5 cm, 1.0 cm x 1.0 cm and 2.0 cm x 1.0 cm for cellulose size and another at 25 mL, 50 mL and 100 mL for the volume of Cr(VI) solution.

The determination of detection limit was performed using 10 replicate blank samples. The absorbance of ten replicate blanks was then measured by UV-VIS spectrophotometer after the immersion of a DPC-CC material for 30 minutes. In addition, another ten replicate Cr(VI) solutions at the concentration of 50 μg/L were prepared for repeatability test. The absorbances of these ten replicate Cr(VI) solutions were also measured as described above at detection limit determination.

The influence of anions and cations on the determination of 100 μg/L of Cr(VI) using DPC-CC materials was also studied.. Examples of ions used in this study were 2.5 g/L of NO_3^-, SO_4^{2-}, PO_4^{2-}, 2 g/L of Cl^- for anion interference and 5 g/L of Na^+ and K^+, 2 g/L of Mg^{2+}, 5 mg/L of Al^{3+}, Cu^{2+}, Zn^{2+}, Pb^{2+} and Fe^{3+} for cation interference. Each salt of cations and anions were separately dissolved in the presence of 100 μg/L of Cr (VI) solution. Then, the Cr (VI) concentration of each co-existing ions solution was examined using DPC-CC materials combined with UV-VIS spectrophotometer.

This present method was also applied for Cr(VI) determination in the extraction solution from unknown chrome plated metallic sheets. For comparison purposes, three sets of each sample solution extracted from chrome plated-metallic sheets were analyzed by the combination method between DPC-CC method and UV-VIS spectrophotometry. Then, these three extracted solutions were analyzed by ICP-MS (ELAN DRC-e, PerkinElmerSCIEX).

Cr(VI) extraction procedure (Environmental Standardization for Electrical and Electronic Products and systems, Draft) : A metal plate sample was cut into small pieces with a dimension of 4 cm x 6 cm and the small pieces were separately boiled in 50 mL deionized water for 10 minutes. Orthophosphoric acid was used to adjust the acidity of solution to pH 2. The final volume of each sample solution was adjusted to 50 mL and, then, each solution was separated into two parts (25 mL/part). The first 25 mL sample solution was introduced for the determination of Cr(VI) by DPC-CC method combined with UV-VIS spectrophotometry and the last were analyzed by ICP-MS technique.

RESULTS AND DISCUSSION

1. Microstructures of cellulose support material

The microstructures bare cellulose and DPC-CC specimens were investigated using SEM technique. Figure 1 shows the SEM images of cellulose materials, it can be clearly seen that DPC was coated on cellulose textile as shown in Figure 1(b).

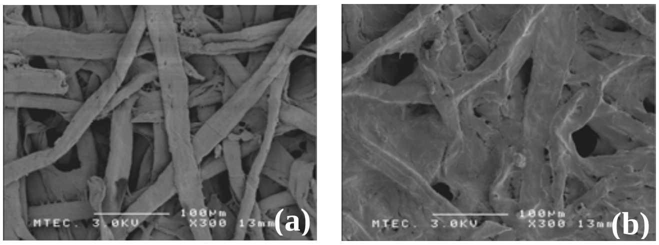


Figure 1 SEM micrographs of cellulose support material; a) uncoated cellulose specimen, b) DPC-CC specimen

2. Hexavalent chromium (Cr(VI)) detection

A piece of DPC-CC sized 1 cm x 1 cm was introduced to detect the Cr(VI) contaminant in a series of 25-mL sample solutions with a range of concentration from 0 µg/L to 100 µg/L at pH 2. The color of sample solutions was gradually developed from colorless to pink-violet color as shown in Figure 2(a). Then, the absorbance of solution was measured at 540 nm using UV-VIS spectrophotometer after 30 minutes left to assure the complete reaction between DPC and Cr(VI) in acidic solution. It should be noted that at the concentration of 10 µg/L Cr(VI) solution, the color development can be distinguished by naked-eyes. The calibration curve can be plotted based on the absorbance of each solution against their concentrations (Figure 2(b)). The correlation (R^2) of the calibration curve was higher than 0.999 that is sufficient and repeatable for quantitative analysis. Effects of the size of cellulose support materials and the volume of Cr(VI) solutions were studied. The influence of co-existing ions was also determined on the determination of 100 µg/L of Cr(VI). This present method was also applied for Cr(VI) determination in the extraction solution from unknown chrome plated metallic sheets. All results are reported and discussed in the following subsections.

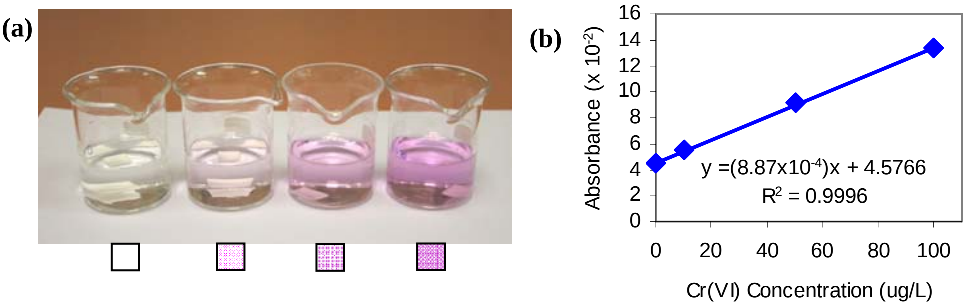
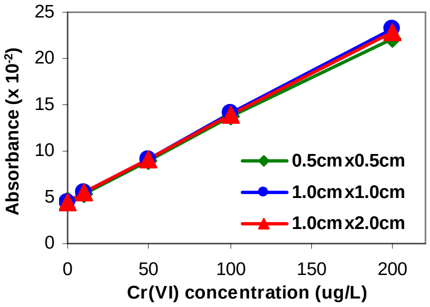


Figure 2 Sample solutions after color development (a) and the plot between absorbance and concentrations of them (b)

2.1 Size effect of cellulose support materials on calibration curve

The size effect of cellulose supporters on the determination of Cr(VI) was studied. The DPC-CC materials sized 0.5 cm x 0.5 cm, 1 cm x 1 cm and 1 cm x 2 cm were prepared and used for Cr(VI) determination at the concentrations of 0 to 200 μg/L. The calibration plots of the absorbance against the Cr(VI) concentrations of these three sizes of cellulose supporters was presented in Figure 3. It was observed that the size of cellulose materials was not significantly affected to the calibration curves obtained from each cellulose support size. The parameter of the linear equation, intercept and slope, presented similar values. It implies that the amount of DPC coated on the smallest cellulose material (0.5 cm x 0.5 cm) should be sufficient to form a complex compound with the 25 mL Cr(VI) solution having the concentration at 200 μg/L. However, for convenience in handling, the cellulose sized 1 cm x 1 cm was chosen for the rest of the experiments.

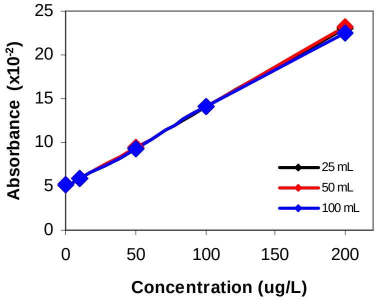


	Cellulose support size (cm x cm)		
	0.5x0.5	1.0x1.0	1.0x2.0
R^2	0.9995	0.9996	0.9998
Intercept (10^{-2})	4.59	4.57	4.61
Slope (x 10^{-4})	8.8	9.4	9.2

Figure 3 The plots between absorbance and Cr(VI) concentration with various sizes of cellulose support, i.e., 0.5 cm x 0.5 cm, 1.0 cm x 1.0 cm and 1.0 x 2.0 cm (sample volume at 25 mL with pH 2)

2.2 Effect of sample volume on calibration curve

The sample volume of 25, 50 and 100 mL was studied on the Cr(VI) determination at the concentration of 0 to 200 μg/L. Figure 4 shows the calibration plots obtained from the determination of Cr(VI) solutions at various volumes of 25, 50 and 100 mL. It was found that the sample volume in the studied range was not affected on the Cr(VI) determination at the concentration of 0 to 200 µg/L. It can be noted that this method was tolerable for the determination of hexavalent chromium even though the measurement volume was deviated from 25 mL to 100 mL.



	Sample volume (mL)		
	25	50	100
R^2	0.9999	0.9998	0.9996
Intercept (10^{-2})	5.00	4.96	5.06
Slope ($x10^{-4}$)	9.0	9.1	8.8

Figure 4 The plots between absorbance and Cr(VI) concentration with various sample volumes of 25 mL, 50 mL and 100 mL (a cellulose supporter sized 1 cm x 1 cm at pH 2)

2.3 Detection limit and precision

The detection limit of the Cr(VI) determination using DPC-CC combined with UV-VIS spectrophotometer was examined by 3-sigma method. With this method, the deviation of detection limit on the conditions of Cr(VI) determination, i.e., cellulose support size and sample volume, were studied. Ten replicates of sample blanks were prepared and the amount of Cr(VI) content using DPC-CC materials was examined. The detection limit of each condition was shown in Table 1. It was found that the detection limits obtained from each condition were not much different from others.

In this study, we also determined the precision in terms of repeatability by measuring 10 replicates of the Cr(V) solutions at the concentration of 50 µg/L using DPC-CC method combined with UV-VIS spectrophotometer (Table 2). The relative standard deviation (RSD) at 3.3% was obtained at this concentration. The low value of RSD indicates high precision of this hexavalent chromium determining method.

Table 1 Detection limit for Cr(VI) determination using DPC-CC material combination with UV-VIS spectrophotometer with various conditions

	Effect of cellulose material size (cm x cm)[1]			Effect of sample volume (mL)[2]		
	0.5 x 0.5	1 x 1	1 x 2	25	50	100
Detection Limit (DL: 3SD)	1.026	1.429	1.142	1.429	1.181	1.249

[1] – Use the sample volume at 25 mL, [2] – Use the cellulose material sized 1 cm x 1 cm

Table 2 Repeatability of Cr(VI) determination using DPC-CC material combination with UV-VIS spectrophotometer

Ten replicates of Cr(VI) solution at the concentration of 50 µg/L					Mean Conc. (µg/L)	SD (µg/L)	%RSD
54.41	54.63	51.22	49.74	50.56	52.09	1.73	3.3
52.85	54.56	53.41	52.07	51.41			

DPC-CC sized 1 cm x 1 cm and sample volume at 25 mL

2.4 Interference of coexisting ions

Interference of some presence ions, both cations and anions, in the Cr(VI) determination was investigated. The interference of typical four anions, NO_3^-, SO_4^{2-}, PO_4^{2-} and Cl^- was studied. It was found that using DPC-CC method, no interference occurred at the presence of 2.5 g/L of NO_3^-, SO_4^{2-} and PO_4^{2-} and 2 g/L of Cl^- in the Cr(VI) solution at the concentration of 100 µg/L. Additionally, the interference of 8 cations on the determination of 100 µg/L Cr(VI) solution using DPC-CC method was also studied. There is no interference in the presence of either 5 g/L of Na^+ and K^+, 2 g/L of Mg^{2+} or 5 mg/L of Al^{3+}, Cu^{2+}, Zn^{2+}, Pb^{2+} and Fe^{3+} by this method. The tolerance of co-existence of eight cations and four anions are summarized and the results are reported in Table 3.

Table 3 The tolerance of ions on the determination of 100 µg/L Cr(VI)

Ions	Ion Concentration	Cr(VI) Concentration found (µg/L)	%Recovery
NO_3^-	2.5 g/L	103.15	103%
SO_4^{2-}	2.5 g/L	107.93	108%
PO_4^{3-}	2.5 g/L	99.37	99%
Cl^-	2.0 g/L	109.26	109%
Na^+	5.0 g/L	96.63	97%
K^+	5.0 g/L	95.37	95%
Mg^{2+}	2.0 g/L	95.00	95%
Al^{3+}	5.0 mg/L	107.37	107%
Fe^{3+}	5.0 mg/L	103.04	103%
Pb^{2+}	5.0 mg/L	114.78	115%
Zn^{2+}	5.0 mg/L	104.82	105%
Cu^{2+}	5.0 mg/L	95.41	95%

2.5 Hexavalent chromium determination with a real sample

The DPC-CC method combined with UV-VIS spectrophotometer was applied to the Cr(VI) determination in a real sample solution extracted from unknown chrome coated aluminum sheets. Table 4 shows the Cr(VI) concentrations determined by this method compared with those determined by ICP-MS method. Three replicates of extracted samples were performed for the study. The results obtained by DPC-CC method combined with UV-VIS spectrophotometer were comparable to ICP-MS method.

Table 4 The Cr(VI) concentration of Cr(VI) solution extracted from real chromium coated aluminum sheets by DPC-CC and ICP-MS

	Cr(VI) concentration (µg/L)	
	DPC-CC method	ICP-MS technique
Replicate 1	24.19	24.67
Replicate 2	24.15	24.52
Replicate 3	24.37	25.04
Average	**24.23 ± 0.12**	**24.74 ± 0.27**

The acidity of sample was adjusted to pH 2 with orthophosphoric acid . The sample volume used in this study was 25 mL.

CONCLUSION

A simple detection method for hexavalent chromium (Cr(VI)) determination was reported. The method is based on the preparation of diphenylcarbazide coated cellulose materials (DPC-CC). Various parameters that might affect on the Cr(VI) determination were studied. The supporting sizes, 0.5 cm x 0.5 cm, 1 cm x 1 cm and 1 cm x 2 cm, and sample volumes, 25, 50 and 100 mL, have no any influence on the Cr(VI) determination by this method. Four typical anions and eight cations at some particular concentrations did not give any interference on the determination of 100 µg/L of Cr(VI) solution. This method could be applied to determine hexavalent chromium in an unknown metal sheet. Based on the Cr(VI) content in water, the detection limit of this method is 1.43 µg/L with the DPC-CC sized 1 cm x 1 cm and tested volume at 25 mL.

ACKNOWLEDGMENTS

The authors would like to acknowledge National Metal and Materials Technology Center for their financial support and would like to thank for Trace Element Analysis Laboratory (TEA-Lab) for her support on the analytical equipment for this research.

REFERENCES

Andrade, F. J., Rocha, J., Pasquini, C. and Baccan, N. (1983). Effect of On-line Complex Formation Kinetics on the Flow Injection Analysis Signal: the Spectrophotometric Determination of Chromium(VI), *The Analyst*, *108*, 621-625.

Andrade**,** F. J.**,** Tudino**,** M. B. and Troccoli**,** O. E. (1996). Flow dissolution of 1,5-diphenylcarbazide for the determination of chromium(VI), *The Analyst*, *121*, 613-616**.**

Capitan-Vallvey, L.F., Fernandez Romos, M. D. and Alvarez de Cienfuegos-Galvez, P. (2002). Optical Test Strip for Calcium Determination Based on a Neutral Ionophore, *Analytica Chimica Acta*, *451*, 231-241.

Environmental Standardization for Electrical and Electronic Products and systems, (Draft). International Electro-technical Commission. *IEC 62321*, 2nd, Annex B, 79-82.

Hua, L., Chan, Y.C., Wu, Y.P. and Wu, B.Y., (2008). The Determination of Hexavalent Chromium (Cr6+) in Electronic and Electrical Components and Products to Comply with RoHS Regulations, *Journal of Hazardous Materials*, inpress.

Meissam, N. and Mozhgan, K. (2003). Application of Potassium Chromate-Diphenylcarbazide in the Quantitative Determination of Ascorbic Acid by Spectrophotometry. *Turk J Chem*., *27*, 717-722

Prestel, H., Gaha, A. and Niessner, R., (2000). Detection of Heavy Metals in Water by Fluorescence Spectroscopy: On the Way to a Suitable Sensor System, *Journal of Analytical Chemistry*, *368*, 182-191.

Qingjun, L., Hua, C., Ying, X., Lidan, X., Mo, Y. and Ping, W. (2007) Detection of heavy metal toxicity using cardiac cell-based biosensor. *Biosensors and Bioelectronics*, *22*, 3224-3229.

U.S Environmental Protection Agency, (1992). Guidance for Data Useability in Risk Assessment, Part A.

Yukiko, T., Hitoshi K., Hachiro N. and Toshishige, S, (2006). Test Strips for Heavy-Metal Ions Fabricated from nanosized Dye Compounds, *Angewandte*, *45*, 913-916.

Yukiko, T., Takashi, H. and Toshishige, S. (2007). Test Strips for Lead(II) Based on a Unique Color Change of PVC-film Containing O-Donor Macrocycles and Anionic Dye, *Anal Sci*., *23*, 147-150.

Zhiqiang, Z., Ruomei, G., Jingtian, L., Suran, L. and Hui, L., (1998). Chromotopic Acid as a Reagent for Ultraviolet Spectrophotometric Determination of Hexavalent Chromium in Water, *Microchemical Journal*, *58*, 1-5.