

## DEVELOPMENT OF CELLULOSIC PAPER-BASED TEST STRIPS FOR Cr(VI) DETERMINATION

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Paper-based test strips specific for the Cr(VI) determination in aqueous solutions were developed by impregnating a color-forming reagent, diphenylcarbazide, and a quaternary ammonium salt (Aliquat 336) into paper sheets. The results showed that the quaternary ammonium salt could effectively retain the colored complex in the paper substrate. This paper-based test strip showed a high selectivity for Cr(VI) in the samples to be tested. The method was applied to synthetic samples, and the results were compared to these from a reference method. The current work demonstrated that with a color chart, this portable paper-based test strip has the potential to be used for field screening or on-site semi-quantitative analysis.

*Keywords:* Cellulosic paper; Test strip; Chromium(VI) determination; Diphenylcarbazide; Quaternary ammonium salt (Aliquat 336); Water analysis

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### INTRODUCTION

Fiber modification (engineering) is the key to changing the pulp and paper industry, and there is a compelling need for fiber engineering that can yield specific fiber attributes required for end-use performance (Baum 2002). Among the different approaches, the modification of fiber surfaces or paper surface either chemically or physically holds great potentials for the development of fiber-based functional products (Baum 2002). For the modification of paper/fibers with chemical additives, various methods can be adopted, such as chemical impregnation (Amelin and Kolodkin 2001; Nadzhafova et al. 2001), sizing followed with plasma treatment (Dushmantha et al. 2006; Xu et al. 2008), coating, and grafting polymerization (Huang et al. 2006). We have recently started a project on fiber modification for the purpose of producing value-added products, including electro-conductive paper (Huang et al. 2005), and cationic fibers (Zheng et al. 2006). In this study, we aimed to develop a paper-based test strip specific for determination of Cr(VI) in aqueous samples, and it was carried out by impregnating polymers containing ammonium groups and color-forming reagent onto paper sheet. Such a product would be similar to pH paper, which could then be used readily in the laboratory and field.

The test strips format as a one-step analytical method, has been widely used (Capitan-Vallvey et al. 2003). The advantages of test strips include lower costs, ease of handling, short analysis time, and the ability to make quick testing (Capitan-Vallvey et al. 2005). Test strips are typically in the form of thin pads or films that contain all of the

reagents required for the analysis. Usually they rely on the color intensity for determining the concentration of the interested species (Capitan-Vallvey et al. 2000; Cano Raya et al. 2006). Currently there are many paper-based test strips prepared by introducing chemicals into this fiber matrix, such as pH paper, KI test paper, and peroxide test strips, all of which are already commercially available. However, paper-based test strips specific for Cr(VI) determination have not been reported in the literature.

Chromium is recognized as one of the most serious pollutants in many water streams due to its carcinogenic potential (Alguacil et al. 2004). The toxicity of chromium depends on its oxidation states. Cr(VI) is approximately 100 times more toxic than Cr(III) due to its high mobility in the surface environment, bioaccumulation, and carcinogenesis (Zachara et al. 1989; Kabay et al. 2003). Currently, the United States Environmental Protection Agency (USEPA) has regulated maximum levels (Mohamed et al. 2006) of 100 and 50 mg·L<sup>-1</sup> of total chromium and Cr(VI) in drinking and ground water, respectively. The conventional method for the Cr(VI) concentration determination is based on a UV-Vis spectrophotometric method (ISO 11083). In recent years, many membrane-based optical sensors for Cr(VI) have been reported in the literature (Scindia et al. 2002; Choi et al. 2005; Jain et al. 2005; Saad Hassan et al. 2005; Güell et al. 2007; Sodaye et al. 2007). Cellulose triacetate (CTA) or polyvinyl chloride (PVC) was used as the substrate material. PVC or CTA membrane is non-porous and non-polar. In such applications, a plasticizer is required for giving high metal flux, which increases the cost. In most cases the plasticized PVC or CTA membrane has a long response time for the diffusion of the concerned species into the membrane, for example, at least 60 min based on the research by Scindia et al. (2004).

Compared with PVC or CTA, paper, consisting of plant fibers, has high porosity, good physical strength, stiffness, and dimensional stability, etc. This material is cheaper due to its low cost of production. Moreover, it is well known that cellulose fiber is a renewable natural resource with superior advantages over other polymeric materials for its environmental friendliness (Huang et al. 2006). No plasticizer is needed, and the response time can be shortened due to the high porosity of paper sheet. In addition, the good stiffness and strength of paper mean that it can serve as a suitable supporter.

In the current work, effective chemicals were impregnated into a paper sheet, which was then dried in an ambient environment. The dried paper products are ready to be used, with a color chart, to determine the Cr(VI) concentration in an aqueous solution. In practice, the incorporation of these effective chemicals may be carried out by following paper surface treatment techniques, such as surface sizing, coating, without much modification to the existing papermaking facility.

The paper-based test strip proposed here for Cr(VI) was based on i) impregnating a color-forming agent, in this study, diphenylcarbazide (DPC), into paper sheet, and ii) producing colored complexes between the color-forming agent in the test paper strip and the chromium in the sample.

## EXPERIMENTAL

### Materials

The paper sample used for the test strip was quantitative filter paper of medium porosity, which was obtained from Fisher Scientific. 1,5-diphenylcarbazide (DPC) was obtained from Sigma-Aldrich. Tricaprylmethylammonium chloride (Aliquat 336 mixture of C<sub>8</sub>-C<sub>10</sub>, where C<sub>8</sub> is dominating) was obtained from Aldrich. The chromium solutions with various concentrations were freshly prepared by diluting a stock solution (1000mg/L) of Cr(VI) (Fisher Scientific). Other solutions, including chloride, sulphate, nitrate, carbonate, bromide, iodide, phosphate, acetate, and bicarbonate were prepared by using analytical reagent grade chemicals and deionized water. The pH was adjusted to 4.0 by adding either diluted HCl or NaOH solution. The spiked water sample of Cr(VI) was prepared by adding Cr(VI) into tap water with its pH adjusted to 4.0.

### Apparatus

A Genesys 10-s UV-Vis spectrophotometer (Thermo Electron Co, USA) was used to determine the absorption of the colored complex solutions. The Cr(VI)-DPC samples or the solutions after contacting with the DPC loaded test strip at different dipping times were directly transferred to a cuvette of the spectrophotometer. A Ultrascan reflector (Hunterlab, model SN-7554, USA) was used for the measurement of color intensity formed on the test strip.

### Test Strip Preparation

The DPC and the quaternary ammonium salt solutions (0.2% and 1.0%, respectively) were prepared in acetone. The filter paper was then immersed into the acetone solution (containing DPC and the quaternary ammonium salt) for 5 min at room temperature. Subsequently, the DPC and the quaternary ammonium salt loaded filter paper was dried in air. The dried filter paper was cut into strips, sealed in a plastic bag, and kept ready to be used.

### Diffuse Reflectance Measurement

The prepared test strip was dipped into the Cr(VI)-containing solution for a few seconds and our experimental results showed that an equilibrium was reached fast between the Cr(VI) concentration on the test strip and that in the solution. After the test, the strip was pulled out of the solution and held 10 minutes in air for the color development. After that, the color intensity that had developed on the test strip was measured using the Ultrascan reflector at a fixed wavelength of 550nm.

## RESULTS AND DISCUSSION

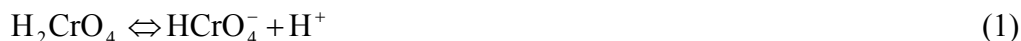
### Development of Paper-Based Test Strip

Paper sheets such as filter paper is composed of fibers that have high specific surface area and plenty of capillary pores; thus, there is high absorbability to inorganic or organic liquids. By impregnating the effective chemicals into paper, a paper-based test

strip specific for the determination of Cr(VI) concentration is expected to be portable, user-friendly, and handy, similarly to pH paper. The specific features of such a test strip would include i) give a visual color response so that the test strip, to be used with color chart, can quantitatively determine the Cr(VI) concentration in the field screening or on site semi-quantitative analysis without excessive laboratory equipment, and ii) have a selectivity for Cr(VI) so that sample pretreatment steps would be minimized.

Based on the above expectation, the technical barriers to be overcome include: i) the effective chemicals need to be loaded into a paper matrix, ii) the color formed in the test strip with Cr(VI) needs to be retained in the test strip without leaching, and iii) high selectivity for Cr(VI) is required.

In an aqueous solution, chromium(VI) exists as chromate ( $\text{CrO}_4^{2-}$ ), dichromate ( $\text{Cr}_2\text{O}_7^{2-}$ ), hydrogen chromate ( $\text{HCrO}_4^-$ ), hydrogen dichromate ( $\text{HCr}_2\text{O}_7^-$ ), trichromate ( $\text{Cr}_3\text{O}_{10}^{2-}$ ), and tetrachromate ( $\text{Cr}_4\text{O}_{13}^{2-}$ ) anions, due to the formation of isopolyacids, depending on the pH of the solution (Sodaye et al. 2007). The last three ions have been detected in solution of  $\text{pH} < 0$  or at Cr(VI) concentration greater than  $1 \text{ mol}\cdot\text{L}^{-1}$ . At  $\text{pH} \leq 1$ ,  $\text{H}_2\text{CrO}_4$  is a significant species; at  $\text{pH} = 2-6$ ,  $\text{HCrO}_4^-$  and  $\text{Cr}_2\text{O}_7^{2-}$  are present, at  $\text{pH} = 2-4$ ,  $\text{Cr}_2\text{O}_7^{2-}$  predominates, while  $\text{HCrO}_4^-$  predominates at  $\text{pH} = 4-6$ ; and at  $\text{pH} > 6$ ,  $\text{CrO}_4^{2-}$  predominates. The following equations describe the distribution of the main chromium species in an aqueous solution (Bhowal et al. 2001; Kabay et al. 2003; Jain et al. 2005):



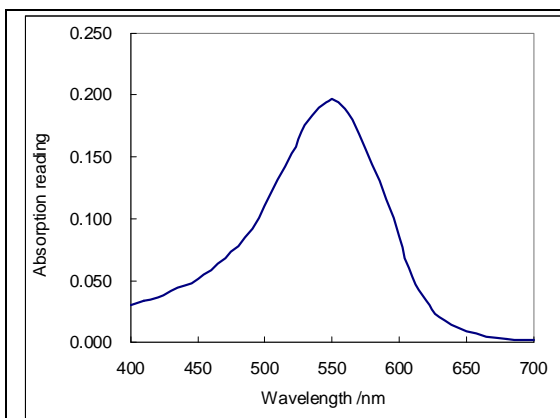
The formation of colored complexes involved two steps (Scindia et al. 2004):

- Cr(VI) oxidizes DPC to diphenylcarbazone(DPCO) and itself is reduced to Cr(III);
- Cr(III) chelates with DPCO to form the Cr(III)-DPCO complex, which has a characteristic magenta color.

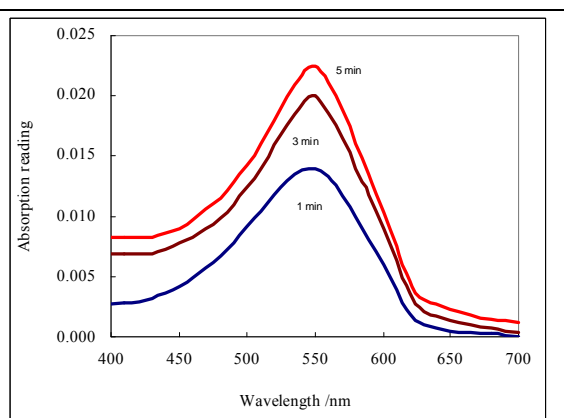
Although the exact structure of this colored complex is not totally known, it appears to be a cationic complex  $[\text{Cr(III)-DPCO}]^{(3-n)+}$ , where n is the number of protons released in complex formation. This complex has an absorption maximum at 550nm, as shown in Fig. 1.

When the DPC loaded test strip was dipped into a Cr(VI) solution, the magenta complexes were developed rapidly on the surface of test strip. However, some of the complexes were leached into the Cr(VI) solution, and this is particularly so if extending the dipping time, as evidenced by the gradual increase of the solution color. We used a UV-Vis test to determine the color increase, as shown in Fig. 2. One can find that with the extending of dipping time from 1 minute to 5 minutes, the absorption at 550nm increased. We estimated that after 10 minutes dipping time, about 40% of the formed complexes were leached into the solution. Certainly this is not acceptable for a test strip

method. Therefore in the next step, we made efforts to minimize the complex leaching effect.



**Fig. 1** Characteristic absorption of Cr(III)-DPCO complex in aqueous solution ( $10 \text{ mg}\cdot\text{L}^{-1}$  Cr(VI), pH 3.0)



**Fig. 2** Absorption spectrogram of solution in contact with DPC loaded test strip at different dipping times ( $10 \text{ mg}\cdot\text{L}^{-1}$  Cr(VI), pH 3.0)

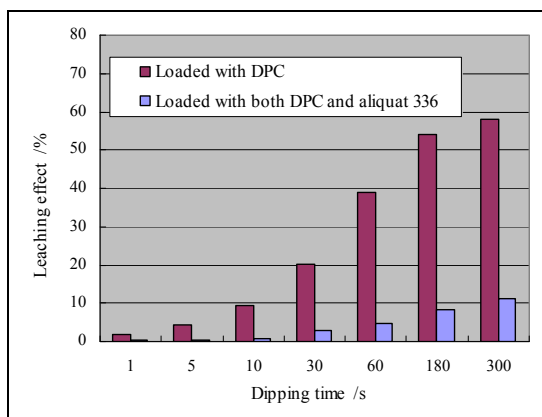
Our approach to solve this technical problem was to identify a suitable hydrophobic polymer, which once on paper surface can decrease the leaching effect of the formed Cr(III)-DPCO complexes. Polymers that have tertiary amines and quaternary ammonium salts, such as tricaprilmethylammonium chloride (Aliquat 336), may serve for this purpose well. The quaternary ammonium salt has been used in membrane-based separation technology for various species, including Cr(VI). In such applications, the quaternary ammonium salt was used as an anion exchange material (Lo and Shiue 1998; Park et al. 2001; Choi and Moon 2004), and was identified as a good choice (Park et al. 2001). We dissolved the quaternary ammonium salt and DPC in the same acetone solution, which were then impregnated into the filter paper. The concentration of the quaternary ammonium salt and DPC in the acetone solution was 1.0% and 0.2%, respectively. As a background experiment, the same quaternary ammonium salt acetone solution without DPC was prepared and loaded into a piece of filter paper. In the presence of the quaternary ammonium salt, we found that the formed Cr(III)-DPCO complexes retained well in the test strip, as shown in Figure 3. The leaching effect was defined as follows,

$$\text{Leaching effect} = \frac{A_1}{A_0} \times 100 \% \quad (5)$$

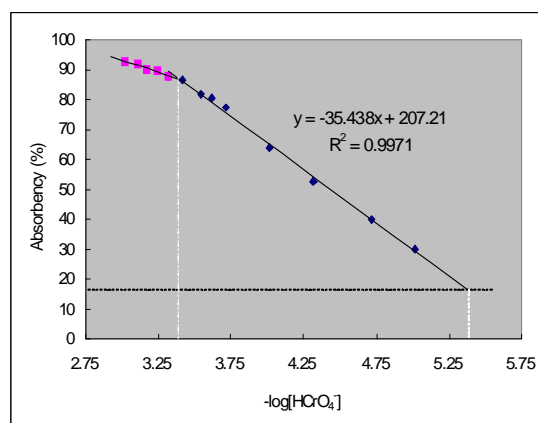
where

$A_1$  = absorption reading at 550nm of Cr(VI) solution after contacting with test strip

$A_0$  = absorption reading at 550nm of Cr(VI) solution containing the same amount of DPC as that in the test strip used in  $A_1$ .



**Fig. 3** Leaching effect of Cr(III)-DPCO complexes of the test strip with or without tricaprilmethylammonium chloride ( $10 \text{ mg}\cdot\text{L}^{-1}$  Cr(VI), pH 3.0)



**Fig. 4** Working range of paper-based test strip (pH 4.0, dipping time 10s, color developing time 10 min)

For the test strip loaded with DPC only, i.e. in the absence of the quaternary ammonium salt, the amount of Cr(III)-DPCO complexes leached into the solution increased significantly as the dipping time was extended. In fact in 10 seconds about 10% was leached out and in 60 seconds, about 40% was leached out. On the other hand, in the presence of the quaternary ammonium salt (it was found that a 1.0% concentration in the acetone solution can serve well for this purpose), the amount of Cr(III)-DPCO complexes leached out was negligible within the first 10 seconds. The possible mechanism of the quaternary ammonium salt is that due to its hydrophobic nature it can form hydrophobic ion-pairs with Cr(III)-DPCO complexes, which are more easily retained in paper strip, so that once the Cr(III)-DPCP complexes are formed, it prevents the colored complexes from leaching into the solution.

### Calibration Curve

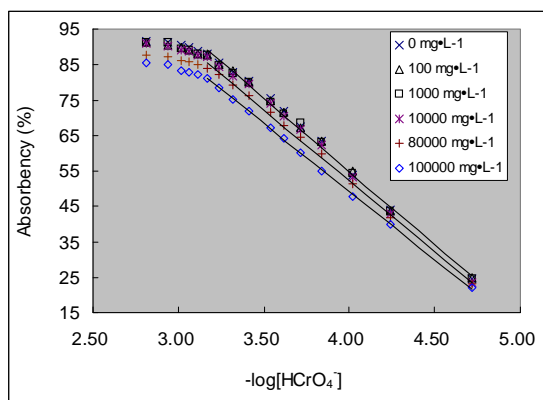
To establish the calibration curve, Cr(VI) solutions with different concentrations were prepared and used. The absorbency as a function of the Cr(VI) concentration is shown in Figure 4. The lower detection limit was determined by the reflectance of blank sample and the upper detection limit was determined by the crossover point of two lines as shown in Fig. 4. It was found that the absorbency had a linear relationship with the logarithm of  $[\text{HCrO}_4^-]$  from its concentration of  $0.38 \times 10^{-5} \text{ mol/L}$  to  $40.4 \times 10^{-5} \text{ mol/L}$ , corresponding to 0.20 - 21.0  $\text{mg}\cdot\text{L}^{-1}$  for Cr(VI) or 0.45 - 47.25  $\text{mg}\cdot\text{L}^{-1}$   $\text{HCrO}_4^-$  in the solution. The lower detection limit was determined by the absorbency of a blank sample, while the upper detection limit was determined by the crossover point of two lines as shown in Fig. 4.

### Selectivity of Paper-Based Test Strip

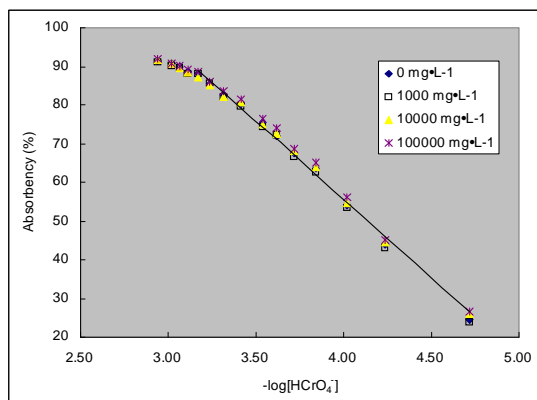
Chloride ( $\text{Cl}^-$ ) and sulfate ( $\text{SO}_4^{2-}$ ) are commonly present in aqueous systems (Jain et al. 2005). Their effects on the Cr(VI) determination are shown in Figs. 5 and 6, respectively. For chloride, if its concentration was less than  $1.0 \times 10^4 \text{ mg}\cdot\text{L}^{-1}$ , its effect was negligible; however, at higher concentration (higher than  $1.0 \times 10^4 \text{ mg}\cdot\text{L}^{-1}$ ), some deviation can be observed, as shown in Fig. 5. For sulfate, the effect was negligible for the entire

range studied (up to  $1.0 \times 10^5$  mg·L<sup>-1</sup>), as shown in Fig. 6. Typically the highest concentration of SO<sub>4</sub><sup>2-</sup> in the waste water is in the range of 5000-6000 mg·L<sup>-1</sup> (Wei et al. 2007). One can conclude that under such a condition, the results on the Cr(VI) determination based on the method developed from this study will not be affected, and there is no need for removal of sulfate.

The selectivity of test strip for Cr(VI) in the presence of other species was also studied. We defined a Tolerance (T) Factor, which gave a deviation in absorbency of less than 1.5%. Table 1 shows the results for chloride, sulfate, nitrate, acetate, bicarbonate, bromide, and iodide. One can find that the Tolerance Factors for all of them were very high, implying that the test strip method developed has a high selectivity in a complex sample system.



**Fig. 5** Effect of Cl<sup>-</sup> concentration in Cr(VI) solution on determination of test strip (dipping time 10s, color developing time 10min)



**Fig. 6** Effect of SO<sub>4</sub><sup>2-</sup> concentration in Cr(VI) solution on determination of test strip (dipping time 10s, color developing time 10min)

**Table 1.** Selectivity of Test Strip for Cr(VI) Determination in the Presence of Other Anions

Species	Cr <sup>6+</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>	NO <sub>3</sub> <sup>-</sup>	CH <sub>3</sub> COO <sup>-</sup>	HCO <sub>3</sub> <sup>2-</sup>	Br <sup>-</sup>	I <sup>-</sup>
Tolerance Factor (T)*	/	6391	10000	918.2	200	800	3500	4515

\* Dipping time: 10s, color developing time: 10min, [Cr(VI)] = 10 mg·L<sup>-1</sup>

### Verification of the Method with Spiked Water Samples

The test strip method developed in this study was used to determine the Cr(VI) concentration in spiked samples. The dipping time and color developing time were fixed at 10 seconds and 10 minutes, respectively, and the calibration curve shown in Fig. 4 was used. The results from the test strip method were compared with those from the UV-Vis spectrophotometric method (ISO 11083) (used as the reference method), shown in Table 2. One can find that there was a very good agreement between these two methods. In addition, the test strip method gave a good standard deviation. Based on these results, we draw the conclusion that the paper test strip method developed in this study is suitable to determine the Cr(VI) concentration in similar samples.

**Table 2.** Determination of Cr(VI) Concentration in a Spiked Tap Water Sample Based on the Test Strip Method

Sample ID	Test strip method		UV-Vis method (referenced)
	[Cr(VI)] / mg·L <sup>-1</sup> *	Standard deviation / mg·L <sup>-1</sup>	[Cr(VI)] / mg·L <sup>-1</sup>
1	5.2	0.20	5.1
2	8.1	0.19	8.2
3	12.5	0.34	12.1
4	15.3	0.45	15.7

\* means average value (n=3)

## CONCLUSIONS

1. A paper-based test strip method specific for the Cr(VI) determination was developed by impregnating the color-forming reagent diphenylcarbazide (DPC) and hydrophobic polymer, a quaternary ammonium salt (Aliquat 336), into paper sheets. Cr(VI) can oxidize DPC to DPCO, while itself is reduced to Cr(III). Subsequently, the Cr(III)-DPCO complexes are formed, which has a characteristic magenta color.
2. Our results showed that the quaternary ammonium salt can retain the colored Cr(III)-DPCO complexes, on which the test strip method is based.
3. The paper-based test strips were found to have a high selectivity to Cr(VI) ion and were found to be effective, stable, and reliable for determination of Cr(VI).
4. The method has high Tolerance Factors for many common species present in water samples such as sulfate and chloride.
5. We found that the paper test strip method, when applied to samples spiked with Cr(VI), gave accurate results in comparison with the conventional method for the determination of Cr(VI). Therefore, we concluded that the paper test strip method developed in this study is an effective, reliable way of determining the Cr(VI) concentration in aqueous sample.

## ACKNOWLEDGMENT

The authors are grateful for the support of the Canada Research Chairs Program, and the Atlantic Innovation Fund Program.

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Article submitted: April 14, 2009; Peer review completed: May 17, 2009; Revised version received and accepted: June 18, 2009; Published: June 20, 2009.