# A FLUOROSENSOR FOR Cr (VI) BASED ON THE INNER FILTER EFFECT

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Abstract. A fluorosensor for Cr (VI) was designed based on the inner filter effect of fluorescence. The sensor employs the reagents, diphenylcarbazide as the primary absorber and coumarin as the fluorophore. The sensor exhibits maximum fluorescence at  $\lambda_{ev/em} = 464 / 493$  nm. At these wavelengths, the fluorescence intensity is dependent on Cr (VI) concentrations.

A kinetic approach was used to relate the fluorescence response of the sensor to Cr (VI) concentrations. The sensor was most sensitive to Cr (VI) at the 0-1 ppm range, with an LOD of 21.3 ppb. The sensor can only be used once. However, different fabricated sensing membranes were found to have a reproducible and stable response. The coefficient of variation was calculated to be 5.03 % (n= 14 membranes) at the concentration of 1 ppm Cr (VI). The sensor also showed a very high selectivity and specificity to Cr (VI). At pH 1, no response of the sensor were detected with the various interfering metal-ions. Assay of synthetic Cr (VI) water samples, revealed a high recovery of the sensor response, ~95-103 %.

Keywords: Chromium (VI), inner filter effect, diphenylcarbazide, coumarin, optical sensing membranes

#### INTRODUCTION

The determination of chromium in water is of major interest because of its demonstrated effects in both water quality and health. The threshold value of chromium in water has been set at 50  $\mu$ g / L [1]. There are two main forms of chromium: Cr (III) and Cr (VI). Of these two forms, Cr (VI) is the most soluble and the one most readily absorbed by the body. In human studies, absorption from the gastrointestinal tract has been shown to be about 10% [2, 3]. Once inside the body, Cr (VI) is readily transported through the cell membrane where it is reduced to Cr (III), the form which binds to macromolecules. (Cr (III) per se does not penetrate the cell membrane). The toxic effects associated with Cr (VI) has been attributed to its ability to oxidize metabolic species and its adverse impact on the lungs, liver and kidneys [4].

The primary source of chromium in most surface water and some shallow groundwater is human industrial activity. Cr (VI) enters into water supplies mainly from effluent discharge of the chrome plating and tanning industries or from industrial cooling towers which use chromic acid and other forms of Cr (VI) [5-7]. Cr (VI) can also enter the drinking water distribution system from the corrosion inhibitors used in water pipes [6].

In order to monitor Cr (VI) in water continuously and remotely, sensitive and reliable optical chemical sensors (OCS) are desired. Only three OCS for Cr (VI) have been reported [8-10]. These sensors employ as sensing materials the absorbance reagent, diphenylcarbazide (DPC) [8] and the fluorescence reagent, rhodamine 6G [9].

In the design of OCS, fluorescence-based sensing materials are preferred owing to the inherent advantages associated with the fluorescence technique. These attractive features include the intrinsic sensitivity of fluorescence, its almost complete inertness to turbidity effects and its flexibility with respect to geometric arrangement. It is difficult however, to obtain a single fluorescent material which has the following characteristics desirable in an OCS: (a) high reactivity, sensitivity and selectivity to the analyte; (b) long wave absorption or excitation wavelength (from 400-700 nm), so as to allow use of inexpensive waveguide optics (optical fibers) and solid-state light sources (LEDs) and detectors; (c) high photostability and chemical stability; and (d) large *Stoke's* shift (difference between excitation and emission wavelengths), so to minimize optical interferences. Thus, some workers in the field [11-13] exploit the so-called 'inner filter effect" of fluorescence. This is one generic strategy for designing fluorescence-based sensors. Here, a combined absorber-fluorophore system is employed, where the absorption band of the absorber overlaps with the excitation or emission band of the fluorophore.

In this paper, we report on a fluorosensor for Cr (VI) which exploits the inner filter effect. A preliminary survey for the possible sensing materials is described and the development and characterization of a sensor based on the chosen reagents is discussed.

# EXPERIMENTAL

**Reagents and Materials.** All inorganic and organic chemicals were of analytical reagent-grade and were obtained mostly from Sigma (St. Louis, USA) and Aldrich Co. (Milwaukee, USA). The reagents investigated for Cr (VI) are summarized in Tables 1 and 2, respectively. All these reagents were used without further purification.

The solid support for immobilizing these reagents was hydrogel D-4 (from Tyndale Plains-Hunter Ltd., New Jersey,

Table 1.	Absorbance Reagents Investigated as Sensing
	Materials for Cr (VI)

Absorbance Reagents	Visual Reactions of Constructed OCS
Alizarin (AZ)	Negative reaction for Cr (VI)
Congo Red (CR)	Negative reaction for Cr (VI)
Crystal Violet (CV)	Negative reaction for Cr (VI)
Diphenylcarbazide (DPC)	Positive reaction only for Cr (VI)
Dithizone (DTZ)	Positive reaction for Cr (VI) and also for metal ions of Ag, Cd, Cu, Hg and Pb.
Ethyl Violet (EV)	Negative reaction for Cr (VI)
Malachite Green (MG)	Positive reaction for Cr (VI) and also for $H^+$
Quinaldine Red (QR)	Negative reaction for Cr (VI)
4- (2-thiazolylazo)	Positive reaction for
resorcinol (TAR)	Cr (VI) and also for $H^{\!\scriptscriptstyle +}$

Fluorescent Reagents	Response Characteristics of the Resulting OCS			
	λmax ex/em	Slope <sup>2</sup>	Stoke's shift	Remarks
	(nm)	( <u>+</u> %)	(nm)	
Coumarin (CM)	464/493	-4	29	Very low sensitivity to Cr (VI)
6, 9-Diamino Ethoxy Acridine Lactate Monohydrate (DEALM)	430/495	+97	65	Unstable with light and air; OCS turns brown after only an overnight storage
Eosin B (EB)	528/553	-5	25	Very low sensitivity to Cr (VI)
Eosin Y (EY)	550/551	-85	1	Very low Stoke' shift; also responds to H <sup>+</sup>
7-Hydroxycoumarin (HCM)	360/470	-61	110	λ <sub>ex</sub> maxima is at very low UV range where commercial LEDs are not readily available
Pyronin Y (PY)	547/563	-40	16	Unstable with light; OCS exhibits photodecomposition
Rhodamine 6G (Rh-6G)	535/560	+19	25	Unstable with light; OCS exhibits photodecomposition
Rhodamine B (Rh-B)	563/583	-18	20	Shows reaction to Cr (VI) and also to metal ions of Hg (II) and Cu (II)

# Table 2. Response Characteristics of OCS from Fluorescence Reagents for CR (VI)<sup>1</sup>

#### Legend:

<sup>1</sup>In preparing stock Cr (VI) solutions, a Clark and Lubs pH 1 buffer (0.2 M KCl-HCl) was used since most of the reagents showed great sensitivity to Cr (VI) at acidic conditions. Also, due to leaching of the reagent materials, fresh batches of OCS were used in each spectral run.

<sup>2</sup>The % slope was taken at emission wavelength maxima ( $\lambda_{ev/em} = 464/493 \text{ nm}$ ) and was calculated based on the formula: % slope = [(F<sub>c</sub> - F<sub>B</sub>) / F<sub>c</sub>] x 100

where;  $F_c$  was the OCS fluorescence due to test Cr (VI) solution, i.e., 1 ppm Cr (VI) in pH 1 buffer and  $F_B$  was the fluorescence due to the blank, i.e., pH 1 buffer only.

A + sign on the % slope denotes  $F_c > F_B$ ; a - sign denotes  $F_c < F_B$ .

USA), a polyurethane type of polymer. Hydrogel D-4 was formulated as a 10% by wt. in 90:10 v/v abs. EtOH:  $H_2O$ solvent. Owing to its inherent permeability, this polymer does not require any plasticizer and thus, allow instantaneous response of the constructed OCS to the Cr (VI) ion. Moreover, its non-toxic alcoholic solvent is highly compatible with many of the investigated Cr (VI) reagents.

All aqueous solutions were prepared using distilled water, deionized from an Alpha-Q water purification system (Millipore, France). The buffer solutions were prepared according to Clark and Lubs. The pH of the buffers was measured with a Metrohm digital pH meter (Model 691). A 1000 ppm Cr (VI) stock solution was prepared by dissolving 35.35 mg  $K_2Cr_2O_7$  (Ajax Chemicals, Australia) in 100 mL buffer solution. Serial dilutions of the stock solution were done to prepare various standards of Cr (VI). Standards solutions were prepared fresh, prior to use.

**Preparation of the Sensing Membrane.** In investigating various sensing materials for Cr (VI), the sensor membrane was composed of  $\sim 1.0 \text{ mg Cr}(\text{VI})$  -sensitive reagent, 0.50 g abolute EtOH solvent, and 1.0 g polymer solution. The membrane components were mixed in the following order: reagent + solvent + polymer. Thorough mixing of the components was accomplished using a Thermolyne vortex shaker (Type 37600). The homogenous cocktail solution was then spin-coated on a planar acetate sheet and the resulting membrane air-dried for  $\sim 30-60$  min at room temperature.

To minimize the leaching of the reagents materials, the membrane was overcoated with another layer of hydrogel D-4 (20 % by weight in 90% v/v abs. EtOH:  $H_2O$  solvent.). Overcoating was done as quickly as possible in order to avoid dissolution of the sensing material. The membrane was then allowed to dry as before. The thickness of the resulting membrane was ~10-20  $\mu$ m.

Spectrophotometric Analysis of the Membrane. Sensing membranes were cut and fitted into the cuvette cell of either, a Perkin-Elmer UV/VIS spectrophotometer (Model Lambda 2) or a Hitachi fluorescence spectrophotometer (Model F-4500).

To compensate for any change in the membrane position, the kinetic method of analysis was adopted. In this method, the sensor response, expressed as fluorescence delta, were taken and related to the Cr (VI) concentration. The fluorescence delta was defined as the difference in the initial (t = 0 sec) and steady-state (t = 300 sec) response of the sensor, upon contact with Cr (VI) solution.

## **RESULTS AND DISCUSSION**

*Screening for Sensing Materials.* In constructing an OCS for Cr (VI), possible sensing materials were explored from various commercially available and reported Cr (VI)-

sensitive reagents. These materials were either absorbanceor fluorescence-based reagents, immobilized in a hydrogel D-4 matrix. As summarized in Tables 1 and 2, respectively, not all of the investigated reagents can be suitable sensing materials for Cr (VI). Among the chromogenic reagents which can be used for absorbance measurement only DPC showed high-selectivity to this metal-ion (Table 1) and can be considered a good sensing material. In the case of fluorescence reagents, all constructed OCS showed positive reactions to Cr(VI). However, various limitations exist in their response characteristics, either with the sensitivity to Cr (VI), the excitation wavelengths, the Stokes' shift, or the chemical stability (Table 2). Thus, among these fluorescence reagents, not one can be singled out as a better sensing material for Cr (VI).

*Exploiting the "Inner Filter Effect".* Among all reagents screened for Cr (VI), DPC was considered the optimum sensing material because of its reactivity and high selectivity to Cr (VI). However, the measurement technique associated with DPC, i.e., absorbance measurement, lacks the advantageous features offered by fluorescence. To make the DPC OCS amenable to fluorescence measurement, fluorophores were co-immobilized with DPC, initially at a 1:1 mol/mol ratio in the hydrogel matrix. These fluorophores were the fluorescence reagents listed in Table 2.

Among the fluorescence reagents tested, coumarin (CM) material coupled to DPC showed a highly sensitive fluorescence response to Cr (VI) (Fig. 1). This is a consequence of the inner filter effect of the CM fluorescence. The CM OCS exhibited an enhancement (more than 30x) in its fluorescence sensitivity to Cr (VI) when coupled with DPC. Both the excitation and emission wavelengths of CM were greatly modulated with the varying absorption of DPC in the



Wavelength/nm

Fig. 1. The excitation and emission spectra ( $\lambda_{max} ex/em = 464/493 \text{ nm}$ ) of the DPC:CM OCS in the (- - -) presence and (—) absence of Cr(VI). The DPC:CM material were optimized at the molar ratio of 16 mol CM per mol DPC.



Fig. 2. Emission fluorescence-time scan of the DPC:CMOCS at various conc'ns of Cr(VI)



Membrane Label



presence of Cr(VI). The maximum excitation and emission wavelengths of CM occur at 464 and 493nm, respectively, while that of DPC (the absorbance maxima) is at 545 nm in the presence of Cr (VI). Although other investigated Cr (VI) fluorescence reagents possess excitation and emission wavelengths closer to the absorption maxima of DPC than CM, the fluorescence detected were depressed in the presence of Cr (VI). Thus, we assumed a dynamic chemical interaction between the absorber and the fluorophore. This interaction can be considered an important factor in designing fluorescence-based sensors using the inner filter effect.

**Performance Characteristics of the DPC:CM OCS.** The OCS based on the CM/OPC reagent required about 5 minutes to reach a steady state in the presence of Cr(VI) DPC:CM OCS (Fig. 2). However at 25 ppm Cr (VI), the response of the sensor was unstable with increasing time. It attained to a minimum steady-state fluorescence and then increased beyond the steady-state. This behavior can be attributed to a greater degree of dissociation of the DPC - Cr (VI) complex at higher concentration of the metal ion.



Fig. 3. Calibration plot of the DPC:CM OCS using the fluorescence delta values



Fig. 5. Storage-stability of the DPC:CMOCS.

The calibration plots for the sensor response exhibited greater linearity and sensitivity to Cr (VI) at the concentration range of 0-1 ppm and 1-5 ppm (Fig. 3). This is supported by the accompanying regression data shown in Table 3. The calculated slope suggested the sensor to be most sensitive to Cr (VI) at the 0-1 ppm range. Also at this range, the LOD was found to be 21.3 ppb Cr (VI) (n = 11 sensor membranes).

The reproducibility and storage-stability characteristics of the sensor were found to be well within the acceptable limit of  $\leq 10\%$ . A total of 14 OCS tested in their fluorescence response to 1 ppm Cr (VI) gave an r. s. d. of 5.03 % (Fig. 4). This observation suggests reproducible response from one OCS to another. In the case of storage-stability, the results are shown in Figure 5. The values depicted are the mean of three trials. The sensor membranes can be stored indefinitely and still retain their original characteristics. Storage of the membranes must be made in air-tight containers to keep them dust- and moisture- free.

# Table 3. Calibration Plot of the DPC:CM OCS Using the Fluorescence Delta Values

Statistical Item	Cr (VI) Concentration Range, in ppm			
<u></u>	0.0-1.0	1.0-5.0	5.0-25.0	
Pearson correlation				
coefficient	0.9960126	1.0	-0.976950	
Slope [Fluorescence				
delta change/ppm				
Cr (VI) change]	936.19780	270.75000	-35.792310	
y-intercept	133.31429	788.25000	2253.23080	

Table 4. Assay of Cr (VI) Using the DPC:CM Sensor

Theoretical (Prepared) ppm Cr (VI)	Experimental (Found) ppm Cr (VI) (% S. R.*)		
0.400	0.410 (102.5 %)		
0.700	0.665 (95.0 %)		
1.600	1.595 (99.7 %)		

\* % Signal Recovery computed as 100-100[{theor'l. ppm Cr (VI) -expt'l. ppm. Cr (VI)}/theor'l. ppm Cr (VI)].

Several interfering ions were tested for their reaction to DPC:CM OCS. These ions were Hg (II), Cu (II), Pb (II), Fe (III), Co (II), Zn (II), Cd (II), Ni (II), Mg (II) and Mn (II); both at 1 and 100 ppm concentrations. At pH 1.0, the condition specified for analyzing Cr (VI), no response was obtained with any of these interfering ions. This confirms the high selectivity and specificity to Cr (VI) of the DPC:CM OCS.

Assay of Cr(VI). Synthetic water samples of Cr (VI) were prepared [15] and analyzed using the DPC:CM OCS. A high signal recovery of around 95 -103 % was exhibited by the sensor. This is depicted in Table 4.

## CONCLUSION

We have designed a fluorosensor for Cr (VI) based on the inner filter effect of fluorescence. The sensor employed DPC as the primary absorber and coumarin as the fluorophore. The sensor exhibited maximum fluorescence at  $\lambda_{ex/em} = 464$  / 493 nm. At these wavelengths, the fluorescence intensity was found to be dependent on Cr (VI) concentrations.

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at the concentration of 1 ppm Cr (VI). The sensor also showed a very high selectivity and specificity to Cr (VI). At pH 1, no response of the sensor were detected with the various interfering metal-ions. Assay of synthetic Cr (VI) water samples, revealed a high recovery of the sensor response, ~95-103 %.

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