9-Acridone-4-carboxylic acid as an efficient Cr(III) fluorescent sensor: Trace level detection, estimation and speciation studies

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ABSTRACT

9-Acridone-4-carboxylic acid has been established as an efficient Cr(III) fluorescent sensor. The binding of this ligand with Cr(III) is confirmed by FTIR, thermal and mass spectral analysis of the product. Based on this chelation assisted fluorescence quenching, a highly sensitive spectrofluorometric method is developed for trace level detection, estimation and speciation studies of chromium in DMF-water. The ligand has an excitation and emission maxima at 408 nm and 498.4 nm, respectively. The equilibrium binding constant of the ligand with Cr(III) is 8.1378×10^4 as calculated using Stern–Volmer equation. Up to 9×10^{-6} mol L^{-1} of [Cr^{3+}], linearity has been observed. The interference of foreign ions has been found to be negligible.

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1. Introduction

Toxicological studies have indicated that the degree of toxicity of metal ions depends on its chemical form. The toxic nature of the Cr(VI) is attributed to its higher oxidation potential and relatively smaller size, which enables it to penetrate through biological cell membranes. Moreover, in air, chromium particulates play an important role in the oxidation of sulfur dioxide, leading to the formation of acidic aerosols responsible for global acid rain [1]. Cr(VI) has an adverse impact on liver, lung, kidney [2] and causes cancer by oxidizing the biological species such as DNA and some proteins [3].Cr(III) may be considered as an essential trace element for the proper functioning of living organisms (mammals), e.g.: for the maintenance of "glucose tolerance factor"; it is thought to be a cofactor for the insulin action and to have a role in the peripheral activity of this hormone. Metallic chromium or its compounds are widely used in anodizing operation in the surface industry, in making alloys, chrome plating, leather tanning, batteries, refractory, mordant dyeing, paints, welding, catalysis, corrosion control, oxidation, wood preservative and various other industrial applications [4,5]. Chromium species can enter into the environment from their discharge. They can also enter into drinking water supply systems from the corrosion inhibitors used in water pipes and contain-

ers. The threshold limit for chromium uptake in air is 0.1 mg m^{-3} and in water is 0.05 mg dm^{-3} [3]. Owing to these two contrasting effects, precise and accurate determination of both species in environmental samples is essential, in addition to the total chromium content [6–8]. Chromium content in natural waters is normally at μg L^{-1} level and there are also severe matrix interferences, which cannot be minimized. Therefore, the direct determination may not be possible with sufficient sensitivity and selectivity even by the methods such as flame atomic absorption spectrometry (FAAS) [9,10], graphite furnace atomic absorption spectrometry (GFAAS) [11,12], inductively coupled plasma atomic emission spectroscopy (ICPAES) [13], X-ray fluorescence spectrometry [14] and electrochemical methods [15,16]. Some forms of preliminary separation and pre-concentration like liquid–liquid extraction [17,18], cloud point extraction [19], ion-exchange [20,21] and solid phase extraction [22–24] are required. Bueno et al. [25] reported direct chromium speciation using X-ray spectrometry allied to chemometrics without separation / preconcentration of Cr species with a detection limits of 17 and 50 μg L^{-1} for Cr(III) and Cr(VI) respectively. X-ray fluorescence spectrometric determination of Cr(VI) after aliquat 336-AC assisted solid phase extraction was carried out by De Vito et al. [26] which required no elution process. Ghaedi et al. [27] reported a new Cr(III) selective electrode based on 1-[(2-hydroxyethyl) amino]-4-methyl-9H-thioxanthen-9-one as a neutral carrier with detection limit of 1.6×10^{-7} mol L^{-1}. Despite having good limits of detection and wide linear ranges, most of these techniques necessitate the use of sophisticated and costly apparatus and complicated operational procedure. Recently, the

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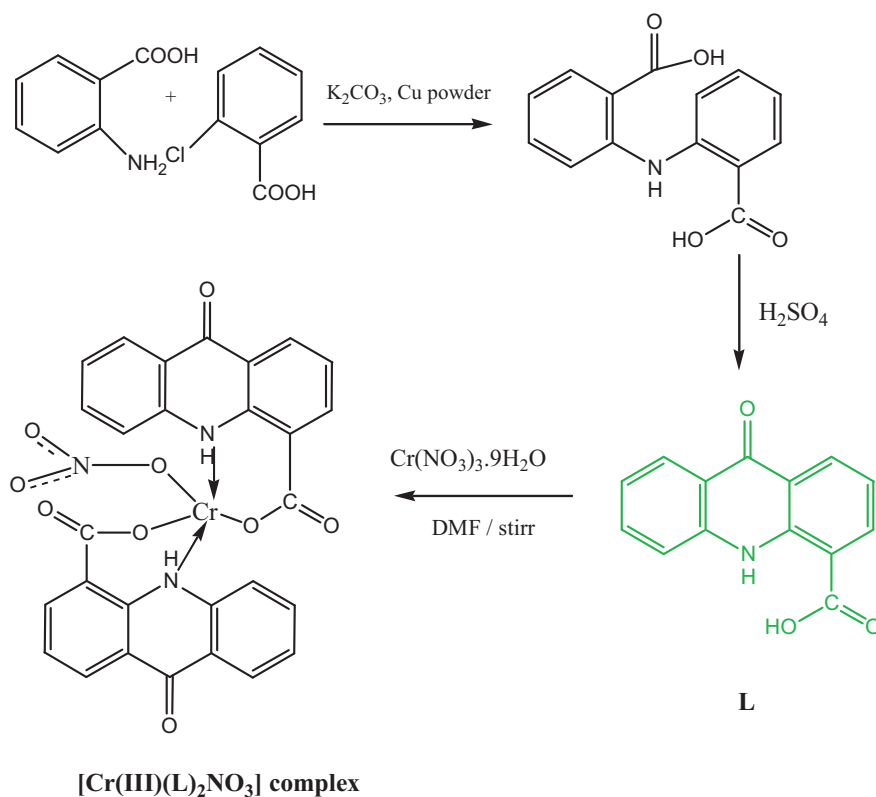
Scheme 1. Synthesis of the fluorescent chemosensor and its Cr(III) complex.

fluorescent method has become very popular due to its operational simplicity, high selectivity, sensitivity, rapidity, nondestructive methodology and direct visual perception [28]. For an efficient fluorescent sensor, in addition to high selectivity towards the ion of interest, a significant change in the fluorescence intensity in presence of the ion and /or a spectral change are required [29,30]. Although, recently a few Cr(III) selective fluorescent sensors [31–33] have been reported but report on trace level speciation and estimation of chromium species without separation of individuals by fluorescence quenching technique are rare [34]. Herein, we report the use of 9-acridone-4-carboxylic acid as an efficient and selective fluorescent sensor for Cr speciation. The developed method is very fast, simple, and inexpensive. The binding of the reagent with Cr(III) is firmly established by the ESI-MS (+) technique and supported by FTIR spectroscopy and binding constant measurement using Stern–Volmer method [35].

2. Experimental

2.1. Materials

2-Chloro benzoic acid (Alfa Aesar, India) and anthranilic acid (SRL, India) were purchased and used as received. All other chemicals and solvents were of analytical grade and used without further purification. Milli-Q 18.2 MΩ cm^{-1} conductivity purification system (Bedford, MA, USA) water was used throughout all the experiments. Cr(III) and Cr(VI) stock solutions were prepared from Cr(NO$_3$)$_3$·9H$_2$O and K$_2$CrO$_4$ (Merck, Dramstadt, Germany) respectively. The solutions 50 mg L^{-1} for Cr(III) and 5 mg L^{-1} for Cr(VI) were prepared in deionised water respectively. These solutions were standardized against standard stock solutions of Cr(III) (1000 mg L^{-1}) supplied by SOLUTIONS plus inc. (Missouri, USA) which were tested $vs.$ NIST SRM # 3108a using AAS. The working solutions of Cr(III) and Cr(VI) were prepared by successive dilution

of the stock solutions. The sources of Na$^+$, K$^+$, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cr^{3+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+} and Pb^{2+} ions are either their chloride or nitrate salts.

2.2. Apparatus

Absorption and fluorescence spectra were recorded on Shimadzu Multi Spec 1501 absorption spectrophotometer and Hitachi F-4500 fluorescence spectrophotometer, respectively. Mass spectrum was recorded in QTOF Micro YA 263 mass spectrometer in ESI positive mode. IR spectra were recorded on a JASCO FTIR spectrophotometer (model: FTIR-H20). Thermogravimetric analysis was performed on a Perkin Elmer TG/DTA lab system l (Technology by SII). A VARIAN (Spectra AA 55) flame atomic absorption spectrophotometer (FAAS) (Australia) was used for measuring concentration of Cr(III) in the isolated Cr complex to confirm the structure of the L–Cr(III) complex. All measurements were performed using integrated absorbance (peak area). Hollow cathode lamp for Cr was operated at 7.0 mA at wave length 357.9 nm and at a slit width of 0.2 nm. Air and acetylene flow rates were maintained at 3.5 and 1.5 L min^{-1} respectively.

2.3. Synthesis of the ligand (L)

Scheme 1 shows the Ullmann condensation of 2-chlorobenzoic acid and 2-aminobenzoic acid followed by cyclization in the presence of sulfuric acid, produced 9-acridone-4-carboxylic acid [36].

2.4. Synthesis and Isolation of Cr(III) complex with L (Scheme 1)

DMF solution of Cr(III) (55.6 mg, 0.139 mmol of Cr(NO$_3$)$_3$·9H$_2$O in 5 mL DMF) was added to a methanolic solution of L (100 mg, 0.418 mmol of L in 10 mL methanol) dropwise, and the mixture was stirred for 1 h (Scheme 1). The green reaction mixture
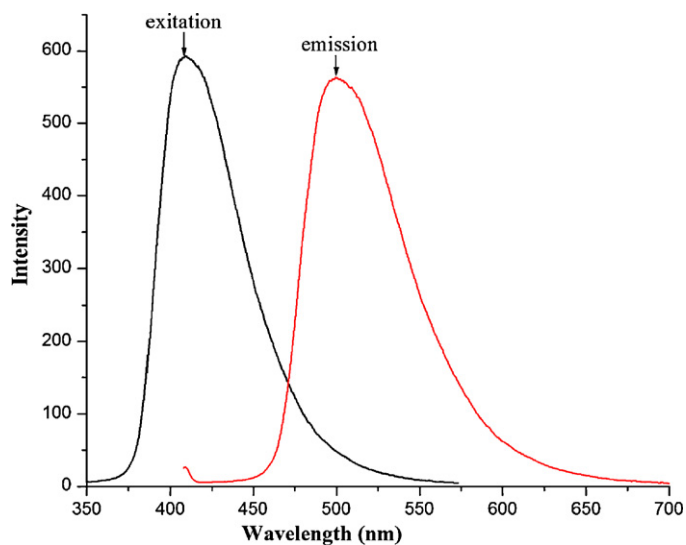
Fig. 1. Excitation and emission spectra of L (1 μM).

Fig. 2. Changes of the fluorescence spectra of L (1 μM, $\lambda_{ex} = 408$ nm, $\lambda_{em} = 498.4$ nm) in DMF/water, 9/1 (v/v) as a function of added [Cr^{3+}].

was kept for 3 days to obtain green crystalline compound which was characterized by ESI-TOF mass spectra and atomic absorption spectrometric analysis of chromium. m/z (ESI TOF positive) for $C_{28}H_{16}$ CrN_3O_9: 590.03 (M); found: 613.03 [M + Na]$^+$ %Cr: Cr, 8.83 (calcd. 8.81). Selective FT-IR data (cm^{-1}): 1325 (ν_s NO$_2$); 1405 (ν_a NO$_2$), 1618 (ν CO).

2.5. Measurement procedures

A 1×10^{-6} mol L^{-1} solution of Cr^{3+} was obtained by serial dilution of the stock solution. A 10^{-6} mol L^{-1} stock solution of L was prepared by dissolving appropriate amount of L in DMF: water (9:1, v/v). The aforementioned solutions of Cr^{3+} and L were mixed in different ratios for subsequent fluorescence measurement. 1.00 cm quartz cell was used for fluorescence measurement.

Thermal studies of the ligand and its Cr(III) complex was also performed to prove the binding event of the ligand with Cr(III).

3. Results and discussion

3.1. Spectral characteristics

Fig. 1 shows the excitation ($\lambda = 408$ nm) and emission ($\lambda = 498.4$ nm) spectra of L. The value of Stokes shift ($\vartheta_A - \vartheta_F$), which is an important parameter indicating the difference in properties and structure between the ground state (S$_0$) and the first excited state (S$_1$) of 9-acridone-4-carboxylic is 4450 cm^{-1}. Upon addition of Cr^{3+} ion, quenching of the fluorescence intensity at 498.4 nm occurs. The changes in the fluorescence emission intensities of L (1×10^{-6} mol L^{-1}) as a function of added [Cr^{3+}] (1×10^{-6} mol L^{-1}–1.8×10^{-5} mol L^{-1}) are presented in Fig. 2. The plot of fluorescence intensities *vs* externally added [Cr^{3+}] (Fig. 3) reveals that after a certain amount of externally added Cr^{3+}, there is no further change in the emission intensity of the system. Up to 9 times (9×10^{-6} mol L^{-1}) of the externally added [Cr^{3+}], we observed linearity. So, by making use of this linear relationship (inset of Fig. 3), one can easily find out the concentration of any unknown Cr(III) species in aqueous solution. If we go to the lower limit of added [Cr^{3+}] the change of fluorescence intensity is detectable upto 0.2 times of L (1×10^{-6} mol L^{-1}) i.e. 2×10^{-7} mol L^{-1} added Cr^{3+}. ESI-TOF mass spectra (Fig. 4), confirmed the structure of the

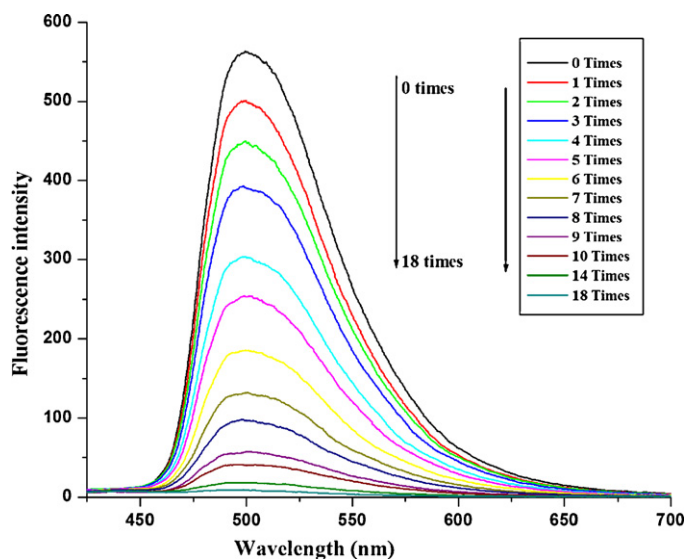Cr(III)–L complex as [Cr(III)(L)$_2$(NO$_3$)]. Fig. 5 supported the 2:1 stoichiometry of ligand (L) and Cr(III) complex [37].

3.2. Calculation of binding constant

The binding constant of the ligand L with Cr^{3+} is 8.1378×10^4 (Fig. 6) as calculated using Stern–Volmer equation, $I_0/(I_0 - I) = 1/A + 1/KA \cdot 1/[Q]$ where, I_0 is the fluorescence intensity of free ligand L, I is the fluorescence intensity of the L–[Cr^{3+}] complex, Q is [Cr^{3+}], A is constant and K is binding constant.

3.3. Selectivity

The selectivity behavior is obviously one of the most important characteristics of an ion-selective chemosensor, which is the relative optode response for the primary ion over other ions present in the solution. Thus, the influence of a number of common metal ions on the fluorescence intensity of the proposed
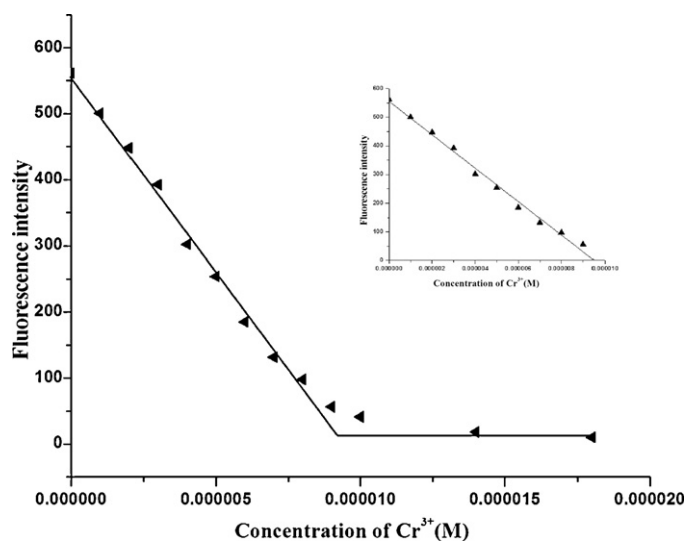

Fig. 3. Fluorescence intensity *vs* [Cr^{3+}], inset shows that upto 9 times [Cr^{3+}] linearity is sustained.
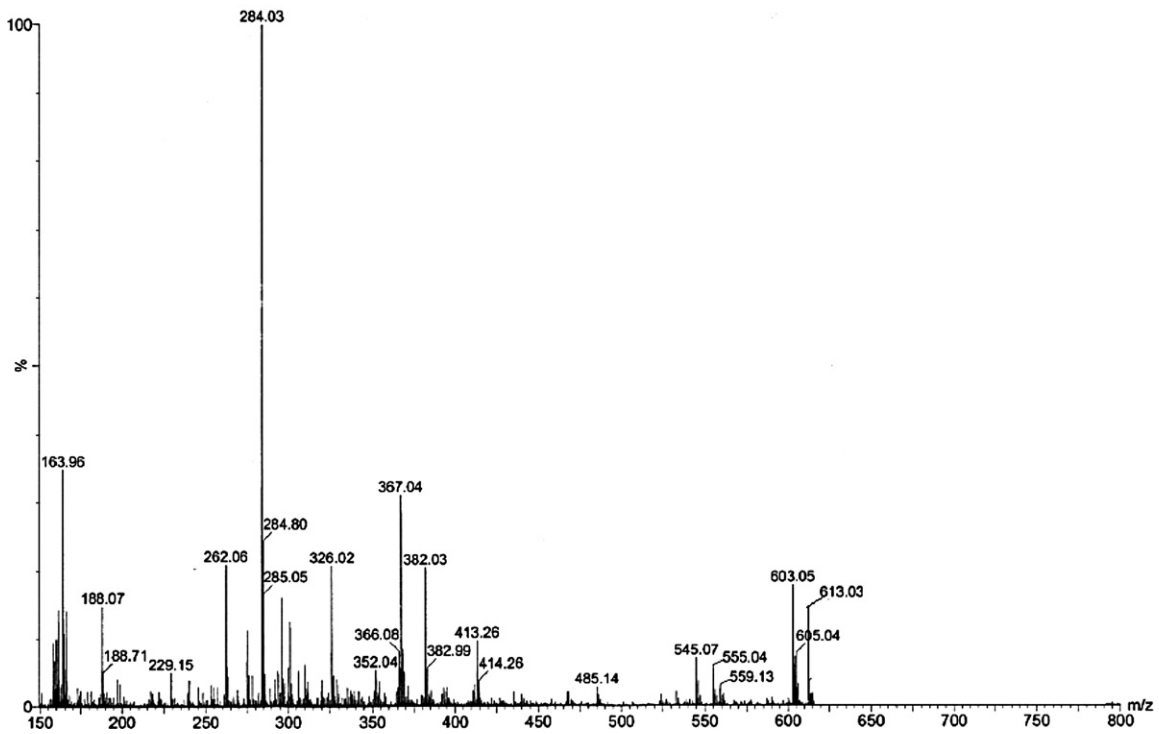
Fig. 4. ESI-TOF mass spectra of the Cr(III) complex with the fluorescent chemosensor.

Cr^{3+} chemosensor was investigated. In Fig. 7 effect of foreign metal ions on the fluorescence intensity of the L–Cr^{3+} system is presented. Increase in the fluorescence intensity compared to the L–Cr^{3+} system upon addition of foreign cations is designated as positive interference and the reverse phenomenon is designated as negative interference. In this study, $[L] = 10^{-6}$ mol L^{-1}, $[Cr^{3+}] = 10^{-6}$ mol L^{-1} and the foreign metal ions are present 10 times of the $[Cr^{3+}]$ i.e. 10^{-5} mol L^{-1}. It is observed from Fig. 7 that Na^+, K^+, Mg^{+2}, Ca^{2+}, Cd^{2+}, Zn^{2+}, Hg^{2+}, Mn^{2+}, Cu^{2+}, Fe^{3+}, Co^{2+}, Pb^{2+}, Ni^{2+} show insignificant positive interferences. Common anions including oxalate, dithionite and dithionate have no interference on the fluorescence intensity of the ligand or L–Cr(III) complex.
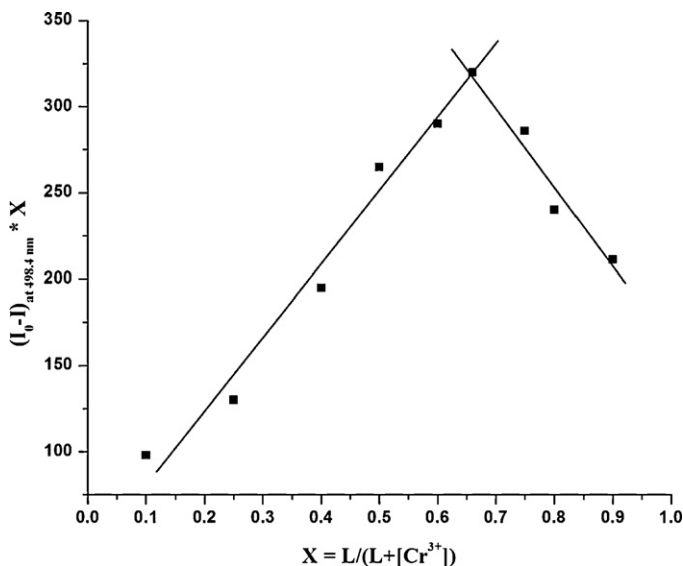
3.4. Thermal studies

Stability of the ligand, L and its Cr(III) complex was studied by thermogravimetry (TGA / DTG) to prove the binding event of the ligand, L with Cr(III) ion. Results are presented in Figs. 8 and 9 respectively. It is clear from the graphs that thermal stability of the Cr(III) complex (up to 150 °C) is more than the free ligand (up to 100 °C)

3.5. Speciation studies

So far we have observed that Cr(III) can quench the fluorescence intensity of the **L** significantly and we can detect and
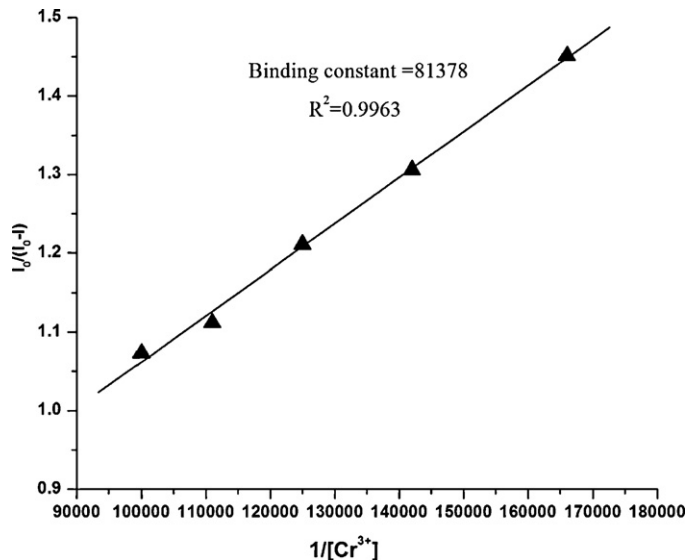

Fig. 5. Job's Plot to determine the stoichiometry of the Cr^{3+}–L complex in solution.

Fig. 6. Stern–Volmer plot for calculating binding constant of the chemosensor with Cr(III).
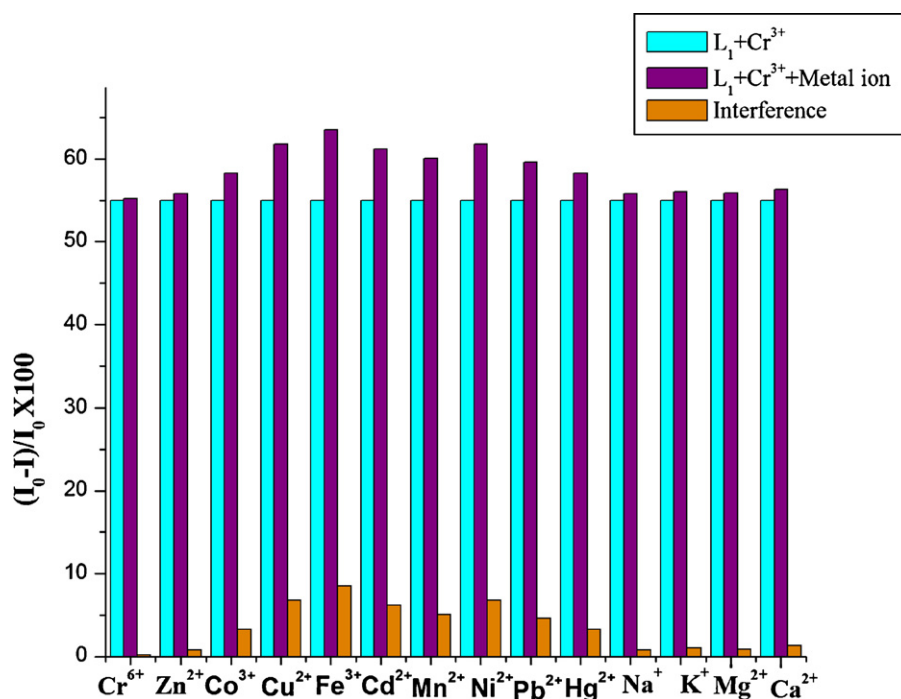
Fig. 7. Interference of foreign cations.

estimate trace level Cr(III) in aqueous solution. But Cr(VI) have no effect on the fluorescence intensity of the L. So we converted Cr(VI) to Cr(III) by using two different reducing agents, *viz.* oxalic acid and sodium dithionite. In our case oxalic acid worked better. We have already mentioned that oxalate or dithionate and dithionite have no adverse effect on the emission intensities of either free ligand or L–Cr(III) complex. So, slight excess of these species in the mixture will not be harmful to our methodology. Only thing, we have to do after oxalic acid assisted reduction of Cr(VI) to Cr(III), the pH of the medium should be neutral as we have observed diminished emission intensity of the lig-

and at acidic pH. Fig. 10 shows the changes of the fluorescence emission intensities of L (1×10^{-6} mol L^{-1}) with the addition of different concentrations of Cr(VI) (1×10^{-6}–6×10^{-6} mol L^{-1}) followed by equivalent amount of oxalic acid. It can be said that with increasing [Cr(VI)], proportionate amount of [Cr(III)] produced *in situ* which reacted with the L and consequently, emission intensities of the system decreased gradually. Thus, in a mixture of Cr(III) and Cr(VI), we can directly measure free [Cr(III)] and total [Cr(III)] (which is sum of free Cr(III) and Cr(III) produced from free Cr(VI) after reduction with oxalic acid). Difference will give free [Cr(VI)].
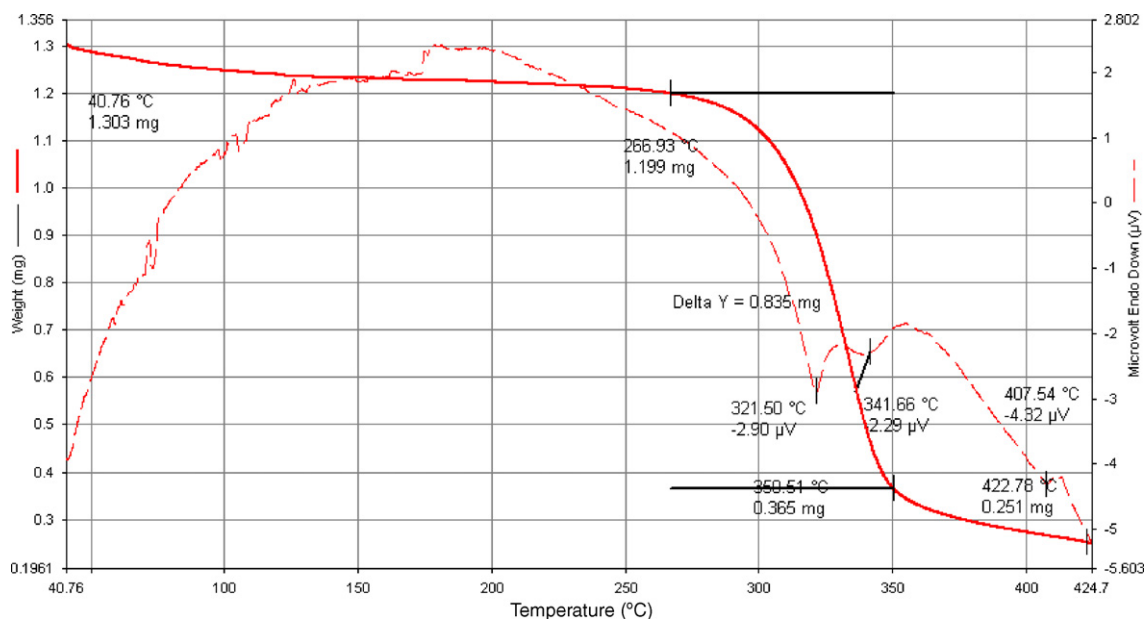

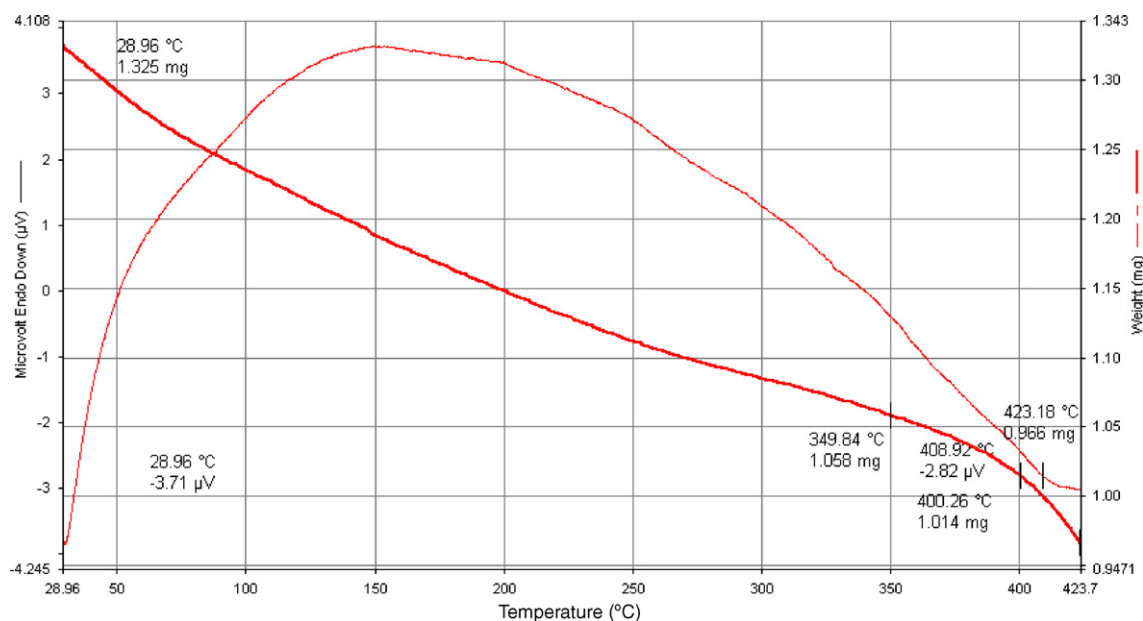**Fig. 8.** Thermal studies of the fluorescent chemosensor.

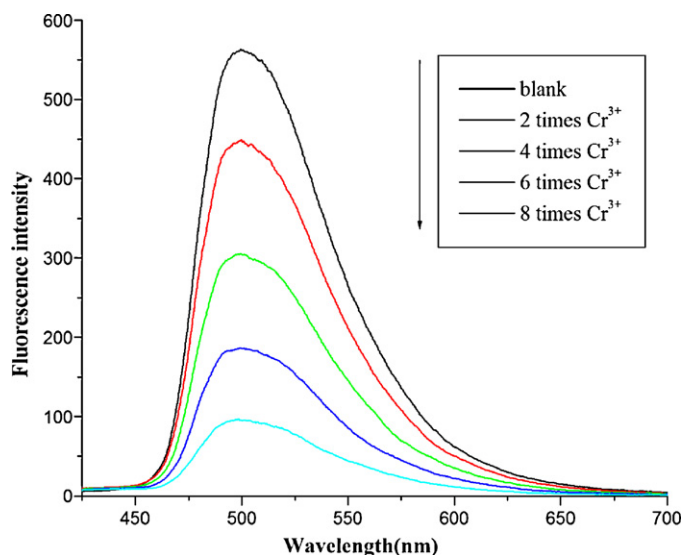**Fig. 9.** Thermal studies of the Cr(III) complex with fluorescent chemosensor.

Fig. 10. Changes of the fluorescence intensities of L (1 μM in DMF/water, 9/1 (v/v) as a function of converted [Cr^{3+}], obtained by the reduction of Cr(VI).

4. Applications

4.1. Estimation of Cr(III) and Cr(VI) from the binary synthetic mixtures

In different sets (each set in duplicate) different amounts of Cr(III) and Cr(VI) were mixed in a total volume of 100 mL. Direct estimation of Cr(III) was performed using our developed method. Reduction of Cr(VI) to Cr(III) by equivalent amount of oxalic acid was performed and total Cr(III) in the solution was estimated as mentioned above. The difference gave free Cr(VI) present in the solution. The results are presented in Table 1.

4.2. Real samples analysis

The waste water samples from different sources (three samples from tannery industrial area, Kolkata and three samples from Durgapur Industrial belt, West Bengal, India) were filtered through a 0.45 μm Milipore membrane filter. They were analyzed as described in the previous section. The results are compared with a reference method [38] and a good agreement is found between the two (t-test, P = 0.06). Results are presented in Table 2.

5. Conclusion

It has been found that 9-acridone-4-carboxylic acid can selectively detect trace level Cr^{3+} by fluorescence quenching method with negligible interference from few cations. The reagent pos-

Table 1
Separation of Cr(III) and Cr(VI) in binary synthetic mixtures.

No. of observations	Amount taken (μg)	Amount found (μg)	Error (%)
1	Cr(III)—50	Cr(III)—51 ± 0.2	1
	Cr(VI)—50	Cr(VI)—49.2 ± 0.06	0.8
2	Cr(III)—30	Cr(III)—29.6 ± 0.5	0.4
	Cr(VI)—50	Cr(VI)—48.2 ± 0.6	1.8
3	Cr(III)—15	Cr(III)—16.2 ± 0.1	1.2
	Cr(VI)—65	Cr(VI)—66.4 ± 0.03	1.4
4	Cr(III)—65	Cr(III)—64.1 ± 0.03	0.9
	Cr(VI)—25	Cr(VI)—26.2 ± 0.09	1.2
5	Cr(III)—30	Cr(III)—31.1 ± 0.5	1.1
	Cr(VI)—45	Cr(VI)—44.4 ± 0.7	0.6

Table 2
Level of Chromium species in environmental samples as determined by present method.

Sample no.	Present method		Reference method [26]	
	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)
1[a]	683.2 ± 0.2	595.9 ± 2	684.2 ± 1	598 ± 3
2[a]	571.6 ± 3	511.2 ± 4	570 ± 4	509 ± 5
3[a]	475 ± 1	417.2 ± 3	472 ± 3	415 ± 2
4[b]	63.3 ± 3	52.5 ± 1	62.3 ± 3	51.3 ± 1
5[b]	43.8 ± 3	44.2 ± 4	41.1 ± 2	43.2 ± 4
6[b]	35.8 ± 4	34.5 ± 5	33.5 ± 0.8	36.0 ± 5

[a] Tannery water.
[b] Industrial water.

sesses high binding affinity towards Cr(III) in aqueous-DMF solution which may be attributed to the affinity of hard binding sites (N, O) of acridone derivative towards hard cation, Cr(III). This fact helped us in Cr speciation without any cumbersome pre-separation process. Analysis of Cr species in different synthetic as well as environmental samples has been successfully performed.

Acknowledgement

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2011.01.110.

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