Application of Organized Media for Rapid Spectrofluorimetric Determination of Trace Amounts of Cr(VI) in the Presence of Cr(III)

Tayyebeh Madrakian,* Abbas Afkhami, and Masoumeh Mohammadnejad

*Faculty of Chemistry, Bu-Ali Sina University, Hamadan, Iran. *E-mail: madrakian@basu.ac.ir*
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A simple, selective and sensitive fluorescence quenching method was developed to the determination Cr(VI). The method is based on the oxidation of I^- to I_3^- by Cr(VI) in sulfuric acid solution followed by immediate formation of ion association compound between I_3^- and rhodamine 6G in Tween-80 micellar media at room temperature. The influence of several surfactants on rhodamine 6G fluorescence signal was studied; particular attention was paid in the aggregation behavior of rhodamine 6G–Tween-80 system. The experimental parameters (*e.g.*, type of surfactant, reagent concentration) were studied and the optimal conditions were established. The linear calibration graph was obtained in the range 2.0 - 100.0 ng mL^{-1} Cr(VI). The detection limit of the method was 0.37 ng mL^{-1}. The relative standard deviation (R.S.D.) is less than 5% (n = 5). The efficiency of the method for the determination of Cr(VI) in the presence of Cr(III) in the sample was investigated. The method was applied successfully to the determination of Cr(VI) and total Cr in water, and liver tissue samples.

Key Words: Spectrofluorimetry, Tween-80, Biological samples, Total Cr

Introduction

Chromium is the metallic element that exists primarily in the mineral chromate, which is present in soils, waters, rocks, fauna and gases. It occurs mainly as a result of human activities through the production of waste water in metal smelting, electroplating, tanning, metallurgy and mining. After processing, chromium occurs in several chemical species and it is not biodegradable in the environment.[1] Trace Cr can be found in different natural waters in two different oxidation states, Cr(III) and Cr(VI).[2] Cr(III) is an essential trace element state for humans and important for glucose tolerance factor,[3,4] but Cr(VI) is a potential carcinogenic agent, moreover, it is easy to absorb and cumulate in the organs[5] causing a variety of clinical problems such as an immediate cardiovascular shock and later effects on kidney, liver, and blood-forming organs. Therefore, many studies have been conducted to selectively determine the Cr(VI). Several methods for determination of Cr(VI) in different sample matrices have been developed. They are mainly spectrophotometry,[6-8] spectrofluorimetry,[9-11] electrometry,[12,13] atomic absorption spectrometry[14,15] and ion chromatography.[16] Some researches have focused on the extraction and detection of chromium species in natural waters.[17-19] In a spectrophotometric method Cr(VI) has been determined based on the formation of ion pair between I_3^- with a basic xanthene dye and decreasing the absorbance of dye after 5 min. Polyvinyl alcohol (an organic solvent) was used for stability of absorbance and avoiding the turbidity of solution.[20]

Jie *et al.* reported a fluorimetric method for the determination of Cr(VI) in the range 8.0 - 80.0 ng mL^{-1} based on the oxidation of rhodamine 6G by Cr(VI) in sulfuric acid solution in boiling water bath.[10] The amount of Cr(VI) was then determined by measuring the unreacted rhodamine 6G fluorimetrically at 545 nm.

The effect of organized media, such as surfactant micelles and cyclodextrins, have been shown to provide increased selectivity and lower detection limits in the spectrofluorimetric analyses of many compounds. Analysis performance is generally improved because the medium shields the analyte from quenchers or because inclusion in the medium increases the efficiency of a requisite interaction, as in the case of energy transfer.[21]

In this paper we propose a simple and rapid method for the determination of Cr(VI) based on the formation of the ion pair between a fluorescent dye, rhodamine 6G, and I_3^- produced from the oxidation of I^- with Cr(VI) in Tween-80 micellar media. Ion pair formation causes a decrease in the fluorescence intensity of rhodamine 6G.

Experimental

Apparatus. A Perkin-Elmer luminescence spectrometer model LS-30, equipped with a xenon lamp; a 7 μL fused silica flow cell and a peristaltic pump was used for recording spectra and fluorescence measurements.

Reagents. All chemicals were of the analytical grade purchased from Merck Company (Darmstadt, Germany). A stock solution of 100.00 mg L^{-1} Cr(VI) was prepared by dissolving 0.0270 g of $K_2Cr_2O_7$ in doubly distilled water and diluting to the mark in a 100 mL volumetric flask. A 1.00×10^{-4} mol L^{-1} of rhodamine 6G (Rh6G) was prepared by dissolving 0.0048 g of reagent in a 100 mL volumetric flask. A solution of 0.100 mol L^{-1} iodide was prepared by dissolving 0.8300 g of KI in water and diluting to the mark in a 100 mL volumetric flask. A 2.00 mol L^{-1} of H_2SO_4 solution and 0.5% (v/v) of Tween-80 solution were prepared.

Procedure. An aliquot of Cr(VI) solution was transferred into a 10 mL volumetric flask containing 1.0 mL of stock Rh6G solution, 0.1 mL of 0.5% Tween-80 solution and 1.1 mL of 2.0 mol L^{-1} H_2SO_4 solution. Then 0.3 mL of 0.1 mol L^{-1} iodide solution was added to the flask, the solution was mixed

completely and finally was diluted to the mark with water. The fluorescence intensity, F, was determined at λ_{em} 550 nm (with excitation at 530 nm). A blank solution was prepared in the same way except that distilled water was used instead of Cr(VI) solution and its fluorescence intensity, F_o, was measured. In the presence of Cr(VI), fluorescence intensity of Rh6G decreases, therefore the fluorescence quenching ($\Delta F = F_o - F$) was recorded as the analytical signal.

Preparation of liver sample. For determination of Cr(VI) in liver tissue, tissue was dissected by plastic knife and 0.1000 g of it, was soaked in 2 mol L^{-1} HNO_3 for 48 h, and rinsed five times with distilled water. Tissue sample was transferred into a 100 mL beaker, then the solution was heated at 200 °C on a hot plate for 3 h, until the solution evaporate slowly to near dryness. Two milliliters of 1.0 mol L^{-1} HNO_3 was added to the residue and the solution was evaporated again on the hot plate. By repeating the additional digestion twice, all organic materials in each sample were completely digested. After cooling, 2.5 mL of HNO_3 was added to digested residue and was transferred to a 50 mL volumetric flask, then diluted to level with water.[24]

Results and Discussion

The high sensitivity of this fluorimetric method obtained in this investigation was resulted from the decrease in the fluorescence intensity of Rh6G solution by formation of ion-association complex of $[Rh6G^+][I_3^-]$. This ion-association complex is formed by using Rh6G and KI as reactants where I_3^- will be generated once an oxidative solute is added.[23]

$$Cr_2O_7^{2-} + 9I^- + 14H^+ \rightarrow 2Cr^{3+} + 3I_3^- + H_2O$$

$$I_3^- + Rh6G^+ \rightarrow [Rh6G^+][I_3^-]$$

The decrease in fluorescent intensity of R6G at 550 nm by formation of ion pair association in the presence of Tween-80 according the above reaction is proportional to the Cr(VI) concentration. Therefore the method can be used to the determination of Cr(VI).

Spectral characteristics. Figure 1 shows the fluorescence emission spectra for the Rh6G, in the optimal conditions in the absence and in the presence of different amounts of Cr(VI) at λ_{ex} 530 nm. As Fig.1 shows, addition of different amounts of Cr(VI) causes a decrease in the fluorescence intensity.

Optimization of the analytical procedure. To take full advantages of the procedures, the reagent concentrations and reaction conditions must be optimized. Various experimental parameters were studied in order to obtain optimized system. These parameters were optimized by setting all parameters to be constant and optimizing one each time.

Effect of type and concentration of surfactant. Several surfactants including anionic (sodium dodecyl sulfate), cationic (cetyltrimethylammounium bromide) and nonionic (Triton X-100, Triton X-114 and Tween-80) were tested in order to increase the sensitivity of the method by increasing interaction between Rh6G and I_3^- for fluorescence quenching. The quenching effect decreased in the presence of SDS because, like I_3^-, this surfactant forms ion pair with Rh6G. In the presence of

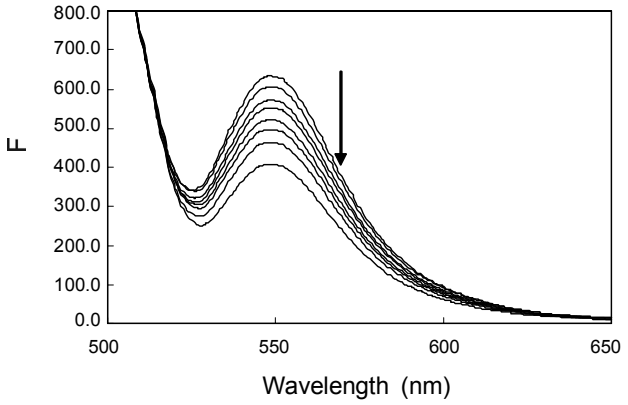

Figure 1. Fluorescence spectra for Rh6G system in the presence of different amounts of Cr(VI). From top to bottom: 0.0, 1.0, 5.0, 10.0, 30.0, 50.0, 70.0, 100.00 ng mL^{-1} Cr(VI). Conditions: 1.00×10^{-7} mol L^{-1} Rh6G, 0.44 mol L^{-1} H_2SO_4 and 3.00×10^{-3} mol L^{-1} iodide and 3.90×10^{-5} mol L^{-1} Tween-80. Excitation wavelength was 530 nm.

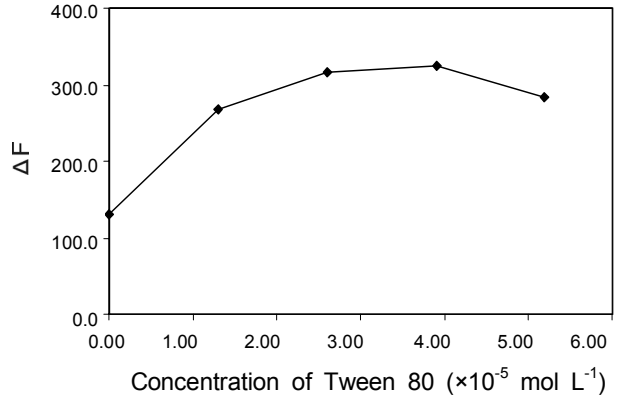

Figure 2. Effect of Tween concentration on the determination of Cr(VI). Conditions: 25.00 ng mL^{-1} Cr(VI), 3.00×10^{-4} mol L^{-1} iodide, 0.44 mol L^{-1} H_2SO_4 and 1.00×10^{-7} mol L^{-1} R6G solution.

CTAB the solutions became turbid after a few minutes. Addition of nonionic surfactants increased the fluorescence quenching (ΔF). Among the nonionic surfactants investigated, Tween 80 was found as the best. It caused the most sensitivity on the determination of Cr(VI). This can be due to the presence of more polyoxyethylene groups and longer alkyl chain (higher hydrophobisity and higher viscosity) in Tween 80 ($C_{64}H_{124}O_{26}$) with respect to Triton X-100 ($C_{14}H_{22}O(C_2H_4O)_n$) (n = 9 - 10) and Triton X-114 ($C_{14}H_{22}O(C_2H_4O)_n$) (n = 7 - 8).

The effect of Tween-80 concentration on the fluorescence quenching (ΔF) of the Rh6G was examined in the range 0.00 - 2.00×10^{-4} mol L^{-1}. The maximum fluorescence quenching occurred at 3.90×10^{-5} mol L^{-1} of this surfactant (Fig. 2).

Effect of reagent concentrations. The effect of Rh6G concentration was studied in the range 1.00×10^{-8} - 5.00×10^{-7} mol L^{-1} and a 1.00×10^{-7} mol L^{-1} was found as optimal.

The effect of iodide concentration on the determination of Cr(VI) was studied in the range 0.00 - 10.0×10^{-3} mol L^{-1}. Figure 3 shows the results. The amount of fluorescence quenching at 550 nm increased by increasing iodide concentration up to 2.00×10^{-4} mol L^{-1} and remained nearly constant at higher concentrations. Therefore, 3.00×10^{-4} mol L^{-1} of iodide was selec-

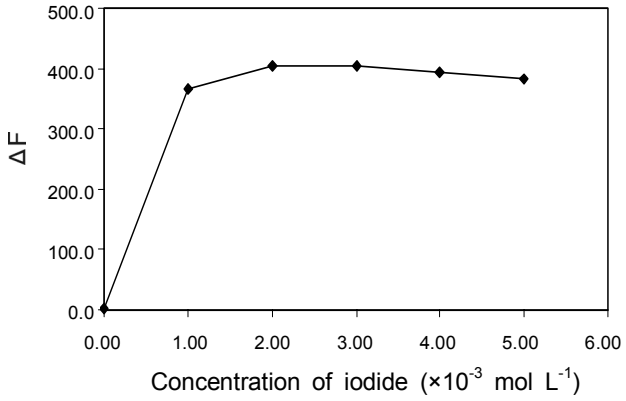

Figure 3. Effect of iodide concentration on the determination of Cr (VI). Conditions: 50 ng mL^{-1} Cr(VI), 0.40 mol L^{-1} H_2SO_4, 7.94×10^{-5} mol L^{-1} Tween-80 and 1.00×10^{-7} mol L^{-1} R6G solution.

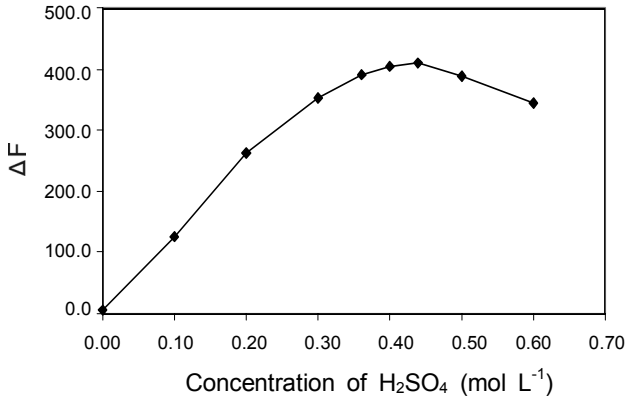

Figure 4. Effect of H_2SO_4 concentration on the determination of Cr(VI). Conditions: 50 ng mL^{-1} Cr(VI), 3.00×10^{-4} mol L^{-1} iodide, 7.94×10^{-5} mol L^{-1} Tween-80 and 1.00×10^{-7} mol L^{-1} R6G solution.

ted as optimum concentration.

The effect of the H_2SO_4 concentration on the fluorescence quenching was studied in the range 0.00 - 0.60 mol L^{-1}. As Fig. 4 shows, the fluorescence quenching increased by increasing H_2SO_4 concentration up to 0.44 mol L^{-1} of H_2SO_4 and then decreased. Therefore 0.44 mol L^{-1} of H_2SO_4 solution was used in the proposed procedure.

Analytical parameters. The calibration graph for the determination of Cr(VI) was constructed under the optimum conditions described above. The linear calibration graph was obtained in the range 2.00 - 100.00 ng mL^{-1} of Cr(VI). The detection limit, based on three times the standard deviation of the blank, was 0.37 ng mL^{-1} of Cr(VI). The linear regression equation was $\Delta F = 1626.31C + 59.75$ with a correlation coefficient of 0.9982, where ΔF is the amount of fluorescence quenching and C is the Cr(VI) concentration in μg mL^{-1}.

Cr(VI) was measured in the presence of different concentrations of Cr(III). Cr(III) did not interfere on the determination of Cr(VI). Therefore, Cr(VI) can be determined in the presence of excess amounts of Cr(III). Cr(III) could be determined by the proposed method after oxidiation to Cr(VI) with MnO_4^- in the acidic media.[21] The excess oxidant is then removed by adding nitrite solution dropwise until decolorization of the pink solution. The calibration curve for Cr(III) was obtained after oxidation to Cr(VI). The linear regression equation was $\Delta F = 1663.12\ C + 59.99$ with a correlation coefficient of 0.9974. The linear range and detection limit for the determination of Cr(III) were the same as those for Cr(VI). The results show the ability of the procedure for determination of Cr(VI) in the presence of Cr(III), Cr(VI) and total Cr in the sample.

Table 1. The recovery of Cr(VI) measured from various synthetic samples

sample[a]	Cr (VI) concentration/ ng mL^{-1}		Recovery (%)	RSD (%) (n = 5)
	present	Foundb		
1	10.00	10.19	99.10-101.30	2.61
2	20.00	20.34	97.40-102.90	3.20

[a]Composition of samples: sample1(Cu(II) 0.410, Zn(II) 0.822, Cr (III) 0.207 μg mL^{-1}) and sample2(Zn(II) 0.621, Ni(II) 0.369, Ca(II) 3.840 μg mL^{-1}).[27] [b]Mean of five determinations.

Table 2. The application of the proposed method for determination of Cr(VI) in real samples.

Sample	Spiked or present in sample (ng mL^{-1})	Found[a] (ng mL^{-1})	Recovery (%)	RSD (%)
Tap water	0.00	ND[b]	–	–
	20.00	19.65 ± 1.17	98.2	5.96
Drinking water	0.00	ND	–	–
	10.00	10.09 ± 0.43	100.9	4.31
Spring water	0.00	ND	–	–
	10.00	10.26 ± 0.49	102.6	4.82
Liver sample	0.00	ND	–	–
	5.00	4.80 ± 0.24	96.0	5.13
	20.00	20.70 ± 1.26	103.5	6.10

[a]Mean of three determinations ± standard deviation, [b]ND: not detected.

Table 3. Determination of 5.0 ng mL^{-1} Cr(VI) in the presence of different amounts of Cr(III) and total chromium in real samples.

Sample	Cr(III)	Total Cr	Recovery (%)
	Added (ng mL^{-1})	Found a (ng mL^{-1})	
Tap water	0.00	4.80 ± 0.31	96.0
	5.00	10.12 ± 0.62	101.20
	10.00	15.44 ± 1.22	102.93
Liver sample	5.00	9.50 ± 0.53	95.00
	10.00	14.60 ± 0.95	97.33

[a]Mean of three determinations ± standard deviation.

Interference of foreign ions. The effect of diverse ions on the determination of 50.00 ng mL^{-1} of Cr(VI) was studied. The tolerance limit of an ion was taken as the maximum amount of the ion causing an error not greater than ± 5%. A 1000-Fold excess of K^+, Na^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Cr^{3+}, HCO_3^-, F^-, SO_4^{2-},CN^-, NO_3^-,PO_4^{3-}; 500-fold excess of Al^{3+}, Cu^{2+}, Mn^{2+}, CO_3^{2-}, $S_2O_8^{2-}$, Cl^-, Br^-; 100-fold excess Pb^{2+} and 10-fold excess of Ag^+ and Fe^{3+} did not interfere on the determination of Cr(VI).

The major interferents were some strong oxidants such as BrO_3^-, IO_3^-, MnO_4^- and NO_2^-, which oxidize I^- to I_3^- or Rh6G. The interfering effect of NO_2^- was successfully removed in the presence of 0.1 mol L^{-1} urea. The interfering effect of MnO_4^- could be removed by dropwise addition of dilute sodium nitrite and urea solution into the sample solutions.[23] By drop-

Table 4. Comparison of the proposed method with other methods

Analytical method	Dynamic range (ng mL^{-1})	Detection limit (ng mL^{-1})	Condition	Ref.
Spectrophotometry	0.0-200.0	-	Poly(vinyl alcohol) forstability of solution and avoiding from the turbidity of solution	18
Spectrofluorimetry	8.0-50.0	0.51	High temperature	23
Spectrofluorimetry	8.0-80.0	0.80	Boiling water bath for 5 min	10
Solid phase extraction/atomic absorption	0.0-250.0	7.70	-	24
Inductively coupled plasma mass spectrometer	1.0-5.0	0.50		28
Cloud point extraction and flame atomic absorption spectrometry	-	0.32	Extraction with Triton X-100	29
Inductively coupled plasma-atomic emission spectrometer	10.0-1000.0	37.0		30
Spectrophotometric flow injection	-	0.70	-	31
catalytic adsorptive stripping voltammetry	0.056-5.6	0.018	Use of polymeric resin for removing interference of organic matter	32
catalytic adsorptive stripping voltammetric	0.011-1.12	-	-	33
Ion Chromatography-ICP-MS	-	0.15	Use of chromatographic separation for removing interferents	35
Spectrofluorimetry	2.0-100.0	0.37	-	Proposed method

wise addition of nitrite solution, MnO_4^- was reduced (decolorize the pink solution), and Mn^{2+} and NO_3^- are produced which could be tolerated at quite high concentrations. The excess amount of NO_2^- was finally decomposed by urea:

$$MnO_4^- + 5NO_2^- + 6H^+ \rightarrow 2Mn^{2+} + 5NO_3^- + 3H_2O$$

$$2NO_2^- + 2H^+ + CO(NH_2)_2 \rightarrow CO_2 + 3H_2O + 2N_2$$

Applications. In order to evaluate the analytical applicability of the proposed method, it was applied to the determination of Cr(VI) in tap water, synthetic samples and a liver sample. The results for the determination of Cr(VI) in synthetic samples are shown in Table 1. The procedure was performed for the determination of Cr(VI) in tap, spring and drinking water samples. The tested water samples were found to be free from Cr(VI) and so synthetic samples were prepared by adding known amounts of Cr(VI) to them. Determination of Cr(VI) in the liver sample, prepared as described in experimental section, was performed and found to be free from Cr(VI), then different amounts of Cr(IV) were added to the liver sample and determination was done. The results are given in Table 2. For determination of total Cr in the samples, appropriate amounts of Cr(III) were added to real samples and analysis was done after oxidation procedure. Table 3 shows the results. The recoveries for the addition of different concentrations of Cr(III) to samples were in the range 95-103%. The results show that the proposed method is suitable for determination of total Cr in samples

Comparison of the method with some previously reported methods for determination of Cr(VI). A comparison between the proposed method with some previously reported methods for determination of Cr(VI) (Table 4) by spectrophotometry,[18,31] spectrofluorimetry,[10,25] flame atomic absorption spectrometry[26,29] and inductively coupled plasma mass spectrometery[28,30] indicates that the proposed method is simpler and provides a lower detection limit and/or a wider linear range. In addition some different methods such as voltammetry,[32-34] Ion chromatography[35] and x-ray fluorescence[36] were reported for determination of Cr with lower detection limit that needs hard instrumentation and conditions.

Conclusions

This method is applicable to the determination of trace amounts of Cr(VI) in the presence of Cr(III) in synthetic, water and liver tissue samples with a detection limit down to 0.065 μmol L^{-1}. The proposed method has the advantage of the simplicity, reproducibility and rapid operation. By oxidation of Cr(III), the proposed method is able to determine Cr in two different oxidation states.

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