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Simple and rapid spectrophotometric determination of trace level chromium using bis (salicylaldehyde) orthophenylenediamine in nonionic micellar media

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Bis(salicylaldehyde)orthophenylenediamine (BSOPD) has been proposed as new analytical reagent for the direct spectrophotometric determination of chromium. It reacts with chromium(VI) in slightly acidic (0.1 – 0.3 M H_2SO_4) micellar medium to form a yellow - orange chelate with a molar ratio 2:3. The most remarkable point of this method is that the molar absorptivities of the Cr^{VI} – BSOPD complex formed in the presence of nonionic TritonX – 100 surfactant are almost 10 times higher than the value observed in aqueous solution. This results in enhancing the sensitivity and selectivity of the method. The reaction is instantaneous and the maximum absorbance was obtained at 482 nm and remains constant for over 24h. The average molar absorption coefficient and Sandell's sensitivity were found 3.5×10^5 L mol^{-1} cm^{-2} and 5 ng cm^{-2} of Cr(VI), respectively. Linear calibration graphs were obtained for 0.01 – 12.0 mgL^{-1} of Cr(VI) with correlation coefficient value 0.9987 for Cr – BSOPD complex. Large excess of over 50 cations, anions and complexing agents do not interfere in the determination. The method was successfully used in the determination of chromium(VI) from synthetic mixture and certified reference materials to test the validity of the method and the results of analyses were found to be in excellent agreement with those of certified values. The developed method was also used for the determination of chromium in some environmental waters (potable and polluted), biological samples (blood and urine) and to determine chromium species. The results of the biological analyses by the proposed method were in good agreement with those of by AAS.

Key Words: Micellar spectrophotometry, chromium determination, Bis(salicylaldehyde)orthophenylenediamine, environmental and biological samples

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Introduction

Chromium plays a dual role in human biochemistry as in trace amounts it is an essential nutrient, while large amounts are toxic and carcinogenic.[1] The essentiality and toxicity of chromium depend on its oxidation states or the forms in which it was supplied. Trivalent chromium is an essential trace element for humans. Together with insulin it removes glucose from the blood, and it also plays a vital role in fat metabolism; chromium deficits may exacerbate diabetes symptoms and heart conditions.[1] Chromium is also found in RNA. Furthermore, hexavalent chromium is known for its negative health and environmental impact, and its extreme toxicity. It causes allergic and asthmatic reactions, is carcinogenic, and is 1000 times as toxic as trivalent chromium. Health effects related to hexavalent chromium exposure include diarrhea, stomach and intestinal bleedings, cramps, and liver and kidney damage.[1] Chromium salts are used extensively in industrial processes and may enter the water supply through the discharge of wastes. Chromium may exist in water supplies in both the hexavalent and the trivalent state although the trivalent form rarely occurs in potable water.[2] Steel works, chrome-plating, and leather tanning industries produce large amounts of chromium. Most countries apply a legal limit of 50 μg L^{-1} of Cr in drinking water. Understanding the behavior of chromium in natural aquatic system is, therefore, of major concern.[3] That is why the determination of chromium(VI) in the environment is of great concern.

A simple sensitive and selective method for determination of trace chromium(VI) has always been required. However, some sophisticated techniques, such as inductively coupled plasma- mass spectrometry (ICP-MS),[4] inductively coupled plasma-atomic emission spectrometry (ICP-AES),[5] electrochemical analysis,[6] spectrophotometry,[7] neutron activation analysis,[8] and atomic absorption spectrophotometry (AAS),[9] are reported for sensitive assays for both species of chromium. These methods are disadvantageous in terms of cost and instruments used in routine analysis; AAS often lacks sensitivity and is affected by matrix conditions of samples such as salinity.[22] 1,5-Diphenylcarbazide is the traditional reagent in the spectrophotometric determination of chromium(VI),[10] but the sensitivity of the method is poor (mg L^{-1}) and the complex is unstable. Spectrophotometric methods for determination of chromium(VI)[11–28] reported so far are listed in Table 1 along with their analytical figures of merit.

In the search for a more sensitive reagent, in this work a new reagent bis(salicylaldehyde)orthophenylenediamine(BSOPD) was synthesized according to the method of Sacconi[29] and a color reaction of BSOPD with Cr(VI). Aqueous micellar media, which are reported to increase the molar absorptivities of spectrophotometric analysis, are also studied to observe the effect of the micellar media on the determination of Cr(VI) using BSOPD as derivatizing reagent.

Experimental

Instrumentation: A Perkin Elmer (Germany) (Model: Lambda-2) double-beam UV/VIS spectrophotometer with 1-cm matched quartz cells was used for all absorbance measurements. A pH-meter, WTW inolab (Germany) (Model: Level-1), with combined electrodes was employed for measuring pH values. A Hitachi Ltd., Model: 180-50, S.N.5721-2 atomic absorption spectrophotometer with a deuterium lamp background corrector, equipped with graphite furnace GA-3, with chromium hollow cathode lamps of Hitachi, and a Hitachi Model: 056 recorder was used for comparison of the results. The experimental conditions were as follows: slit width:

Table 1. Review of reagents for spectrophotometric determination of chromium.

Reagent	$\lambda_{max/nm}$	ϵ ($Lmol^{-1}cm^{-1}$)	Beer's Law mg L^{-1}	Remarks	Ref.
O-chlorophenylfluorone (0-CI-PF) and tetradecylpyridinium chloride (TDPC)	445	1.2×10^5	0.03-0.36	1. Time & temp. dependant 2. Reagent blank has high absorbance. 3. Nitrocellulose membrane was used.	11
N-Hydroxy-N,N'-Diphenylbenzamidine (HDPBA)	395	1.4×10^4	0.2-3.6	1. Solid phase extraction was used 2. Reagent blank has high absorbance.	12
Chromotropic acid	355	1.76×10^4	0-1	1. Time & temp. dependant 2. UV – range.	13
Propylene carbonate	362	1.95×10^3	0-100	1. Less sensitive. 2. Solid phase extraction. 3. UV – range.	14
Trifluoperazine hydrochloride (TFPH)	505	2.08×10^4	0.2-1.8	1. Based on oxidation of (TFPH). 2. Color stable for 2 h.	15
Diphenylcarbazide citrate	540	3.32×10^4	0.03-2	1. Color stable for 1 h. 2. Mg^{+2}, Ca^{+2}, Cs^{+}, Cu^{+2} & Mo^{VI} interfere seriously	16
Cationic yellow 42(CY42)	430	3.8×10^4	0-2	1. Indirect extraction method 2. Color stable for 5 h.	17
Cyanine dye	560	3.6×10^5	0.0-2.1	1. Sensitive but lengthy 2. Solid phase extraction was used	18
Iodonitrotetrazolium chloride (INT)	250	3.7×10^4	-------	1. UV range. 2. Hg interfered seriously.	19
Nitrotetrazolium Blue	260	8.2×10^4	0.01-0.4	1. Solvent extractive. 2. UV range.	20
Leuco xylene cynaol FF	615	8.23×10^4	0.05-0.45	1. Time & temp. dependent. 2. Interferences of Ce^{VI} & Mn^{VII} overcome by extraction of Cr^{VI}.	21
1,4,8,11-tetraazacyclotetradecane (cyclam)	379	1.5×10^4	0.2-20	1. Time & temp. dependant 2. UV range	22
Vaiamine Blue	615	8.2×10^3	0.0003-15	1. Less sensitive. 2. Cu^{II} and Ce^{IV} interfere seriously	23
Prochlorperazine dimaleate	535	2.09×10^4	0.2-2	1.Color stable for 2 h only 2.Less selective	25
Perphenazine	526	1.87×10^4	Up to 0.4	1. Absorbance was stable for 30 min only. 2. Less sensitive	26
Iodonitrotetrazolium chloride and Tetrazolium Violet	250 230	8.8×10^4 12.2×10^4	------- -------	1. UV range. 2. Hg interfered seriously. 3. Organic solvent was used.	27
Ferron	510	-------	5-70	1. Solvent extractive method. 2. Carcinogenic chloroform solvent was used.	28
Bis (salicylaldehyde)-orthophenylenediamine	482	3.5×10^5	0.01-12	1. Highly selective 2. Highly sensitive 3. Aqueous reaction medium 4. Less toxic surfactant 5. Color stable for 24 h at ±25 °C	Present method

1.3 nm; lamp current: 7.5 mA; wavelength: 357.9 nm; cuvette: cup; carrier gas (argon): 200 mL min^{-1}; sample volume: 10 μL.

Chemicals and reagents

All chemicals/solvents used were of analytical reagent grade or the highest purity available. Doubly distilled de-ionized water, which is non-absorbent under ultraviolet radiation, was used throughout. Glass vessels were cleaned by soaking in acidified solutions of $KMnO_4$ or $K_2Cr_2O_7$, followed by washing with concentrated HNO_3 and rinsed several times with de-ionized water.

Standard Chromium(VI) solution: A stock solution (1000 mg L^{-1}) was prepared by dissolving 0.565 g of $K_2Cr_2O_7$ in 100 mL of water. The stock solution was further diluted as needed.

Standard Chromium(III) solution: A stock solution (1000 mg L^{-1}) was prepared by dissolving 0.304 g of chromium(III) chloride in 100 mL of water. The stock solution was further diluted as needed.

Bis(salicylaldehyde)orthophenylenediamine(BSOPD)(1.58 $\times$ $10^{-3}M$) :The reagent was synthesized according to the method of Sacconi[29] and the structure of the reagent is shown in Figure 1. The solution was prepared by dissolving the requisite amount of BSOPD in a known volume of doubly distilled ethanol (Merck, Darmstadt, Germany). More dilute solution of the reagent was prepared as required.

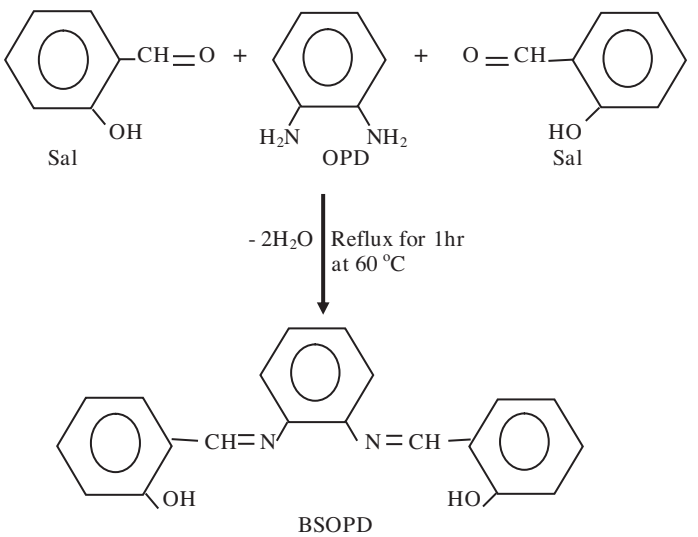

Figure 1. Synthesis of bis (salicylaldehyde) orthophenylenediamine (BSOPD).

Polyoxyethylene octylphenyl ether (TX-100) (10%): A 500 mL TX-100 solution was prepared by dissolving 50 mL of pure polyoxyethylene-octylphenyl ether (E. Merck, Darmstadt, Germany) in 250-300 mL in doubly distilled de-ionized water, sonicated for 15 min, and diluted up to the mark with de-ionized water when it became transparent.

Aqueous ammonia solution: A 100 mL solution of aqueous ammonia was prepared by diluting 10 mL of concentrated NH_3 (28%-30%) ACS grade to 100 mL with de-ionized water. The solution was stored in a polypropylene bottle.

EDTA solution: A 100 mL stock solution of EDTA (0.1% w/v) was prepared by dissolving 128 mg of ethylenediaminetetraacetic acid, disodium salt dehydrate (Merck) in (100 mL) de-ionized water.

Other solutions: Solutions of a large number of inorganic ions and complexing agents were prepared from their AnalaR grade, or equivalent grade, water-soluble salts. In the case of insoluble substances, a special dissolution method was adopted.[30]

General procedure

A series of standard solutions of a neutral aqueous solution containing 0.1-120 μg of chromium(VI) in a 10 mL calibrated flask was mixed with 40- to 165-fold molar excess of the BSOPD solution (preferably 1.0 mL of 1.58 $\times$ 10^{-3} M) BSOPD reagent, 0.5-3.5 mL (preferably 1 mL) of 10% TX-100 solution, and 1-3 mL (preferably 1 mL) of 1M H_2SO_4. The mixture was diluted to the mark with de-ionized water. After standing for 1 min the absorbance was measured at 482 nm against a corresponding reagent blank. The chromium content in an unknown sample was determined using a concurrently prepared calibration graph.

Results and discussion

The absorption spectra of brownish yellow color of the Cr(VI)-BSOPD system in a 1 M sulfuric acid medium were recorded using a spectrophotometer. The absorption spectra of the Cr(VI)-BSOPD is a symmetric curve with the maximum absorbance at 482 nm and an average molar absorption coefficient of 3.5 $\times$ 10^5 L mol^{-1} cm^{-1} in aqueous micellar media (Figure 2). The reagent blank exhibited negligible absorbance, despite having a wavelength in the same region. In all instances, measurements were made at 482 nm against a reagent blank.

Composition of the Complex: Job's method[31] of continuous variation and the molar-ratio method were applied to ascertain the stoichiometric composition of the complex. Cr-BSOPD (2:3) complex was indicated by the molar-ratio method (Figure 3).

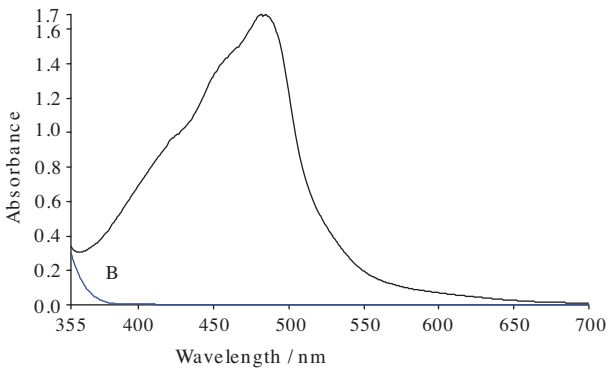

Figure 2. A and B absorption spectra of Cr(VI)-BSOPD system and the reagent blank (λ_{max} = 482 nm) in nonionic micellar media of polyoxyethylene-octylphenyl-ether (TX-100).

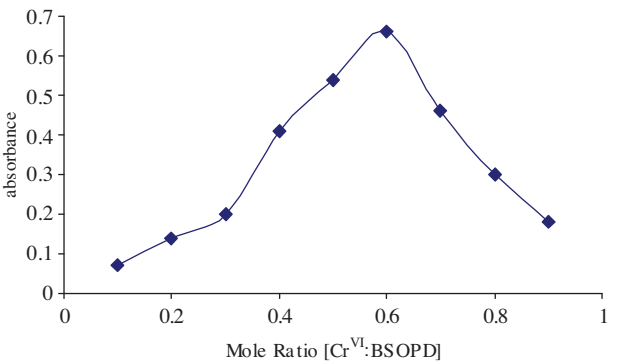

Figure 3. Composition of Cr(VI)-BSOPD complex by the mole ratio method in micellar media.

Optimization of some parameters on the absorbance

Effect of surfactant: Various surfactants [nonionic {polyoxyethylenedodecylether (Brij-35), polyoxyethylene sorbitan monopalmitate (Tween-40), polyoxyethylene sorbitan mono-oleate (Tween-80), Triton X-100}; cationic {cetyltrimethylammonium bromide (CTAB), cetylpyridinum chloride (CPC)}; and anionic {sodium dodecyl sulfate (SDS)}] were studied. In the 10% Triton X-100 medium, however, the maximum absorbance was observed; hence, this solution was used in the determination procedure.

Different volumes of 10% Triton X-100 were added to a fixed metal ion concentration, in a 10 mL volumetric flask, and the absorbance was measured according to the standard procedure. Effect of surfactant concentration was studied on the absorbance of 1.0 mg L^{-1} Cr-chelate complex, and 0.5-3.5 mL of 10% Triton X-100 produced a constant absorbance of the Cr-chelate. Outside this range of surfactant (i.e. 10%-35% of total volume) the absorbance decreased (Figure 4). The effect may be due to inadequate interactions of surfactant at lower concentrations, while the micellar dilution effect is responsible for decreases in the absorbance at higher surfactant concentrations.[32] For all subsequent measurements, 1 mL of 10% Triton X-100 (i.e. 20% of total volume) was added.

Effect of acidity: Of the various acids (nitric, sulfuric, hydrochloric and phosphoric) studied, sulfuric acid was found to be the best acid for the system. The absorbance was at a maximum and constant when a 10 mL of solution (1.0 mg L^{-1}) contained 1-3 mL of 1M (0.1-0.3 M) sulfuric acid (or pH 1.39-2.23) at room temperature (25 ± 5 °C) (Figure 5). For all subsequent measurements, 1 mL of 1M (i.e. 0.1M) sulfuric acid (or pH 1.39) was added.

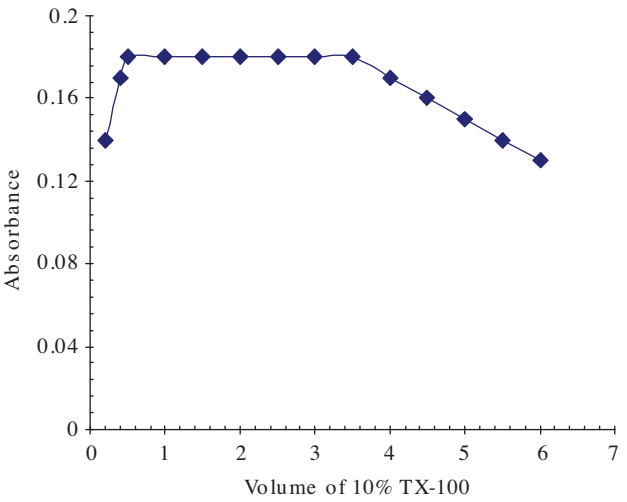

Figure 4. Effect of a surfactant on the absorbance of the Cr(VI)-BSOPD system.

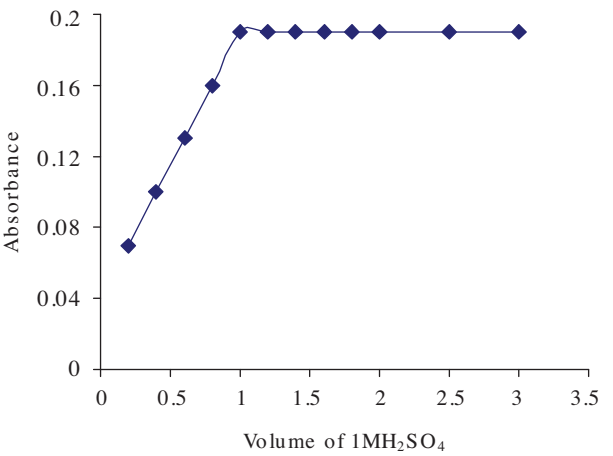

Figure 5. Effect of the acidity on the absorbance of the Cr(VI)-BSOPD system.

Effect of time: The reaction is fast and a constant maximum absorbance was obtained just after 1 min of the dilution to volume at room temperature (25 ± 5 °C), and remained strictly unaltered for 24 h.

Effect of temperature: The absorbance at different temperatures (0-40 °C) of a 10 mL solution (1.0 mg L^{-1}) was measured according to the standard procedure. The absorbance was found to be strictly unaltered throughout the temperature range of 10-40 °C. Therefore, all measurements were performed at

room temperature (25 ± 5 °C).

Effect of the reagent concentration: Different molar excesses of BSOPD were added to a fixed metal-ion concentration, and the absorbances were measured according to the general procedure. It was observed that at 0.5 mg L^{-1} Cr-metal (optical path length, 1 cm), reagent molar ratios 1:40 and 1:165 produced a constant absorbance of the Cr-chelate (Figure 6). The effect of reagent at different concentrations of Cr(VI) (1 mg L^{-1}) was also studied but a similar effect was observed. For all subsequent measurements, 1.0 mL of 1.58 × 10^{-3} M BSOPD reagent was added.

Analytical performance of the method

Calibration curve: The effect of metal concentration was studied over 0.01-100 mg L^{-1}, distributed in 4 different sets (0.01-0.1, 0.1-1, 1-10, 10-100 mg L^{-1}) for convenience of measurement. The absorbance was linear for 0.01-12 mg L^{-1} of chromium(VI) in aqueous surfactant media. From the slope of the calibration graph, the average molar absorption coefficient was found to be 3.5 × 10^5 L mol^{-1} cm^{-1} in aqueous micellar media. Of the 4 calibration graphs, the one showing the limit of the linearity range is given in Figure 7; the next 3 were straight-line graphs passing through the origin (R^2 = 0.9987). The selected analytical parameters obtained with the optimization experiments are summarized in Table 2.

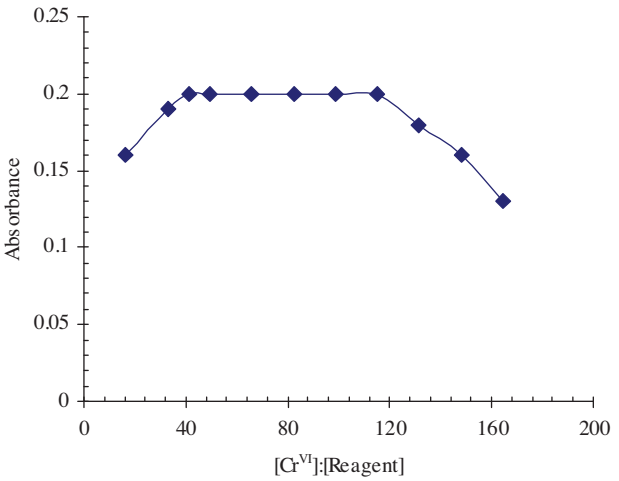

Figure 6. Effect of reagent [BSOPD:Cr(VI) molar concentration ratio] on the absorbance of Cr(VI)-BSOPD system in micellar media.

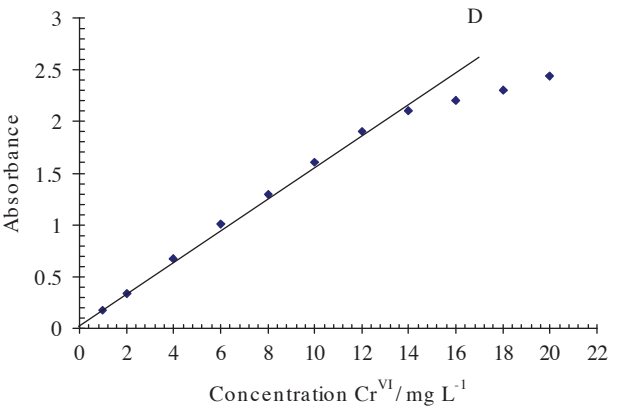

Figure 7. Calibration graph: D, 1-12 mg L^{-1} of Cr (VI).

Precision and accuracy: The precision of the present method was evaluated by determining different concentrations of chromium (each analyzed at least 5 times). The relative standard deviation (n = 5) was 2.5%-0% for 0.1-120 μg of Cr(VI) in 10 mL, indicating that this method is highly precise and reproducible. The detection limit (3s/S) and Sandell's sensitivity[33] (concentration for 0.001 absorbance unit) for Cr(VI) were found to be 1.5 μg mL^{-1} and 5 ng cm^{-2}, respectively. The method was also tested by analyzing several synthetic mixtures containing Cr(VI) and diverse ions as shown in Table 4. The results of the total chromium in a number of real samples were in good agreement with the certified values (Table 5). The reliability of

our Cr-chelate procedure was tested by recovery studies. The average percentage recovery obtained for the addition of a Cr(VI) spike to some environmental water samples and industrial effluent were quantitative, as shown in Table 6. The results of biological and geological analyses by the spectrophotometric method were in excellent agreement with those obtained by AAS (Table 7). Hence, the precision and accuracy of the method were excellent. With suitable masking, the reaction can be highly selective and the reagent blank does not show any absorbance. The method is very reliable, and a concentration in the μg g^{-1} range in aqueous medium at room temperature (25 $\pm$ 5 °C) can be measured in a very simple and rapid way.

Table 2. Selected analytical parameters obtained with optimization experiments.

Parameter	Studied range	Selected value
Wavelength, λ/nm	200-800	482
Acidity / M H_2SO_4	0.01-1.0	0.1-0.3 (preferably 0.1)
pH	0.93-2.23	1.39-2.23 (preferably 1.39)
Surfactant /10% TX-100/mL	0-7	0.5-3.5 (preferably 1.0)
Time / h	0-72	1 min-24 h (preferably 1 min)
Temperature / °C	0-70	10-40 (preferably 25 $\pm$ 5)
Reagent (fold molar excess, M : R)	1: 1-1:410 0	1:40-1:165 (preferably 1:80)
Linear range / mg L^{-l}	0.01-100	0.01-12
Molar absorption coefficient / L mol^{-1} cm^{-1}	2.3×10^5-3.8×10^5	3.5×10^5
Sandell's sensitivity / ng cm^{-2}	1-100	5
Detection limit / μg L^{-1}	1-100	1.5
Reproducibility (% RSD)	0-10	0-2
Correlation coefficient (R^2)	0.9945-0.9999	0.9987

Effect of foreign ions

The effect of over 50 cations, anions, and complexing agents on the determination of only 1 mg L^{-1} of Cr(VI) was studied. The criterion for interference[34] was an absorbance value varying by more than 5% from the expected value for Cr alone. There was no interference from the following 1000-fold amount of chlorides or sulfate; a 500-fold amount of EDTA, carbonate, bicarbonate, citrate, or tetra sodium pyrophosphate(TSPP); a 300-fold amount of acetate; a 200-fold amount of ascorbic acid, phosphate, or nitrate; a 100-fold amount of fluoride; or a 10-fold amount of azide. EDTA prevented the interference of 50-fold of ammonium(I), copper(II), or lead(II), and a 10-fold amount of iron(III) or tin(II). Interferences of 50-fold of manganese(VII) were completely removed by using citrate as masking agent. A 5-fold amount of gold(III) was completely removed by using azide as

masking agent. However, for those ions whose tolerance limit has been studied their tolerance ratios are given in Table 3.

Table 3. Table of tolerance limits of foreign ions.[a]

Species x	Tolerance ratio[b] x/Cr(VI)	Species x	Tolerance ration x/Cr(VI)
Acetate	300	Lanthanum(III)	100
Ascorbic Acid	200	Lead(II)	20[d]
Azide	10	Lithium	100
Bicarbonate	500	Iron(II)	50[d]
Carbonate	500	Iron(III)	10[c]
Chloride	1000	Manganese(VII)	50[f]
Citrate	500	Magnesium	100
Fluorides	100	Molybdenum(VI)	50
EDTA	500	Mercury (I)	100
Nitrate	200	Mercury(II)	100
Phosphate	200	Neodymium(III)	100
Sulfate	1000	Nickel (II)	100
TSPP	500	Potassium	100
Ammonium(I)	50[c]	Rhodium(III)	100
Aluminum(III)	100	Ruthenium(III)	100
Arsenic(III)	100	Selenium(IV)	100
Barium	30	Silver(I)	50
Beryllium(II)	100	Sodium	100
Bismuth(III)	50	Strontium	100
Cadmium(II)	100	Thallium(I)	100
Calcium	100	Tin(II)	10[c]
Cadmium(II)	100	Titanium(IV)	25
Chromium(III)	100	Tungsten(VI)	100
Cobalt(II & III)	100	Zirconium(IV)	100
Copper(II)	50[d]	Zinc	100
Gold(III)	5[e]		

[a]Tolerance limit defined as ratio that causes less than 5% interference.

[b]Tolerance ratio, [Species (x)] / Cr^{VI} (w/w)].

[c]With 100 mg L^{-1} EDTA.

[d]With 500 mg L^{-1} EDTA.

[e]With 10 mg L^{-1}azide.

Applications

The present method was successfully applied to the determination of chromium in a series of synthetic mixtures of various compositions (Table 4), and also in a number of real samples, e.g. several standard reference materials (Table 5). The method was also extended to the determination of chromium in a number of environmental, industrial effluent samples. In view of the unknown composition of environmental water samples, the same equivalent portions of each sample were analyzed for chromium content; the recoveries in both the "spiked" (added to the samples before the mineralization or dissolution) and the "unspiked" samples are in good agreement (Table 6).

Determination of chromium(VI) in synthetic mixtures

Several synthetic mixtures of varying compositions containing chromium(VI) and diverse ions of known concentrations were determined by the present method using EDTA as a masking agent; and the results were found to be highly reproducible. The results are shown in Table 4. Accurate recoveries were achieved in all solutions.

Table 4. Determination of chromium(VI) in some synthetic mixtures.

Sample	Composition of mixture / mg L^{-1}	Chromium(VI) / mgL^{-1}		Recovery ± s(%)
		Added	Found[a]	
A	Cr^{VI}	0.50	0.49	98 ± 0.5
		1.00	0.99	99 ± 1.00
B	As in A + Na (25) + Be^{2+} (25)	0.50	0.49	98 ± 1.5
		1.00	1.00	100 ± 0.0
C	As in B + Zn^{2+} (25) + Ni^{2+} (25) + EDTA(50)	0.50	0.52	104 ± 1.0
		1.00	1.02	104 ± 1.3
D	As in C + Co^{2+}(25) + Ca(25)	0.50	0.53	106 ± 1.5
		1.00	1.05	105 ± 1.6
E	As in D + Mn^{2+}(25) + Hg^{+2}(10)	0.50	0.54	108 ± 1.7
		1.00	1.04	108 ± 1.8
F	As in E + As^{3+}(25) + Cd^{2+}(25)	0.50	0.54	108 ± 1.9
		1.00	1.10	110 ± 2.0

[a] Average of 5 analyses of each sample

Determination of chromium in alloys and steels, soil, whole meal flour, and human hair (certified reference materials)

A 0.1 g amount of a alloy and steel in 50-mL, 0.5 g amount of soil, whole meal flour, or human hair sample was accurately weighed and placed in a 50-mL Erlenmeyer flask following a method previously recommended.[35] To this were added 10 mL of concentrated HNO_3 and 5 mL of concentrated HCl, carefully covering the flask with a watch glass until the brisk reaction subsided. The solution was heated and simmered gently after the addition of 5 mL of concentrated HNO_3, until all carbides were decomposed. The solution was evaporated carefully to

dense white fumes to drive off the oxides of nitrogen and then cooled to room temperature (25 ± 5 °C). After suitable dilution with de-ionized water, the contents of the Erlenmeyer flask were warmed to dissolve the soluble salts. The solution was then cooled and neutralized with a dilute NH_4OH solution. The resulting solution was filtered, if necessary, through a Whatman No. 42 filter paper into a 25-mL calibrated flask. The residue was washed with a small volume of hot water and the volume was made up to the mark with de-ionized water.

A suitable aliquot (1-2 mL) of the above solution was taken into a 10-mL calibrated flask and chromium content was determined as described under a procedure, using EDTA or citrate as masking agent. The results are shown in Table 5. The certified chromium value in alloys and steels, soil, whole meal flour, and human hair were obtained from a calibration graph. The results for total chromium were in good agreement with certified values (Table 5).

Table 5. Determination of chromium in certified reference materials.

Certified Reference Materials	Cr (mg kg^{-1})		Relative standard deviation (%)
	Certified value	Found (n = 5)	
BCR-185R (Bovine liver)	0.026 – 0.088	0.045	0.2
BCR-189 (Whole meal flour)	0.057	0.056	2.0
BCR-484 (Soil)	> 0.06 – 0.09	0.075	1.0
BCR-397 (human hair)	91	90	1.5

Table 6. Determination of chromium in some environmental water samples.

Sample		Chromium / μg L^{-1}		Recovery ± s (%)	s_r^b (%)
		Added	Found[a]		
Tap water		0.0	8.0		
		100	107.0	99 ± 0.2	0.35
		500	508	99.8 ± 0.3	0.38
River Water (Indus)		0.0	12.0		
		100	113.0	100.8 ± 0.5	0.31
		500	510.0	99.6 ± 0.4	0.28
Mancher Lake[c]			25.00		
		100	127.0	102 ± 0.5	0.19
		500	525.0	100 ± 0.0	0.00
Drain water	Aral wah[d]	0.0	45.00		
		100	143.0	98.6 ± 0.6	0.27
		500	550.0	100.9 ± 0.8	0.34
	Fiber tex[e]	0.0	75.00		
		100	180	102.8 ± 1.0	0.35
		500	585.0	102 ± 0.90	0.37

[a] Average of 5 replicate determinations.

[b] The measure of precision is the relative deviation (s_r).

[c] The Manchar Lake, Dadu, Sindh.

[d] Aral wah, Dadu, Sindh.

[e] Kotri, Hyderabad.

Table 6a. Determination of chromium in some industrial water samples.

Samples	Chromium / μg L^{-1}		Recovery ± s (%)	s_r^b (%)
	Added	Found[a]		
1[c]	0.0	75.0		
	100	172.0	98 ± 0.3	0.31
	500	580.0	100.8 ± 0.5	0.35
2[d]	0.0	65.0		
	100	168.0	98 ± 0.5	0.30
	500	565.0	100 ± 0.0	0.00
3[e]	0.0	96.0		
	100	200.0	98 ± 0.6	0.21
	500	595.0	100 ± 0.4	0.10
4[f]	0.0	87.0		
	100	190.0	98.4 ± 0.7	0.45
	500	592.0	99 ± 0.5	0.29
5[f]	0.0	125.0		
	100	230.0	98 ± 0.8	0.49
	500	620.0	100.8 ± 0.9	0.46

[a] Average of 5 replicate determinations.

[b] The measure of precision is the relative deviation (s_r).

[c] Mian M. Shafee Leather Tanning Industry Karachi.

[d] Fasto Leather Workers Karachi.

[e] Atama Leathers Karachi.

[f] National Electroplating Industry Karachi.

Determination of chromium in environmental water and industrial effluent samples

Each filtered (with Whatman No. 42) environmental water sample (100 mL) evaporated nearly to dryness with 10 mL of concentrated HNO_3 in a fume cupboard and was heated with 10 mL of de-ionized water in order to dissolves the salts. The solution was then cooled and neutralized with dilute NH_4OH solution. The resulting solution was then filtered and quantitatively transferred into a 25-mL calibrated flask and made up to the mark with de-ionized water. The effluent from the common tannery treatment plant of a tannery complex in Karachi was taken and diluted as per requirement.

An aliquot (1-2 mL) of this solution was pipetted into a 10-mL calibrated flask, and the chromium content was determined as described under a procedure using EDTA as a masking agent. The analysis of environmental water samples from various sources for chromium was performed and the results are given in Table 6 and 6a.

Determination of chromium in biological samples

Human blood (2-3 mL) or serum (1-2 mL) was collected in polyethane bottles from the affected persons. The samples were taken into a 100-mL micro-Kjeldahl flask. A glass bead and 10 mL of concentrated nitric acid were added and the flask was placed on the digester under gentle heating following a method recommended by Stahr.[36] When the initial brisk reaction was over, the solution was removed and cooled. Five milliliters of concentrated HNO_3 was added carefully, followed by the addition of 0.5 mL of 70% $HClO_4$, and heating was continued to dense white fumes, repeating HNO_3 addition if necessary. Heating was continued for at least 30 min, followed by cooling. The content of the flask was filtered and neutralized with dilute ammonia. The resultant solution was then transferred quantitatively into a 25-mL calibrated flask and made up to the mark with de-ionized water.

A suitable aliquot (1-2 mL) of the final solution was pipetted out into a 10-mL calibrated flask, and the chromium content was determine as described in the Procedure section, using EDTA, ascorbic acid, and azide as a masking agent. The samples were also measured by atomic absorption spectrometry for comparison of the results. The results of biological (human fluids) analyses by the spectrophotometric method were found to be in excellent agreement with those obtained by AAS. The results are given in Table 7.

Table 7. Determination of chromium in some human fluids.

S. No.	Sample	Chromium / mg L^{-1}		Sample source[b]
		AAS	Proposed method[a]	
1	Blood	0.35	0.37 ± 0.03	Diabetic patient (Male)
2	Blood	0.26	0.23 ± 0.04	Diabetic patient (Female)
3	Blood	0.45	0.42 ± 0.05	Diabetic patient (Female)
4	Blood	0.84	0.87 ± 0.06	Cardiovascular (Female)
5	Blood	0.95	0.98 ± 0.07	Cardiovascular (Male)
6	Blood	1.25	1.21 ± 0.05	Cardiovascular (Male)
7	Blood	1.46	1.51 ± 0.08	Cardiovascular (Female)
8	Serum	0.64	0.65 ± 0.06	Cardiovascular (Female)
9	Serum	0.96	1.00 ± 0.03	Cardiovascular (Male)
10	Blood	0.75	0.77 ± 0.05	Normal adult (Male)

[a] Average of five replicate determination ± s

[b] Samples were from NIMRA Hospital, Jamshoro

Determination of Cr(III) and Cr(VI) speciation in mixtures

Suitable aliquots (1-2 mL) of Cr(III) +Cr(VI) mixtures (preferably 1:1, 1:5, 1:10) were taken in a 25-mL conical flask. A few drops of 1 M H_2SO_4 and 1-2 mL of 1% (w/v) potassium permanganate solution were added to oxidize the trivalent chromium. Then 5 mL of water was added to the mixture, which was subsequently heated on water bath for 10-15 min, with occasional gentle shaking, and then cooled at room temperature. Then 3-4 drops of freshly prepared sodium azide solution (1% w/v) were added and heated gently with the further addition of 2-3 mL of water, if necessary, for 5 min to drive off the azide cooled to room temperature. The

reaction mixture was neutralized with dilute NH_4OH and transferred quantitatively into a 10 mL volumetric flask; then 1 mL of 10% TX-100 was added, followed by addition of 1 mL of 1 M H_2SO_4 and 1 mL of 1.58 $\times$ 10^{-3} M BSOPD reagent solution. The absorbance was measured at 482 nm against reagent blank. Then the total chromium content was determined according to the general procedure with the help of the calibration graph.

An equal aliquot of the above chromium(III) + chromium(VI) mixture was taken into a 10-mL volumetric flask; then 1 mL of 10% TX-100 was added, followed by addition of 1 mL of 1M H_2SO_4 and 1 mL of 1.58 $\times$ 10^{-3} M BSOPD reagent, and made up to the volume with de-ionized water. The absorbance was measured against the reagent blank, as before. The chromium concentration was calculated in mg L^{-1} or μg L^{-1} with the aid of the calibration graph. This gave a measure of the chromium(VI) originally present in the mixture. The value was subtracted from that of the total chromium to determine the chromium(III) present in the mixture. The results were found to be highly reproducible and are shown in Table 8. The mean errors for Cr(VI) and Cr(III) were found to be $\pm$0.016 and $\pm$0.013, respectively, and the corresponding standard deviations for Cr(VI) and Cr(III) were found to be $\pm$0.015 and $\pm$0.016, respectively. The occurrence of such reproducible results is also reported for different oxidation states of chromium.[37]

Table 8. Determination of chromium(III) and chromium(VI) speciation in mixtures.

S. No.	Cr(VI): Cr(III)	Cr, taken (mg L^{-1})		Cr, Found (mg L^{-1}) (n = 5)		Error (mg L^{-1})	
		Cr(VI)	Cr(III)	Cr(VI)	C(III)	Cr(VI)	Cr(III)
1	1:1	1.00	1.00	0.98	0.99	0.02	0.01
2	1:1	1.00	1.00	1.00	1.02	0.00	0.02
3	1:1	1.00	1.00	0.97	0.99	0.03	0.01
Mean error: Cr(VI) = $\pm$0.016 , Cr(III) = $\pm$ 0.013-; s: Cr(VI) = $\pm$ 0.015, Cr(III) = $\pm$0.011							
1	1:5	1.00	5.00	0.98	4.98	0.02	0.02
2	1:5	1.00	5.00	0.99	4.99	0.01	0.01
3	1:5	1.00	5.00	0.98	4.98	0.02	0.02
Mean error: Cr(VI) = $\pm$ 0.016; Cr(III) = $\pm$ 0.016; s: Cr(VI) = $\pm$ 0.0058, Cr(III) = $\pm$ 0.006							
1	1:10	1.00	10.00	0.99	9.99	0.01	0.01
2	1:10	1.00	10.00	0.98	9.98	0.02	0.02
3	1:10	1.00	10.00	0.98	9.98	0.02	0.02
Mean error: Cr(VI) = $\pm$ 0.0016, Cr(III) = $\pm$ 0.002; s: Cr(VI) = $\pm$ 0.015, Cr(III) = $\pm$ 0.015							

Conclusions

In the present work, a simple, sensitive, selective, and inexpensive micellar method with the Cr(VI)-BSOPD complex was developed for the determination of chromium in industrial and environmental samples. The presence of a micellar system (altered environment) avoids the previous steps of solvent extraction, and reduces the cost and toxicity while enhancing the sensitivity, selectivity, and molar absorptivity. The molar absorptivities of the chromium-BSOPD complex formed in presence of the nonionic TX-100 surfactant are almost 10 times

higher than the value observed in the aqueous solution, resulting in an increase in the sensitivity and selectivity of the method. The apparent molar absorptivities were found to be 3.5×10^5 L mol^{-1} cm^{-1} aqueous micellar media. Compared with other methods in the literature (Table 1), the proposed method has several remarkable analytical characteristics:

1. the proposed method is highly sensitive with molar absorptivity of the complex of 3.5×10^5 L mol^{-1} cm^{-1}. Thus, amount of ng g^{-1} of chromium can be determined without pre-concentration;
2. the proposed method is very simple, rapid, and stable. The reaction of chromium(VI) with BSOPD is completed rapidly in micellar medium within 1 min at room temperature so it does not involved any stringent reaction conditions and offer the advantages of high complex stability (24 h).
3. the method has the added advantage of determining individual amounts of Cr(VI) and Cr(III).

With suitable masking, the reaction can be made highly selective. The proposed method using BSOPD in the presence of aqueous micellar solutions not only is one of the most sensitive methods for the determination of chromium but also is excellent in terms of selectivity and simplicity. Therefore, this method will be successfully applied to the monitoring of trace amounts of chromium in real, environmental, industrial effluent and biological samples.

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