

Notes

Spectrophotometric determination of chromium using saccharin

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A sensitive and selective spectrophotometric method for the indirect determination of trace amounts of chromium(VI) is described. Chromium(VI) oxidizes hydroxylamine in acetate buffer of pH 4.0 to nitrite, which then diazotizes *p*-nitroaniline or sulphanilamide to form diazonium salt. These diazonium salts are then coupled with saccharin in an alkaline medium, which gives azo dye with absorption maximum at 372 and 390 nm for *p*-nitroaniline and sulphanilamide, respectively. The method obeys Beer's law in the concentration range of 1-16 $\mu\text{g mL}^{-1}$ for chromium with *p*-nitroaniline-saccharin and 0.6-14 $\mu\text{g mL}^{-1}$ of chromium with sulphanilamide-saccharin couples. The molar absorptivity, Sandell's sensitivity of the systems with *p*-nitroaniline-saccharin and sulphanilamide-saccharin couples were found to be $5.41 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$, $1.93 \times 10^{-3} \mu\text{g cm}^{-2}$ and $2.63 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$, $3.9 \times 10^{-3} \mu\text{g cm}^{-2}$, respectively. The optimum reaction conditions and other analytical parameters were evaluated. The effect of interfering ions on the determination was described. Chromium(III) can be determined after it is oxidized with bromine water in an alkaline medium to chromium(VI). The developed method has been successfully applied to the analysis of the chromium in alloy steel, pharmaceutical samples, natural water and soil samples.

Keywords: Chromium determination, spectrophotometry, *p*-nitroaniline, sulphanilamide, saccharin

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Chromium is one of the most widely distributed heavy metals in the earth's crust. It is normally found in two stable oxidation states i.e. Cr(III) and Cr(VI). Chromium is required in small quantities as an essential trace metal, nutrient and its deficiency may result in several physiological disorders. Most of the biological tissues contain Cr(III) which is usually nontoxic, whereas Cr(VI) is highly toxic to the organisms. It is known that an increase in the content of this element in soils makes them infertile and that the toxic effect depends to some extent on the oxidation state of chromium. On the other hand, the

introduction of chromium salts into soils has some positive effects due to activation of some biochemical processes¹. Cr(III) is an essential nutrient for maintaining normal physiological function², whereas Cr(VI) is toxic³. The determination of chromium by spectrophotometric method based on oxidation of organic compounds⁴⁻⁶ and on formation of ion associates⁷ has the disadvantage of a high blank value. The most widely used reagent for chromium(VI) determination is diphenylcarbazide^{8,9}, but it suffers serious interference from Fe(III), Mo(VI), Cu(II), and Hg(II)¹⁰ and also the formed complex is stable only for 30 min in the presence of a phosphate buffer¹¹. Other reagents used for spectrophotometric determination of chromium include 4-(2-pyridylazo)-resorcinol¹², phenylarsenazo¹³, gallaceto-phenone oxime¹⁴, citrazinic acid¹⁵, trifluoroperazine hydrochloride¹⁶, leuco xylenecyanol FF¹⁷ and 4-(2-thiazolylazo)-resorcinol¹⁸. Of these reagents, some are reported to be carcinogenic, while few others are less selective. The need for a sensitive, simple, reliable method for the determination of chromium is therefore clearly recognized.

The aim of the present work is to provide a simple and sensitive method for the indirect determination of chromium(VI) based on the oxidation of hydroxylamine to nitrite, which then diazotizes with *p*-nitroaniline or sulphanilamide. These diazonium salts are then coupled with saccharin in an alkaline medium, to form an azo dye. Chromium(III) is determined after it is oxidized to chromium(VI) by bromine water. The proposed method has been used to determine chromium in alloy steel, pharmaceutical preparations, natural water and soil samples.

Experimental Procedure

Apparatus

A secomam Anthelie NUA 002 UV-visible spectrophotometer with 1 cm quartz cell was used for the absorbance measurements and a WTW pH 330, pH meter was used.

Reagents

All chemicals used were of analytical grade (Merck) and distilled water was used for dilution of reagents and samples. Standard chromium(VI) stock

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solution ($1000 \mu\text{g mL}^{-1}$) was prepared by dissolving 0.2829 g of $\text{K}_2\text{Cr}_2\text{O}_7$ in 100 mL of water. The stock solution was further diluted as needed. Standard chromium(III) stock solution ($1000 \mu\text{g mL}^{-1}$) was prepared by dissolving 0.2829 g of $\text{K}_2\text{Cr}_2\text{O}_7$ in 50 mL of water, adding 1 mL saturated sodium sulphite solution, acidifying with 1 mL of 2.5 M sulphuric acid, and then boiling for 2 min to remove excess SO_2 and diluting with water to 100 mL. Suitable volume of this solution was diluted to obtain the working standard. *p*-nitroaniline (0.05%) and sulphanilamide (0.05%) were prepared in acid medium. Saccharin (0.1%) was prepared by dissolving 0.1 g of saccharin in 2 mL of 4 M NaOH and diluted to 100 mL with water. The following reagents were prepared by dissolving appropriate amounts of reagents in distilled water: hydroxylamine hydrochloride (0.1%), bromine water (saturated), sulphosalicylic acid (5%), KOH (4.5 M), sulphuric acid (2.5 M), HCl (5 M) and NaOH (4 M).

Determination of chromium

An aliquot of a sample solution containing known amount of chromium(VI) was transferred into a series of 10 mL calibrated flasks. A volume of 0.5 mL of acetate buffer (pH 4.0) and 0.5 mL of 0.1% hydroxylamine hydrochloride solution were added. After 2 min, 0.5 mL of 0.05% *p*-nitroaniline or sulphanilamide was added to each flask and the mixture was allowed to stand for 2 min. Then, 2 mL of 0.1% saccharin and 1.5 mL of 4 M sodium hydroxide solution and contents were diluted to the mark with distilled water and mixed well. After 5 min, absorbance of the azo dye was measured at 372 (nitroaniline) or 390 nm (sulphanilamide) against reagent blank. For the determination of chromium(III), suitable volume of an aliquot of sample solution of chromium(III) was transferred into a series of 10 mL calibrated flasks. 0.5 mL of saturated bromine water and 0.5 mL of 4.5 M KOH solution were added to each flask and allowed to stand for 5 min. Then 0.5 mL of 2.5 M sulphuric acid and 0.5 mL of 5% sulphosalicylic acid were added and then above procedure for chromium(VI) was followed. The absorbance of the resulting solution was measured at the abovementioned wavelength against reagent blank.

Analysis of chromium steels

Known quantity of alloy steels were dissolved in approximately 8 mL of aqua regia. It was evaporated

nearly to dryness on a sand bath, sulphuric acid (1-2 mL, 1:1) was added and evaporated until salts crystallized, to this 10 mL of water was added. The solution was warmed, filtered, and treated with 10 mL of HCl and 10 mL of methyl isobutyl ketone to extract iron^{1,21}. The aqueous layer was separated and diluted to a known volume with water. Suitable aliquots of sample solutions were analysed according to the procedure for chromium(III).

Analysis of pharmaceutical samples

Samples of the finely ground multivitamin-multimineral tablets containing chromium(III) were treated with 5 mL of nitric acid, and the mixtures were evaporated to dryness. The residue was leached with 5 mL of 0.5 M H_2SO_4 . The solution was diluted to a known volume with water. Suitable aliquots of the sample solution were analyzed according to the procedure for chromium(III).

Determination of chromium in natural water samples

An aliquot of the natural water samples containing not more than $12 \mu\text{g mL}^{-1}$ chromium was treated with 0.5 mL, 1 M NaOH and 0.5 mL 0.2 M EDTA. Any precipitate formed was centrifuged off. The solution was transferred into a 10 mL standard volumetric flask and the chromium content determined as per the method discussed above. Solutions were also analysed according to the standard diphenylcarbazide method²².

Determination of chromium in soil samples

A known amount of (1 g) air dried homogenized soil samples, spiked with known amounts of chromium(VI) was taken and then fused with 5 g anhydrous sodium carbonate in a silica crucible and evaporated to dryness after the addition of 25 mL of water. The dried material was dissolved in water, filtered through Whatman no. 40 filter paper into 25 mL calibrated flask and neutralized with dilute ammonia. It was then diluted to a known volume with water. An aliquot of this sample solution was analysed for chromium(VI). Solution was also analysed according to the standard diphenylcarbazide method²².

Effect of the reagent concentration

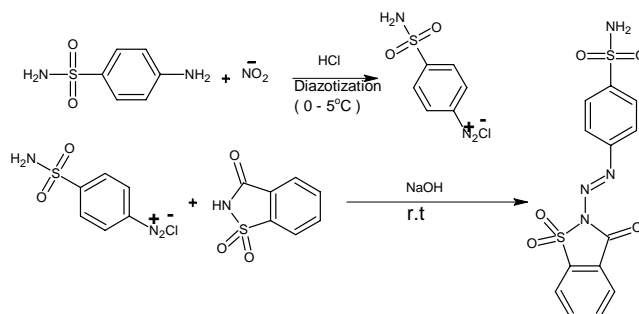
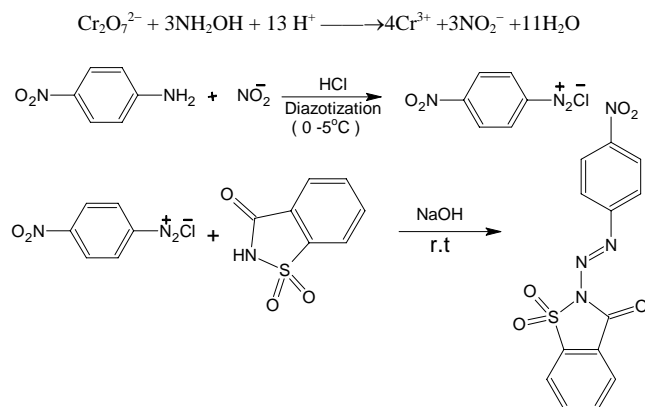
The nitrite generated by the oxidation of hydroxylamine by chromium(VI) was determined by treating it with 0.5 mL of each *p*-nitroaniline (0.05%) and sulphanilamide (0.05%) in 4 M HCl to form diazonium chloride, which then coupled with

saccharin in an alkaline medium. A volume of 0.5 mL of 0.1% saccharin and 1.5 mL of 4 M NaOH in a total volume of 10 mL were found to be sufficient to provide maximum absorbance by forming the dye. The increase in concentration of the saccharin had no effect on intensity of the colour. The colour of the system was stable for more than 6 h. In the present investigation, bromine water in an alkaline medium was used to oxidize Cr(III) to Cr(VI) and the excess oxidizer was removed by the addition of sulphosalicylic acid (5%).

Results and Discussion

The nitrite generated by the oxidation of hydroxylamine with chromium(VI) is used to diazotize *p*-nitroaniline or sulphanilamide. The diazonium salt formed is coupled with saccharin in an alkaline medium. Diazotization and coupling reactions are found to be temperature dependent. Diazotization reaction was carried out at 0-5°C and coupling reaction was carried out at room temperature, above 35°C there is a decrease in intensity of the colour. Analytical parameters are optimized by measuring absorbance of the azo dyes at 372 and 390 nm, respectively.

Chromium(VI) effectively oxidizes hydroxylamine to nitrite in the pH range 3.5-4.5. The oxidation of hydroxylamine to nitrite is fast and complete within 2 min. This reaction is similar to the oxidation of hydroxylamine to nitrite by iodine^{19,20}. Quantitative oxidation of hydroxylamine to nitrite by chromium(VI) was confirmed by performing the diazo-coupling reaction with a standard nitrite solution. In this reaction, the generated nitrite can be determined easily by diazo-coupling reaction. The scheme of the reaction is as follows:



Analytical data

The adherence to Beer's law was studied by measuring the absorbance values of solutions varying chromium concentration. A straight line graph was obtained by plotting absorbance against concentration of chromium. This method obeys Beer's law in the concentration range of 1-16 $\mu\text{g mL}^{-1}$ for chromium with *p*-nitroaniline-saccharin and 0.6-14 $\mu\text{g mL}^{-1}$ for chromium with sulphanilamide-saccharin couples. The molar absorptivity, Sandell's sensitivity of coloured system with *p*-nitroaniline-saccharin and sulphanilamide-saccharin couples were found to be $5.41 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$, $1.93 \times 10^{-3} \mu\text{g cm}^{-2}$ and $2.63 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$, $3.9 \times 10^{-3} \mu\text{g cm}^{-2}$, respectively. The detection limit ($D_L = 3.3\sigma/S$) and quantitation limit ($Q_L = 10\sigma/S$) [where σ is the standard deviation of the reagent blank ($n=5$) and S is the slope of the calibration curve] for the chromium determination with *p*-nitroaniline-saccharin couple and sulphanilamide-saccharin couple were found to be 0.06, 0.18 and 0.04, 0.107 $\mu\text{g mL}^{-1}$, respectively.

Table 1—Effect of interfering ions on the determination of 2 $\mu\text{g mL}^{-1}$ of Cr(VI)

Ion	Tolerance level ($\mu\text{g mL}^{-1}$)	Ion	Tolerance level ($\mu\text{g mL}^{-1}$)
Al ³⁺	500	Ce ⁴⁺	20
Ca ²⁺	100	MoO ₄ ²⁻	1000
Fe ³⁺	15	Citrate	1500
Fe ²⁺	20	F ⁻	3000
Na ⁺	3000	CO ₃ ²⁻	3000
Co ²⁺	100	Tartarate	2000
Pb ²⁺	600	Sulfite	3000
Ni ²⁺	100	Oxalate	2000
Cu ²⁺	50	Sulfate	3000
Ag ⁺	80	Chloride	3000
Mg ²⁺	200	Nitrate	2000
Cd ²⁺	500	PO ₄ ³⁻	2000
Hg ²⁺	30	Acetate	3000
Ba ²⁺	2000	AsO ₃ ³⁻	2000
Bi ³⁺	1500	EDTA	5000
Mn ²⁺	500		

Effect of interfering ions

The effect of various ions at $\mu\text{g mL}^{-1}$ levels on the determination of chromium(VI) was examined. The tolerance limits of interfering species were established at those concentrations that do not cause more than $\pm 2.0\%$ error in absorbance values of chromium(VI) at $2 \mu\text{g mL}^{-1}$ (Table 1). The results indicated that Fe(III), Ce(IV) and Hg(II) interfere in the determination of chromium. However, the tolerance level for these ions

may be increased by the addition of 1 mL of 1% EDTA.

Applications

The proposed method was applied to the quantitative determinations of chromium in standard alloy steels, pharmaceutical samples, natural water samples and soil samples. The results are presented in Tables 2-4. Statistical analysis of the results by *t* and

Table 2—Determination of chromium in water and soil samples

Sample	Chromium added	PNA-S method					SA-S method					Reference method ²²		
		Chromium found ^a	Recovery (%)	RSD (%)	<i>t</i> ^b test	<i>F</i> ^c test	Chromium found ^a	Recovery (%)	RSD (%)	<i>t</i> ^b test	<i>F</i> ^c test	Chromium found ^a	Recovery (%)	RSD (%)
Natural water	4.0	3.98±0.04	99.5	1.0	0.79	1.0	3.96±0.02	99.0	0.51	0.50	4.0	3.97±0.04	99.25	1.0
	6.0	5.97±0.02	99.5	0.34	0.62	2.25	5.98±0.04	99.7	0.67	0.89	1.78	5.96±0.03	99.33	0.50
Soil	4.0	3.99±0.05	99.8	1.2	0.69	1.56	3.98±0.05	99.5	1.26	0.35	1.56	3.97±0.04	99.25	1.0
	6.0	5.98±0.04	99.7	0.67	0.75	4.0	5.99±0.06	99.8	1.0	0.89	1.78	5.95±0.08	99.17	1.34

Concentration is expressed in $\mu\text{g mL}^{-1}$

a. Mean \pm standard deviation (n=5)

b. Tabulated *t*-value for 8 degree of freedom at P(0.95) is 2.306

c. Tabulated *F*-value for (4,4) degree of freedom at P(0.95) is 6.39

PNA (*p*-nitroaniline), S (Saccharin), SA (sulphanilamide)

Table 3—Determination of chromium in alloy steels

Sample	Chromium certified	PNA-S method			SA+S method		
		Chromium (%)	Recovery found (%) ^a	<i>t</i> -test ^b (%)	Chromium found (%) ^a	Recovery (%)	<i>t</i> -test ^b
GKW Steel, India (0.05g/100mL); C 0.54, Mn 0.89, S 0.018, P 0.034, Si 0.33, V 0.13 ^c	1.02	1.016±0.04	99.6	0.22	1.012±0.02	99.2	0.89
Stainless steel no.394(0.05g/100mL); Ni 8.12, Fe 70-71 ^d	18.0	17.97±0.03	99.8	2.23	17.95±0.06	99.5	1.86

a. Mean \pm standard deviation (n=5)

b. Tabulated *t*-value for four degree of freedom at P (0.95) is 2.78

c. Diluted to 10 times before analysis

d. Diluted to 20 times before analysis

PNA (*p*-nitroaniline), S (Saccharin), SA (sulphanilamide)

Table 4—Determination of chromium in pharmaceutical preparations

Sample	Chromium certified (mg/tablet)	PNA-S method			SA+S method		
		Chromium found ^a (mg/tablet)	Recovery (%)	<i>t</i> -test ^b	Chromium found ^a (mg/tablet)	Recovery (%)	<i>t</i> -test ^b
Optisulin [Sai Mira Innopharm Pvt. Ltd., India]	0.500	0.496±0.009	99.2	0.99	0.494±0.007	98.8	1.92
Chromoplex [Aristo Pharmaceuticals Ltd., India]	0.200	0.195±0.006	97.5	1.86	0.197±0.005	98.5	1.34

a. Mean \pm standard deviation (n=5)

b. Tabulated *t*-value for four degree of freedom at P (0.95) is 2.78

F-tests showed no significant difference in accuracy and precision of the proposed and reference method²². The precision of the proposed method was evaluated by replicate analysis of samples containing chromium at three different concentrations.

Conclusions

The proposed method for the determination of chromium(VI) is facile, rapid, sensitive and has a reasonable analytical range without the need of extraction or heating. The developed method does not involve any stringent reaction conditions and offers the advantages of high colour stability (more than 6 h) compared to the standard diphenylcarbazide method (30 min). The proposed method has been successfully applied to the determination of trace amounts of chromium in alloy steels, pharmaceutical samples, natural water and soil samples.

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