

Application of Electrophilic Coupling Reactions as a Novel Spectrophotometric Methods for the Determination of Chromium (VI)

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Abstract: Phenoxazine (PNZ), 2-chlorophenoxazine (CPN), 2-trifluoromethylphenoxazine (TPN) were used as new spectrophotometric reagents for the determination of trace amounts of chromium(VI) in presence of cisapride (CSP) and metaclopramide (MCP) as new electrophilic coupling reagents. The methods are based on the oxidation of CSP or MCP by chromium(VI) in hydrochloric acid medium and coupling with PNZ, CPN or TPN to yield red colored derivatives, which are stable for about 24 h and have an absorbance maximum in the range 520-530 nm. Beer's law is obeyed for chromium(VI) in the concentration range 0.22-1.19 $\mu\text{g mL}^{-1}$ and 0.16-1.20 $\mu\text{g mL}^{-1}$ for phenoxazine- cisapride and phenoxazine - metaclopramide, respectively. The methods were successfully applied in the analysis of chromium(VI) in water, soil and some biological samples. Applying standard addition method indicated that there is not significant difference between the proposed methods and the standard reference spectrophotometric method.

Key words: Spectrophotometry • Chromium(VI) • Phenoxazines • Cisapride • Metaclopramide • Environmental samples

INTRODUCTION

One of the important features that distinguish metal pollutants from other pollutants is that they are not biodegradable. Once they enter the environment their toxicity potential is determined to a large extent by their chemical form [1, 2]. Consequently much attention is focus to heavy metals in biological and environmental matrices during the last three decades mainly because of occupational exposures to heavy metals in several chemical industries and the disposal of metal-rich wastes, mostly solid residues or sludges from industrial effluent treatment plants or use of non-treated industrial waste waters in irrigation [1, 2]. Large amounts of digested sewage sludge are disposed off to land, as this is easy and inexpensive method of disposal. However, most of the sludges contain certain toxic metals which are in higher amounts of than the receiving soils and it is necessary that these toxic metals should be properly monitored prior to disposal to land [1, 2].

Chromium is a critical metal used in a number of products that we use every day, but it is seldom used alone. The most common application is in metallurgical

end uses in alloys, consuming 90% of virgin chromium [3]. The addition of chromium adds corrosion and helps in oxidation resistance to metals [3]. Corrosion resistance extends the life of products, allows industrial activities to occur in harsh environments and with harsh chemicals and reduces replacement costs. In addition to metallurgical uses, chromium is also used in refractories and foundry sands for its heat resistance and in chemicals for leather tanning, pigmentation and wood preservation [3]. The increase in use of chromium in industry and its unregulated application has led to the contamination of soil, sediment, surface and ground waters thereby causing serious health hazards to humans and animals [4]. Soil conditions like electron donor availability, soil texture, competing ions, adsorption capabilities, pH, moisture content, temperature and the presence of vegetation are the major factors that govern the mobility of chromium in soil [5-9].

Chromium exists mainly in its III and VI oxidation states in soil. Hexavalent chromium is an oxyanion commonly occurring as relatively water soluble chromate or dichromate. In temperate region soil, where negatively charged clay minerals predominate, chromium(VI) anions

are repelled by the negative charge of soil particles. Due to this hexavalent chromium is quite mobile in soils [10]. Chromium(VI) is a strong oxidizing agent and this makes it toxic to biological systems [11-15]. The toxicity of hexavalent chromium is further enhanced by its ability to diffuse through cell membranes and oxidize biological molecules.

Chromium determination at trace level is of paramount importance because of the contrasting biological effects of its chromium(III) and chromium(VI) oxidation states. Chromium(III) is an essential element in maintaining normal physiological functions [16], unlike, chromium(VI), which is toxic [17]. Chromium salts are used extensively in industrial processes and they may enter water supply systems through the waste discharges.

Chromium(VI) is a major water pollutant, which usually results by some industrial pollutions including tanning factories, steel works, dyeing, chromium plating, wood preservation and artificial fertilizers [18, 19]. For this, determination of chromium(VI) in soil, water and biological samples has gained paramount importance.

Significant attention has been paid to the adverse health effects of chromium, which are highly dependent on its oxidation state. There is sufficient evidence to demonstrate carcinogenicity in humans due to hexavalent chromium in the chromate, chromate pigment and chromium plating industries; limited evidence for carcinogenicity of chromic acid and sodium dichromate; and inadequate evidence for metallic and trivalent compounds. Harmful effects of hexavalent chromium exposure include respiratory cancer, kidney damage and skin irritation [3].

There are various methods proposed for the determination of metals in varied biological and environmental matrices and these include electroanalytical [20], radioanalytical [21] and chromatography [22]. However, these methods have limitation with regard to specificity, sensitivity, simplicity and analysis time. For example, polarography demands extensive selectivity regarding the pH and the supporting electrolytes, to avoid interference by various metal ions, which may likely to be encountered. The ions may also directly interfere if they are polarographically active in the potential range of interest and indirectly interfere by co-precipitation of metals with hydroxides of polyvalent cation formed at medium pH. Radioanalytical methods are reliable for arcane academic purpose, but are seldom used for routine analysis of metals in environmental samples such as sludges and industrial effluents. Chromatographic

methods are valuable for speciation studies rather than routine quantitative analysis. Further, the cost of the instrument is relatively high and its maintenance demands sophistication.

Optical methods such as inductively coupled plasma atomic emission spectrometry, electrothermal atomization atomic absorption spectrometry (ETAAS) and atomic absorption spectrometry (AAS) have been used for detection, quantitation and characterization of these metals. ETAAS presents problems owing to the formation of metal carbide during ashing and atomization thereby decreasing the sensitivity. These instruments are highly costly and besides their maintenance cost is high and is not completely free from various types of inherent interferences.

Amongst the optical methods visible spectrophotometry seems to be the most appropriate for the determination of toxic metals, as it provides sensitive, precise and accurate measurements of analytes and offers practical and economical advantages over other methods. Besides, visible spectrophotometric detection is much more viable as a useful technique to develop a portable on-line or at-line system.

In the literature survey we found recommendation of diphenyl carbazide as a spectrophotometric reagent for the determination of hexavalent chromium in acid solution [23]. In this method, a red violet colour of unknown composition is produced and many cations seriously interfere. In our earlier communication, we have reported the use of iminodibenzyl and 3-chloroiminodibenzyl as reagents for the determination of mercury(II), nickel(II), copper(II) and cobalt(II) in the presence of electrophilic coupling reagents, namely 3-methyl-2-benzothiazoline hydrazone hydrochloride hydrate (MBTH) and 4-amino antipyrine (AAP) [24].

In this research first-ever usage of phenoxazine (PNZ), 2-chlorophenoxazine (CPN) and 2-trifluoromethylphenoxazine (TPN) as chromogens and also cisapride (CSP) and metaclopramide (MCP) as electrophilic coupling reagents for determining chromium(VI) in selected water, soils, sludge samples, plating bath solutions and urine samples are reported. The chemical reactions involved have been studied and the results showed that the methods are simple, rapid and sensitive. Very besides, PNZ, CPN and TPN along with CSP and MCP (Fig.1) offer clear advantages over most of the chromogenic reagents currently used for the purpose and the procedure shows positive features over the existing methods.

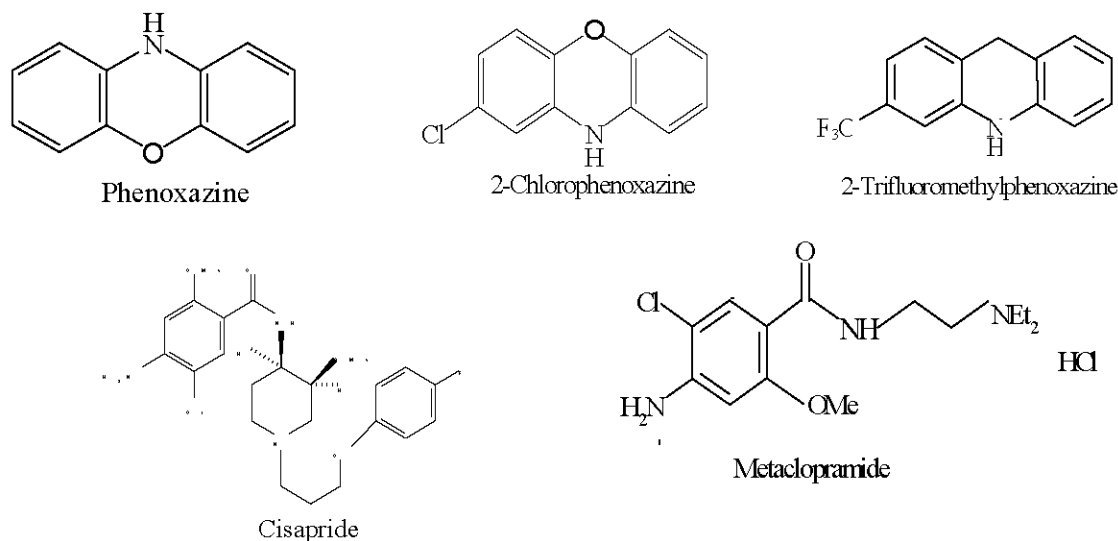


Fig. 1: Structure of phenoxazine derivatives, cisapride and metaclopramide

Experimental

Materials and Solutions: All solutions used were prepared from analytical grade chemicals unless specified otherwise. The reagents were used without further purification. Phenoxazine (PNZ), 2-chlorophenoxazine (CPN) and 2-trifluoromethylphenoxazine (TPN) were obtained from Aldrich, India. CPN and TPN were synthesized as per the procedure [25, 26] described in the literature. CSP from USV Ltd. (India) and MCP from Ipca Laboratories Ltd. (India) were received as gift samples and were used as working standards without further treatment.

Synthesis of CPN and TPN: 2-Substituted phenoxazines are not commercially available. Hence, synthesis of 2-chlorophenoxazine (CPN) and 2-trifluoromethylphenoxazine (TPN) were carried out by pyrolytic condensation of *o*-chloronitrobenzene derivatives with sodium *o*-bromophenolate followed by reduction with stannous chloride and subsequent cyclization of ether using anhydrous potassium carbonate, cupric carbonate and *p*-xylene to yield intermediate products. The intermediates were refluxed with potassium hydroxide. The final products were purified with *n*-hexane. A yield of 75% (CPN) and 70% (TPN) were obtained. The purity of the products was checked by thin layer chromatography and further purified by column chromatography and used for spectral studies.

Stock solution (1000 $\mu\text{g mL}^{-1}$) of chromium(VI) was prepared by dissolving known quantity of potassium chromate in 100 mL of distilled water. Solutions of required strength as needed in the analyses were prepared by diluting the stock solution with distilled water.

Fresh solutions of 0.025% (w/v) PNZ, CPN and TPN were prepared by dissolving 25 mg of each sample separately in 100 mL distilled ethyl alcohol. The 0.05% (w/v) solutions of CSP and MCP were prepared by dissolving 50 mg of the samples individually in 100 mL of distilled water.

Apparatus: Specord 50 UV-vis spectrophotometer with 1.0-cm silica quartz matched cell (Jasco, Tokyo, Japan) was used for measuring the absorbance.

General Procedure: To a series of 25 mL calibrated flasks, 1.5 mL of 0.05% (w/v) CSP or MCP, different aliquots of standard solutions of chromium(VI), 2.0 mL of 0.025% (w/v) PNZ/ CPN/ TPN and 1.0 mL of 2 N hydrochloric acid were added. The contents were mixed thoroughly and allowed for 5 min to complete the reaction. The resultant red colored solutions in the standard flasks were made up to the volume with distilled water. Absorbance was measured at 520 nm for PNZ-CSP/MCP and 530 nm for CPN/TPN-CSP/MCP with 1.0-cm quartz cell against the corresponding reagent blank, which was prepared similarly except for the addition of chromium(VI). The optical characteristics for determination of chromium(VI) with PNZ, CPN and TPN using CSP and MCP are shown in Table 1.

Table 1: Spectral data for determination of chromium (VI) using CSP/MCP as electrophilic coupling agents and PNZ, CPN or TPN as chromogens

Parameters	CSP			MCP		
	PNZ	CPN	TPN	PNZ	CPN	TPN
Color	Red	Red	Red	Red	Red	Red
λ_{\max} (nm)	520	530	530	520	530	530
Stability (h)	24	24	24	24	24	24
Beer's law ($\mu\text{g mL}^{-1}$)	0.22-1.19	0.42-2.75	0.48-3.52	0.16-1.20	0.32-2.35	0.48-1.92
Recommended ion concentration ($\mu\text{g mL}^{-1}$)	0.54	1.27	1.60	0.65	1.07	1.12
Molar absorptivity ($\text{L mol}^{-1}\text{cm}^{-1}$) $\times 10^4$	2.85	1.26	1.06	2.70	1.52	1.17
Sand ell's sensitivity ($\mu\text{g cm}^{-2}$) $\times 10^{-3}$	1.82	4.13	4.90	1.92	3.42	4.45
Regression equation ^a :						
Slope (a)	0.676	0.269	0.205	0.617	0.304	0.393
Intercept (b)	-0.058	-0.025	0.001	-0.052	-0.014	-0.122
Correlation coefficient	0.9885	0.9950	0.9897	0.9892	0.9960	0.9886
Reaction time (min)	5	5	5	5	5	5
RSD% (n=7)	0.45	0.62	0.51	0.51	0.21	0.57

^aRegression curve: $y = ax + b$ where x is the concentration of Chromium(VI) in $\mu\text{g mL}^{-1}$ and y is absorbance

RESULT AND DISCUSSION

Phenoxazine derivatives are nervous system depressants particularly with sedative, antiepileptic, tranquillizing [27], spasmodic [28], antitubercular [29] and anthelmintic activities [30]. Phenoxazine derivatives exist in neutral form, as monocations, as dications and even as trications depending on the environment [31]. Their molecular structure and luminescent properties have been studied to a great extent [32]. Besides, they have impressive applications as biological stains [33], as laser dyes [34] and as redox indicators [35]. In recent years phenoxazine derivatives are reported to be potential chromophoric compounds in host-guest artificial photonic antenna systems [36].

Cisapride (CSP) and metaclopramide (MCP) are substituted benzomides. They belong to the class of gastro-intestinal drugs. Both benzamides stimulate gastro-intestinal motility. They are also used to stimulate gastric emptying during radiographic examinations [37]. Major parameters that influence the performance of the proposed methods were studied in order to establish the optimum working configurations. All data given are on% R.S.D. in optimization steps for both physical and chemical parameters and are the mean values obtained from successive determinations. All optimization steps were carried out with a chosen chromium(VI) concentration as mentioned in Table 1.

Absorption Spectra of Colored Derivatives: In order to have minimum interferences, it was necessary to identify optimum wavelength for chromium(VI) determination in

the proposed methods. This wavelength must be specific for the quantitative and specific monitoring of the CSP/MCP-Cr(VI)-PNZ, CPN and TPN. The wavelength of maximum absorbance was identified by scanning the product of CSP/MCP-Cr(VI)-PNZ-HCl, CSP/MCP-Cr(VI)-CPN-HCl or CSP/MCP-Cr(VI)-TPN-HCl over the range 300–800 nm with a specord 50-UV-vis spectrophotometer. Wavelengths of 520, 530 and 530 nm were found optimum to get best results (Fig. 2 and 3).

Under the optimized conditions, although the color developed almost instantaneously, but 5 min were allowed to have maximum and constant absorbance in both the methods. The red color derivative was stable up to 24 h. The absorbance varied by $\pm 2\%$ within a day. The color development was influenced by temperature in the range of 20–35°C and the most good results were obtained in this temperature range. At higher temperature the absorbance values decreased; which indicates the dissociation of color on prolonged heating.

Reaction Mechanism: The chemical reaction in the spectrophotometric study, involved the reduction of chromium(VI) by CSP/MCP and the latter's subsequent oxidative coupling with PNZ/ CPN/TPN in an acidic medium to form a red product. The factors affecting the color development, reproducibility, sensitivity and adherence to Beer's Law were investigated by optimizing the analytical variables.

Effect of Reagents and Acid Concentration: The effect of PNZ, CPN and TPN reagents at 0.025% (w/v) solution in the range of 0.10–10.0 mL was studied to find the volume

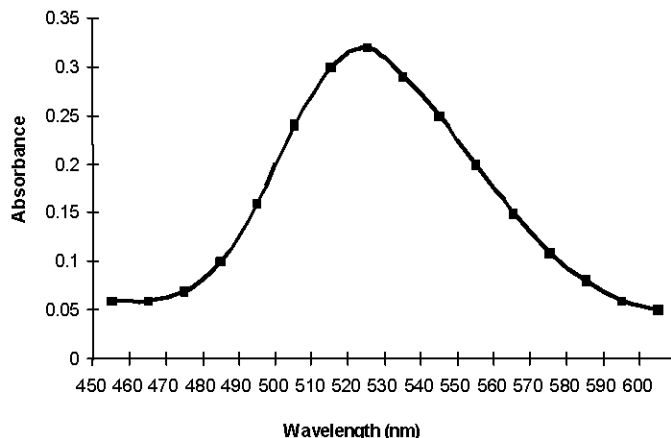


Fig. 2: Absorption spectra of MCP -Cr(VI)-PNZ

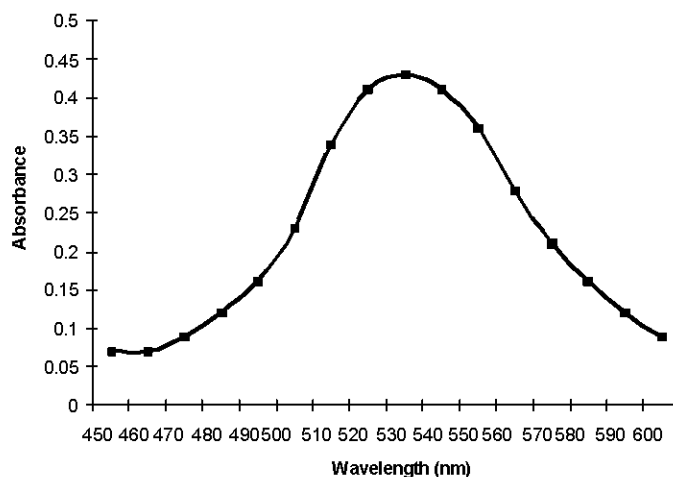


Fig.3: Absorption spectra of MCP- Cr(VI)-CPN/TPN

needed to get maximum color intensity. The results indicated that 1.0-3.0 mL of the solution was necessary. Hence, 2.0 mL of 0.025% (w/v) solution in a 25mL standard flask was taken. Maximum intensity of the red color was achieved in hydrochloric acid medium. Preliminary investigations showed that hydrochloric acid was better than sulphuric, phosphoric or acetic acid. Maximum intensity of the red color was achieved in the range of 0.5-1.5 mL of 1M HCl. Therefore, 1.0 mL of 1M HCl in 25 mL was used for getting best results. Similarly, the amount of CSP or MCP needed for constant and maximum color development was investigated. It was found that 1.0-2.0 mL of the solution was necessary. Hence, 1.5 mL of 0.05% (w/v) CSP or MCP solution was selected to get reproducible results.

Experiments were carried out to optimize temperature and time of the reaction. It was found that maximum color intensity developed within 5 min at room temperature

and remained almost stable for about 24 h. Increase in reaction temperature decreased the intensity of red color. Hence, 5 min reaction was selected for the routine analysis.

Order of Addition of Reactants: During the investigation it was observed that the sequence of addition of reactants was also important as it influenced the intensity and the stability of the color of the product to a great extent. The sequences (i) PNZ, CPN or TPN-HCl-Cr(VI)-CSP or MCP and (ii) Cr(VI)-HCl- PNZ, CPN or TPN-CSP or MCP gave less intense and unstable color. While, more intense and stable red color was obtained with sequence (iii) CSP or MCP-Cr(VI)-PNZ, CPN or TPN-HCl. This was expected as the reaction (i) and (ii) produced radical cation while, in (iii) electrophilic reaction was involved.

Linearity, Accuracy and Precision: The spectrophotometric methods were further evaluated under the optimum conditions with regard to linearity, accuracy and precision, molar absorptivity and Sandell's sensitivity.

The linearity of the spectrophotometric method for the determination of chromium(VI) was evaluated under the optimum conditions. The regression calibration equation obtained under optimum conditions for chromium(VI), PNZ and MCP was: $Y = -0.052 + 0.617X$; $r = 0.9892$ and $n = 5$.

Where, Y is the absorbance and X the chromium(VI) concentration in mg/L. The calibration curve was linear over the range 0.16-1.20 mg/L.

The molar absorptivity was $2.70 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ for PNZ - MCP complex and Sandell's sensitivity was $0.00192 \text{ mg cm}^{-2}$.

The accuracy of the method was evaluated by taking real environmental samples like lake, well and tap waters and soil samples and the results were compared with standard spectrophotometric method results. The results obtained in the proposed spectrophotometric methods compared very well with those from the standard method [38]. The % R.S.D. was found to be < 0.8 ($n = 5$).

To further confirm the validity and accuracy of the proposed method, recovery tests were performed by standard addition method. Each test was repeated five times. The results presented in Tables 3-12 indicate very good recoveries and non-interference from commonly encountered constituents normally present in the environmental samples.

Effect of Diverse Ions: In order to establish the analytical potentiality of proposed method, the effect of some possible interfering ions, which often accompany chromium(VI) was examined by the proposed methods. Any ion is considered to be interfering with the determination, only when the absorbance value differs by $\pm 3\%$ from that of chromium(VI) alone. Metals such as iron(III), vanadium(V), manganese(VII) and cerium(IV) and non metals like bromate, iodate and periodate were found to interfere severely as they caused low recovery of chromium(VI). Masking agents like EDTA, tartrate and citrate have not interfered in the determination of chromium(VI). Therefore, 2.0 mL of 2% EDTA were used to mask the interference by the above stated interfering metals. During the interference studies, if any precipitate was formed, it was overcome by centrifugation. The possible interference and the maximum tolerable concentrations are listed in Table 2.

Applications

Collection, Preparation and Determination of Chromium(VI) in Soil Samples: Soil samples were collected about 0.5 km away from the waste treatment plant of a factory, which used chromium extensively in plating baths. The treated industrial effluents (water) from the factory were released into the neighboring fields, which were ultimately used for irrigation purposes. By following static sampling procedure six soil samples were collected at random from a depth of 0-20 cm with a distance of about 50 m between each sampling site.

Known amount of soil (5.0 g) was taken in a platinum crucible and heated for 3 h in a muffle furnace at 550°C . After cooling, transferred the sample into a platinum basin and added 2.0 mL of double distilled water, 1.0 mL of concentrated sulphuric acid and 10.0 mL of concentrated hydrofluoric acid and heated on a sand-bath until vapours of SO_3 appeared. The residue was dissolved in 5.0 mL of double distilled water, acidified to pH 3 with hydrochloric acid [39]. The pH of the solution was raised to ~ 10 by adding concentrated ammonia solution to precipitate the iron and aluminum. Filtered off the precipitate and washed with 3 mL portions of double distilled water. Evaporated the filtrate to $\sim 20\text{ mL}$, cooled, acidified the solution to pH 3 with hydrochloric acid, transferred into a 25 mL standard flask and diluted up to the mark with distilled water. Iron, which is commonly found in most of the soil samples, interferes and its elimination is necessary. Methods reported for the elimination of iron(III), include either precipitation as hydroxide or use of masking agents; the former is extensively used [40]. A simple spot test to find out the presence of iron(III) in filtrate is reported, which involves the use of KSCN solution, which gives red color with iron(III) [41] (1 drop of filtrate + 1 drop of $2\text{N H}_2\text{SO}_4$ + 1 drop of thiocyanate, HCl is avoided as it gives dense fumes of NH_4Cl). Absence of red color indicates that iron(III) precipitation is almost complete. About 2.5 ml of the above prepared solution was taken and was oxidized from chromium(III) to chromium(VI) [42] by adding 3 ml of bromine water and boiling for 3 min to ensure the complete oxidation of chromium. The solution was cooled and diluted to volume in a 100-ml standard flask. An aliquot of this solution was used for analysis by the proposed method. The results indicate that there is no significant difference between the proposed method and the reference method as indicated by t and f values at 95% confidence limit. The results are presented in Tables 3-8.

Table 3: Determination of chromium(VI) in selected soils by PNZ method

Soil ^a	Proposed method ^b ($\mu\text{g mL}^{-1}$)	Reference ^[38] method ($\mu\text{g mL}^{-1}$)	<i>t</i> -value ^c	<i>F</i> -value ^d
Leached chernozem	2.01±0.3	2.06±0.2	1.10	2.10
Podzolized black	2.12±0.1	2.10±0.6	0.90	0.80
Brown	3.10±0.5	3.21±0.3	0.80	1.15
Gray forest	3.23±0.4	3.27±0.7	0.70	1.20

^a Obtained from Mineralogical Institute Museum, University of Mysore.^b Average of five determination±relative standard deviation.^c Tabulated *t*-value at 95% confidence level is 2.78.^d Tabulated *F*-value at 95% confidence level is 6.39.

Table 4: Determination of chromium(VI) in selected soils by CPN method

Soil ^a	Proposed method ^b ($\mu\text{g mL}^{-1}$)	Reference ^[38] method ($\mu\text{g mL}^{-1}$)	<i>t</i> -value ^c	<i>F</i> -value ^d
Leached chernozem	2.10±0.3	2.06±0.2	0.9	1.4
Podzolized black	2.08±0.5	2.10±0.6	1.3	2.2
Brown	3.20±0.2	3.21±0.3	1.9	1.5
Gray forest	3.23±0.4	3.27±0.7	0.7	0.8

^a Obtained from Mineralogical Institute Museum, University of Mysore.^b Average of five determination±relative standard deviation.^c Tabulated *t*-value at 95% confidence level is 2.78.^d Tabulated *F*-value at 95% confidence level is 6.39.

Table 5: Determination of chromium(VI) in selected soils by TPN method

Soil ^a	Proposed method ^b ($\mu\text{g mL}^{-1}$)	Reference ^[38] method ($\mu\text{g mL}^{-1}$)	<i>t</i> -value ^c	<i>F</i> -value ^d
Leached chernozem	2.12±0.3	2.06±0.2	1.9	2.2
Podzolized black	2.23±0.5	2.10±0.6	2.1	1.8
Brown	3.24±0.2	3.21±0.3	1.4	1.5
Gray forest	3.22±0.4	3.27±0.7	0.9	0.8

^a Obtained from Mineralogical Institute Museum, University of Mysore.^b Average of five determination±relative standard deviation.^c Tabulated *t*-value at 95% confidence level is 2.78.^d Tabulated *F*-value at 95% confidence level is 6.39.

Table 6: Determination of chromium(VI) in different water and soil samples using MCP and PNZ

Sample	Proposed method			Reference method ^[38]		<i>t</i> -Value ^b	<i>F</i> -Value ^c
	Cr(VI) added ($\mu\text{g mL}^{-1}$)	Cr (VI) recovered ($\mu\text{g mL}^{-1}$)	Recovery (%) ±R.S.D. ^a	Cr(VI) recovered ($\mu\text{g mL}^{-1}$)	Recovery (%) ±R.S.D. ^a		
Well Water	0.300	0.304	101.33±0.62	0.296	98.66±0.83	1.76	5.21
	0.650	0.640	98.46±0.91	0.645	99.23±0.50	1.53	2.90
	0.900	0.901	100.11±0.31	0.895	99.44±0.35	1.40	4.38
Lake water	0.300	0.296	98.66±0.82	0.304	100.33±0.61	2.43	4.10
	0.650	0.654	100.61±0.50	0.660	101.54±0.72	1.70	3.86
	0.900	0.910	101.11±0.67	0.912	101.33±0.48	1.10	5.70
Tap water	0.300	0.301	100.33±0.72	0.303	101.00±0.54	2.25	2.80
	0.650	0.648	99.69±0.67	0.648	99.60±0.75	2.00	2.65
	0.900	0.896	99.55±0.82	0.921	102.33±0.82	1.53	4.98
Farmland soil	0.300	0.295	98.33±0.58	0.307	101.47±0.53	1.90	5.64
	0.650	0.646	99.38±0.43	0.640	98.46±0.65	2.20	4.70
	0.900	0.913	101.44±0.56	0.890	98.88±0.57	1.70	2.65
Road side soil	0.300	0.305	101.66±0.75	0.294	98.00±0.40	1.64	5.60
	0.650	0.656	100.92±0.78	0.658	101.23±0.84	1.88	3.86
	0.900	0.920	102.22±0.49	0.907	100.77±0.62	1.60	1.96
Manured garden soil	0.300	0.292	97.33±0.21	0.310	103.33±0.48	2.20	2.30
	0.650	0.640	98.46±0.58	0.670	103.07±0.32	1.98	4.25
	0.900	0.890	98.88±0.39	0.932	103.55±0.91	1.76	2.58

^a - Average of five-determination. RSD - relative standard deviation.^b - Tabulated *t*-value at 95% confidence level is 2.78.^c - Tabulated *F*-value at 95% confidence level is 6.39.

Table 7: Determination of chromium(VI) in different water and soil samples using MCP and CPN

Sample	Proposed method			Reference method ^[38]		<i>t</i> -Value ^b	<i>F</i> -Value ^c
	Cr(VI) added ($\mu\text{g mL}^{-1}$)	Cr (VI) recovered ($\mu\text{g mL}^{-1}$)	Recovery (%) \pm R.S.D. ^a	Cr(VI) recovered ($\mu\text{g mL}^{-1}$)	Recovery (%) \pm R.S.D. ^a		
Well water Mysore	0.540	0.535	99.07 \pm 0.64	0.542	100.37 \pm 0.28	2.50	2.80
	1.070	1.065	99.53 \pm 0.43	1.073	100.28 \pm 0.43	1.10	4.10
	1.700	1.695	99.71 \pm 0.38	1.687	99.23 \pm 0.54	1.88	2.65
Lake water	0.540	0.548	101.48 \pm 0.73	0.546	101.11 \pm 0.63	1.50	3.25
	1.070	1.058	98.88 \pm 0.65	1.045	97.66 \pm 0.78	1.64	4.98
	1.700	1.694	99.65 \pm 0.37	1.695	99.70 \pm 0.46	1.35	3.25
Tap water	0.540	0.538	99.63 \pm 0.67	0.535	99.07 \pm 0.93	1.80	5.64
	1.070	1.085	101.40 \pm 0.52	1.080	100.93 \pm 0.63	2.10	2.70
	1.700	1.705	100.29 \pm 0.48	1.703	100.17 \pm 0.78	1.69	1.90
Farmland soil	0.540	0.537	99.44 \pm 0.32	0.552	102.22 \pm 0.37	0.95	2.31
	1.070	1.100	102.80 \pm 0.26	1.058	98.88 \pm 0.56	1.35	4.38
	1.700	1.746	102.70 \pm 0.31	1.708	100.47 \pm 0.63	1.23	1.76
Road side soil	0.540	0.553	102.41 \pm 0.84	0.532	98.52 \pm 0.25	2.60	5.32
	1.070	1.098	102.61 \pm 0.43	1.109	103.64 \pm 0.32	1.56	5.60
	1.700	1.675	98.53 \pm 0.65	1.695	99.70 \pm 0.37	1.70	2.65
Manured garden soil	0.540	0.558	103.33 \pm 0.91	0.560	103.70 \pm 0.73	2.00	5.62
	1.070	1.084	101.31 \pm 0.74	1.090	101.87 \pm 0.52	1.60	1.77
	1.700	1.689	99.35 \pm 0.36	1.754	103.17 \pm 0.61	1.43	5.40

a - Average of five-determination. RSD - relative standard deviation.

b - Tabulated *t*-value at 95% confidence level is 2.78.c - Tabulated *F*-value at 95% confidence level is 6.39.

Table 8: Determination of chromium(VI) in different water and soil samples using MCP and TPN

Sample	Proposed method			Reference method ^[38]		<i>t</i> -Value ^b	<i>F</i> -Value ^c
	Cr(VI) added ($\mu\text{g mL}^{-1}$)	Cr (VI) recovered ($\mu\text{g mL}^{-1}$)	Recovery (%) \pm R.S.D. ^a	Cr(VI) recovered ($\mu\text{g mL}^{-1}$)	Recovery (%) \pm R.S.D. ^a		
Well water Mysore	0.800	0.812	101.50 \pm 0.67	0.815	101.87 \pm 0.72	1.10	3.86
	1.120	1.110	99.11 \pm 0.53	1.130	100.89 \pm 0.41	0.95	5.64
	1.600	1.623	101.44 \pm 1.20	1.628	101.75 \pm 0.48	1.80	5.32
Lake water	0.800	0.794	99.25 \pm 0.25	0.786	98.25 \pm 0.57	2.50	5.20
	1.120	1.121	100.09 \pm 0.62	1.140	101.78 \pm 0.82	2.30	4.30
	1.600	1.632	102.00 \pm 0.82	1.634	102.12 \pm 0.65	1.83	4.98
Tap water	0.800	0.796	99.50 \pm 0.31	0.812	101.50 \pm 0.41	1.76	3.86
	1.120	1.130	100.89 \pm 0.78	1.110	99.11 \pm 0.31	0.68	2.31
	1.600	1.597	99.81 \pm 0.57	1.594	99.62 \pm 0.50	1.90	2.30
FarmLand soil	0.800	0.810	101.25 \pm 0.75	0.806	100.75 \pm 0.30	1.60	4.10
	1.120	1.124	100.36 \pm 0.65	1.150	102.68 \pm 0.28	1.80	3.40
	1.600	1.618	101.12 \pm 0.82	1.583	98.94 \pm 0.53	1.35	4.38
Road side soil	0.800	0.822	102.75 \pm 0.41	0.804	100.50 \pm 0.61	2.00	2.56
	1.120	1.123	100.26 \pm 0.67	1.125	100.44 \pm 0.62	2.10	3.51
	1.600	1.581	98.81 \pm 0.91	1.562	97.62 \pm 0.82	1.90	4.90
Manured garden soil	0.800	0.786	98.25 \pm 0.37	0.812	101.50 \pm 0.75	1.21	5.60
	1.120	1.090	97.32 \pm 0.82	1.090	97.32 \pm 0.58	1.30	5.32
	1.600	1.649	103.06 \pm 0.53	1.651	103.18 \pm 0.38	1.53	4.64

a - Average of five-determination. RSD - relative standard deviation.

b - Tabulated *t*-value at 95% confidence level is 2.78.c - Tabulated *F*-value at 95% confidence level is 6.39.

Table 9: Determination of chromium(VI) in plating baths solutions by PNZ method

Sample ^a	Proposed method ^b ($\mu\text{g mL}^{-1}$)	Reference ^[38] method ^b ($\mu\text{g mL}^{-1}$)	t-test	F-test
Plating bath 1	1.10 \pm 0.20	1.12 \pm 0.10	2.2	1.1
Plating bath 2	1.21 \pm 0.10	1.32 \pm 0.09	1.1	1.2
Plating bath 3	0.98 \pm 0.10	1.12 \pm 0.1	0.9	1.1

^aBath 1, 2 and 3 refer to samples from three different factories.^bMean of five determinations \pm S.D.

Table 10: Determination of chromium(VI) in plating baths solutions by CPN method

Sample ^a	Proposed method ^b ($\mu\text{g mL}^{-1}$)	Reference ^[38] method ^b ($\mu\text{g mL}^{-1}$)	t-test	F-test
Plating bath 1	1.02 \pm 0.10	1.12 \pm 0.10	1.3	1.1
Plating bath 2	1.15 \pm 0.20	1.32 \pm 0.09	0.9	0.8
Plating bath 3	1.10 \pm 0.10	1.12 \pm 0.10	1.0	1.5

^aBath 1, 2 and 3 refer to samples from three different factories.^bMean of five determinations \pm S.D.

Table 11: Determination of chromium(VI) in plating baths solutions by TPN method

Sample ^a	Proposed method ^b ($\mu\text{g mL}^{-1}$)	Reference ^[38] method ^b ($\mu\text{g mL}^{-1}$)	t-test	F-test
Plating bath 1	1.15 \pm 0.10	1.12 \pm 0.10	2.0	1.4
Plating bath 2	1.30 \pm 0.09	1.32 \pm 0.09	1.3	1.1
Plating bath 3	1.05 \pm 0.10	1.12 \pm 0.10	0.9	0.7

^aBath 1, 2 and 3 refer to samples from three different factories.^bMean of five determinations \pm S.D.

Table 12 Results of chromium(VI) analysis in urine sample

	Cr(VI) added added($\mu\text{g mL}^{-1}$)	Proposed method		Reference method ^[38]		t-Value ^c	F-Value ^d
		Cr(VI) recovered ($\mu\text{g mL}^{-1}$)	Recovery (%) \pm R.S.D. ^b	Cr(VI) recovered ($\mu\text{g mL}^{-1}$)Sample ^a	Recovery (%) \pm R.S.D. ^b		
1	0.300	0.305	101.66 \pm 0.48	0.310	103.33 \pm 0.82	2.10	4.20
2	0.650	0.646	99.38 \pm 0.25	0.639	98.31 \pm 0.72	1.70	5.25
3	0.900	0.908	100.88 \pm 0.53	0.885	98.33 \pm 0.91	1.84	3.40

^aSamples obtained from healthy persons who were kept on diet free of vitamin C, meat, fish, peroxidase containing vegetables and drugs.^bAverage of five determination \pm relative standard deviation.^cTabulated t-value at 95% confidence level is 2.78.^dTabulated F-value at 95% confidence level is 6.39.

Waste Waters from Plating Baths: The solution obtained from waste chromium baths was determined for chromium by taking 2.5 mL of the sample and diluting to 25 mL with distilled water. Bromine water (3 mL) was added and boiled for 3 min to convert all chromium(III) to oxidized chromium(VI). The solution was cooled and made up to 100 mL in a standard flask. Standard addition test was conducted to enhance the reliability of the method. The results are presented in Tables 9, 10 and 11.

Biological Samples

Direct Determination in Human Urine: As mentioned earlier, chromium is an essential trace nutrient for humans. Mertz [43] has proposed urinary chromium excretion as a means of assessing the chromium nutritional status of individuals. Most of the values for chromium in urinary samples lie in the 2 - 20 ng mL⁻¹ range; this presented a

serious dilemma from the nutritional standpoint. The amount of chromium absorbed had been determined by radiotracer experiments to be of 0.5-1.0% [44], so daily urinary excretions of the order of 10 $\mu\text{g/day}$ meant that dietary chromium intake should be more than 1 mg/day.

So far, no reasonable diets in the U.S. could supply more than 100 $\mu\text{g/day}$. Hence, chromium is being supplemented through B-complex vitamin formulations.

Sampling: Urine samples were collected from healthy individuals and also from those who are on supplementary chromium intake in the form of vitamin B-complex formulation. These samples varied considerably in color, salt content and chromium concentration and are considered to be representative samples from various types of subjects, who are usually encountered in metabolic studies.

Procedure: Urine is a biological fluid, which contains many inorganic and organic constituents and most reliable results are obtained when subjected to the method of addition. Five mL of each urine sample were transferred into five, 25 mL standard flasks. Of these, to four standard flasks, 1.0 mL each of the solutions containing 0.30, 0.65 and 0.90 μg chromium mL^{-1} was added. Aliquots of these solutions were analyzed by the proposed method and the results are presented in Table 12.

CONCLUSION

Most of the spectrophotometric methods for chromium(VI) determination have draw backs such as high reagent cost, instability and impossibility of regeneration of reagent for re-use, small dynamic linear range, low sensitivity and selectivity. Use of phenoxazine (PNZ), 2-chlorophenoxazine (CPN) and 2-trifluoromethylphenoxazine (TPN) along with cisapride (CSP) and metaclopramide (MCP) offers clear advantages over most of the chromogenic reagents currently being used for the purpose and the proposed procedure shows definite positive features compared to the existing methods.

In order to overcome the difficulties with the existing systems, the proposed methods have such advantages as high reliability, reproducibility, sensitivity and selectivity. The proposed methods have been successfully applied for the determination of chromium(VI) in soil, water and selected biological samples. Statistical analysis of the results proved that the proposed methods give very accurate and reproducible values comparable to standard reference method. Application of these methods in the determination of chromium(VI) in a variety of real samples has demonstrated their practical utility.

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