Phenoxazines as new spectrophotometric reagent for the determination of chromium(VI) in environmental samples

The name chromium was derived from the Greek word chrooma which means color, in reference to the fact that chromium is known to cause a number of colors in a variety of materials. For example, the green color of emerald is caused by the presence of very small amounts of chromium in the crystal. Geologists estimate that there are about 11 billion tons of chromium ore (chromite) in the world that could be mined.

Chromium is present in the environment in several different forms, the most common being trivalent chromium and hexavalent chromium. Trivalent compounds do not cause any serious damage. The toxic action of chromium is confined to the hexavalent compound, which is a highly toxic carcinogen and may cause death to humans and animals if ingested in large doses. Chromium(VI) exists in food, air, water and soil as a result of human activities. Cr(VI) is quite soluble and is readily leached from soil to groundwater or surface water.

Abstract

Phenoxazine (PNZ), chlorophenoxazine (CPZ) and trifluoromethylphenoxazine (TMP) were investigated as new class of spectrophotometric reagents for determination of chromium(VI) in presence of sulfa drugs as new electrophilic coupling reagent. The reaction was carried out in hydrochloric acid medium. The red color formed indicated maximum absorbance at 540 nm for PNZ and 510 nm for CPZ, TMP method. The methods obeyed Beer’s law. The color developed was stable for 4 h at room temperature. The molar absorptivity and Sandell’s sensitivity gave different values with different reagents. Interference was not observed for the most common ion present in different environmental samples. The methods showed good reproducibility and can be satisfactorily applied for the determination of chromium in different environmental and biological samples.

Keywords: Spectrophotometry; phenoxazines; sulphonamidazoles; sulphonamides; sulphanamethoxazole; sul-phadoxine; chromium(VI)

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V.1. Introduction

Chromium(VI) as toxicant enters the environment from industrial effluent or waste disposal sources, such as waste from steel works, electroplating and tanning industries [1]. Traces of chromium may also enter drinking water supply system. chromium(III) and (VI) are two common forms of inorganic chromium in the environment. There are many differences in their biochemical properties and toxicity. For example, a trace amount of Cr(III) is essential to human beings. While, Cr(VI) is harmful to human health, especially because it is known to be a strong oxidizing agent, posing a high risk to humans and animals due to its carcinogenic and mutagenic properties [2]. Thus, the determination of chromium in environmental and biological samples is of great interest.

The methods proposed for the determination of metals in soil and industrial effluent matrices include electroanalytical [3], radioanalytical [4] and chromatography [5]. However, these methods have proved to be deficient with respect to specificity, sensitivity, simplicity and analysis time. Various 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.

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 suitable 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.

We propose an analytical procedure for determination of chromium(VI) in environmental and biological sample by spectrophotometry, using phenoxazines as chromogenic agent in presence of sulfanilamide (SAA), sulfadoxine (SDX), sulfamethoxazole (SMX) or sulfadazine (SDZ). Different experimental conditions like wavelength, the effect of acid, the amount of chromogenic agent, the effect of coexistence ions, limit of detection and the ranges of applicability of Beer's law on the determination of chromium(VI) have been studied
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V.2. Experimental

V.2.1. Apparatus

Specord 50 UV-Vis spectrophotometer with 1.0 cm silica quartz matched cell was used for measuring the absorbance.

V.2.2. Reagents

Phenoxazines (Aldrich, India), sulphanilamide (SAA), sulphadoxine (SDX), sulphamethoxazole (SMX) and sulfadazine (SDZ) (Smithkline Beecham, India) were used as received. Distilled water was used to prepare all solutions. Distilled ethyl alcohol was used to dissolve PNZ and a minimum amount of 1M hydrochloric acid was used to increase solubility of SDX and SMX.

Stock solutions of chromium(VI) (1000 µg ml\(^{-1}\)) was prepared by dissolving known amount of potassium chromate in 100 ml of distilled water. Solutions of required strength were prepared by diluting the stock solution with distilled water. Fresh solutions of phenoxazine (PNZ), chlorophenoxazine (CPZ) and trifluoromethylphenoxazine (TMP) (0.025%, w/v) were prepared by dissolving 25 mg each of the sample in 100 ml distilled ethyl alcohol. Aqueous solutions of SAA, SDX, SMX and SDZ (0.05%, w/v) were prepared by dissolving 50 mg each and diluting quantitatively to 100 ml with distilled water. Two ml of 1M hydrochloric acid was added during the preparation of SDX and SMX to enhance the solubility. Solutions of diverse ions were prepared by dissolving their corresponding salts. All reagents used were of analytical grade and were used as received.

V.2.3. Analytical Procedure

To a series of 25 ml standard flasks, 2 ml of SAA/SDX/SMX/SDZ (0.05 %, w/v), 1 ml of 2 M HCl, 2 ml of PNZ/CPZ/TPM (0.025%, w/v) and different aliquots of standard solutions of chromium(VI) were added. The solutions were mixed well and allowed to stand for 5 min at room temperature. The solutions were made up to the mark with distilled water. The absorbance was measured at 540 nm for PNZ and 510 nm for CPZ/TMP method against the corresponding reagent blank prepared under identical conditions but without chromium.
The calibration graph was constructed. Concentration of chromium in test solution was calculated from the regression equation computed from the Beer's law data as a reference. The optical characteristics for the determination of chromium with PNZ/CPZ/TMP using SAA/SDX/SMX/SDZ are detailed in the Table V.1.

V.3. Results and discussion

Phenoxazine is an isolog of phenothiazine. It is a part of the chemical structure of actinomycin D, which is known to exert intensive anticancer activity on malignant tumors in children [6] and is reported to be more potent and less toxic chemosensitizer [7]. Phenoxazine derivatives exist in neutral form, as monocations, as dications and even as trications depending on the environment [8]. Their molecular structure and luminescent properties have been studied to a great extent [9]. Besides, they have impressive applications as biological stains [10], as laser dyes [11] and as redox indicators [12]. Phenoxazine derivatives are nervous system depressants particularly with sedative, antiepileptic, tranquillizing activity [13], spasmylytic activity [14], antitubercular activity [15] and anthelmentic activity [16]. In recent years phenoxazine derivatives are reported to be potential chromophoric compounds in host-guest artificial photonic antenna systems [17].

The sulphonamides are analogues of p-aminobenzoic acid and are known since 1932. Though, a large number of sulphonamides are synthesized and reported in the literature, only about two dozens of them have been used in clinical practice [18]. They differ only slightly in their antimicrobial activity but vary greatly in their pharmacokinetic properties, or rate of excretion. Accordingly, they are classified as short-, medium-, long- and ultra-long acting drugs [19]. Sulfanilamide (SAA) belongs to short-acting, sulfamethoxazole (SMX) medium-acting and sulfadoxine (SDX) and sulfadazine (SDZ) ultra-long acting sulphanomides.

V.3.1. Spectral characteristics

The absorption spectrum of the red colored products with chromium shows a wavelength of maximum absorption at 540nm for PNZ and 510 nm for CPZ/TMP. The reagent blank shows negligible absorption at these wavelengths.
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V.3.2. Optimization of analytical variables

The reagent concentrations and quantity needed as also the reaction conditions were optimized to arrive at a standard procedure. Each parameter was optimized by setting other parameters constant.

V.3.3. Order of addition

During the course of the investigation, it was observed that the sequence of addition of reactants was also important as it influence the intensity and the stability of the color of the product to a great extent. The sequence (i) Cr(VI) + SAA/SDX/SMX or SDZ + PNZ/CPZ/TMP + HC1 and (ii) Cr(VI) + PNZ/CPZ/TMP + HC1 + SAA/SDX/SMX or SDZ gave less intense and unstable color. While, (iii) SAA/SDX/SMX or SDZ + HC1 + Cr(VI) + PNZ/CPZ/TMP gave more intense and stable red color.

V.3.4. Effect of reagents

The effect of PNZ/CPZ/TMP reagent was studied in the range of 0.10 - 5.00 ml of (0.025%, w/v) solution of each to achieve the maximum color intensity, volume of 0.50 - 3.00 ml of the solution gave good result. Hence, 2 ml of (0.025 %, w/v) PNZ/CPZ/TMP solutions in 25 ml standard flask was selected for further studies, under optimized conditions. The 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 1.0-6.0 ml of 2M HCl. Therefore, 1 ml of 2M HCl in 25 ml standard flask was used for getting the best results. Similarly, the same procedure was adopted to ascertain the amount of SAA, SDX, SDZ and SMX required for getting constant and maximum color intensity. It was found that 0.50 - 3.00 ml of the solution were needed to get good result. Hence, 2 ml of (0.05%, w/v) SAA, SDX, SMX or SDZ solutions are sufficient to get reproducible results.

Experiments were carried out to optimize temperature and time of the reaction. It was found that the maximum color developed in 5 min at room
temperature and remained almost stable for about 4 h. Increase in the temperature decreased the intensity of the red color.

**V.3.5. Analytical Parameters**

The colored products obeyed Beer’s law. The optical characteristics such as optimum range for the determination of the ions, molar absorptivity, sandell’s sensitivity, slope, intercept, correlation coefficient are presented in Table V.1 which conclusively proves that the reagents are sensitive.

The accuracy and precision of the proposed methods were evaluated by performing recovery tests by standard addition method. These tests were performed by adding known amounts of standard solutions at different concentration levels to a fixed amount of real samples and the mixtures were analyzed by the proposed procedures. Each test was repeated five times.

**V.3.6. Interferences**

In order to establish the analytical potential of proposed method, the effect of some possible interfering ions which often accompany chromium(VI) was examined in presence of number of other ions by the proposed methods. An ion was considered to be interfered with the determination if the obtained absorbance values differed by more than ±3% from that of chromium(VI) alone. Metals such as iron(III), vanadium(V) manganese(VII) and cerium(IV), non metals such as bromate, iodate and periodate were found to interfere severely and caused low recovery of chromium(VI). However, using appropriate masking agents could eliminate the interference from these ions. Masking agents such as EDTA, tartrate and citrate did not interfered in the determination of chromium(VI). The use of a mixture of tartaric acid (50 mg) and citric acid (5 mg) has been found to have effective masking action on a large number of foreign metal ions. During the interference studies, if a precipitate was formed, it was removed by centrifugation. The maximum tolerable concentrations of different ions (defined as the foreign-ion concentration causing an error smaller than ±3% for determining the analyte of interest) in the determination of chromium(VI) are listed in Table V.2.
<table>
<thead>
<tr>
<th>Parameters</th>
<th>SMX</th>
<th>SAA</th>
<th>SDX</th>
<th>SDZ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PNZ</td>
<td>CPZ</td>
<td>TMP</td>
<td>PNZ</td>
</tr>
<tr>
<td>λmax (nm)</td>
<td>540</td>
<td>510</td>
<td>510</td>
<td>540</td>
</tr>
<tr>
<td>Stability (h)</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Beer's law range (µg ml⁻¹)</td>
<td>0.2-1.4</td>
<td>0.9-3.5</td>
<td>0.9-3.2</td>
<td>0.4-2.1</td>
</tr>
<tr>
<td>Recommended ion concentration (µg ml⁻¹)</td>
<td>0.6</td>
<td>1.9</td>
<td>1.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Molar absorptivity (L mol⁻¹ cm⁻¹) x 10⁵</td>
<td>4.9</td>
<td>1.8</td>
<td>1.9</td>
<td>3.4</td>
</tr>
<tr>
<td>Sandell's sensitivity (µg ml⁻¹)</td>
<td>0.001</td>
<td>0.002</td>
<td>0.002</td>
<td>0.001</td>
</tr>
<tr>
<td>Regression equation *</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope (a)</td>
<td>0.2678</td>
<td>0.1473</td>
<td>0.1561</td>
<td>0.220</td>
</tr>
<tr>
<td>Intercept (b)</td>
<td>0.1110</td>
<td>0.0026</td>
<td>-0.006</td>
<td>0.051</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9925</td>
<td>0.9915</td>
<td>0.998</td>
<td>0.996</td>
</tr>
<tr>
<td>R.S.D. **% (n=5)</td>
<td>0.92</td>
<td>0.06</td>
<td>1.00</td>
<td>0.98</td>
</tr>
</tbody>
</table>

* y = ax + b where x is the concentration of Cr(VI) in µg ml⁻¹
** Relative Standard Deviation,
 n = number of the replicates
Table V. 2. Effect of diverse species in the determination chromium(VI)

<table>
<thead>
<tr>
<th>Foreign ions</th>
<th>Tolerance limit (µg ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bi³⁺, Ba²⁺, Ti⁴⁺, EDTA, Br⁻, citrate, tartarate, oxalate</td>
<td>200</td>
</tr>
<tr>
<td>Na⁺, Mg²⁺, Al³⁺, Zn²⁺, Pb²⁺, Cd²⁺, K⁺, Hg²⁺, Ni²⁺, CH₃C0O⁻</td>
<td>100</td>
</tr>
<tr>
<td>NO₃⁻, Cl⁻, Br⁻, SO₄²⁻, NH₄⁺, CO₂⁺, Cu²⁺, PO₄³⁻</td>
<td>50</td>
</tr>
<tr>
<td>Chloramine-T, Chloramine-B, IO₃⁻, IO₄⁻, BrO₃⁻</td>
<td>0.2</td>
</tr>
</tbody>
</table>

V.3.7. Applications

V.3.7.1. Collection, preparation and determination of soil samples

Soil samples were collected about 0.5 km. away from 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. Static sampling procedure was adopted and six soil samples were collected at random from depth of 0-20 cm with a distance of about 50 m between each sampling site.

Known amount of soil sample (5.0 g) was taken in a platinum crucible and then heated it 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 sulfuric acid and 10.0 ml of concentrated hydrofluoric acid and heated on a sand-bath until vapours of SO₃ appear. The residue was dissolved in 5.0 ml of double distilled water acidified with hydrochloric acid to pH 3 [20]. The pH of the solution was raised to ~10 by the addition of concentrated ammonia solution to precipitate the iron and aluminum. Filtered off the precipitate with a suitable filter paper and washed it with 3-ml portions of double distilled water. Evaporated the filtrate to ~ 20 ml, cooled, acidified the solution to pH 3 with hydrochloric acid, transferred it 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 in the literature for the elimination of iron(III) interference, include: either precipitation as hydroxide or use of masking agents. The former method is extensively used [21]. We have reported a simple spot test to know the presence of iron(III) in filtrate. This involves the use of KSCN solution which gives red color with iron(III) [22] (1 drop of filtrate + 1 drop of 2N H$_2$SO$_4$ + 1 drop of thiocyanate, HCl is avoided as it gives dense fumes of NH$_4$Cl). Absence of red color is an indication that iron (III) precipitation process is almost complete.

About 2.5 ml or other suitable aliquot of the above prepared solution was taken and oxidized from chromium(III) to chromium(VI) [9] by adding 3 ml of bromine water and boiled for 3 min to ensure that all chromium(III) was oxidized to chromium(VI). The solution was cooled and diluted to volume in a 100-ml standard flask. An aliquot of this solution was taken for analysis by the proposed method. The results are presented in Table V.3.

**V.3.7.2. Waste waters from industries (plating baths)**

The solution were obtained from three different waste chromium baths and was determined 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 ensure that all chromium (III) was oxidized to chromium(VI). The solution was cooled and made up to 100 ml in a standard flask. To enhance the reliability of the method standard addition test was conducted.

The above solutions were transferred to ten 25-ml standard flasks and were analyzed by proposed methods. The results are presented in Table V.3.

**V.3.7.3. Biological samples.**

As mentioned earlier, chromium is an essential trace nutrient for humans. Mertz [23] has proposed urinary chromium excretion as a means of accessing the chromium nutritional status of individuals. Determination of chromium in biological materials is a serious problem and the reported values for the same are different matrices differed by over an order of magnitude.
Table V.3. Determination of chromium(VI) in soil and waste waters from industries (plating baths)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Proposed method</th>
<th>Reported method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cr added (µg/ml)</td>
<td>Cr recovered (µg/ml)</td>
</tr>
<tr>
<td>Soil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample-1</td>
<td>0.600</td>
<td>0.592</td>
</tr>
<tr>
<td>Sample-2</td>
<td>0.600</td>
<td>0.590</td>
</tr>
<tr>
<td>Sample-3</td>
<td>0.600</td>
<td>0.599</td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample-1</td>
<td>0.600</td>
<td>0.605</td>
</tr>
<tr>
<td>Sample-2</td>
<td>0.600</td>
<td>0.609</td>
</tr>
<tr>
<td>Sample-3</td>
<td>0.600</td>
<td>0.616</td>
</tr>
</tbody>
</table>

Table V.4. Determination of chromium(VI) in biological sample

<table>
<thead>
<tr>
<th>Subject&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Proposed method</th>
<th>Reported method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cr added (µg/ml)</td>
<td>Cr recovered (µg/ml)</td>
</tr>
<tr>
<td>1</td>
<td>0.600</td>
<td>0.596</td>
</tr>
<tr>
<td>2</td>
<td>0.600</td>
<td>0.609</td>
</tr>
<tr>
<td>3</td>
<td>0.600</td>
<td>0.590</td>
</tr>
</tbody>
</table>

<sup>a</sup>Samples obtained from healthy persons who were kept on diet free of vitamin C, meat, fish, peroxidase containing vegetables and drugs.

<sup>b</sup>Average of five determination ± relative standard deviation

<sup>c</sup>Tabulated t-value at 95% confidence level is 2.44

<sup>d</sup>Tabulated F-value at 95% confidence level is 4.28
Most of the values 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 to be of the order of 0.5-1% by radiotracer experiments [24], so daily urinary excretions of the order of 10 µg/day meant that dietary chromium intake had to be more than 1 mg/day. So far no reasonable diets in the U.S. were found which could supply more than 100 µg/day. Hence, chromium is supplemented through B-complex formulations.

V.3.7.4. Sampling

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

Five ml of each of urine sample were transferred into five 25-ml standard flasks. To four standard flasks 1 ml each of the solutions containing 1.2, 1.3, 1.4 and 1.5 µg Cr ml$^{-1}$ was added. Aliquots of this solution were analyzed by proposed method. The results are presented in Table V.4.

V.6. Conclusion

The proposed methods are simple, inexpensive, sensitive and precise also has the advantage of determination without the need for extraction or heating. The method does not involve complicated reaction conditions. This is the first time that spectrophotometric methods are being reported using phenoxazines as chromogenic agent and SAA, SDX, SMX and SDZ as electrophilic coupling agent for determination chromium(VI) in environmental and biological samples. Statistical analysis of the results revealed that the proposed method yield accurate and reproducible values in the determination of chromium(VI) in various soils and industrial effluent matrices. Applications of the proposed method in the determination of chromium(VI) in a variety of real samples have demonstrated their practical utility.
References