Liquid Waste Management
Phenoxazines: a new class of reagent for spectrophotometric determination of manganese

Manganese is one of the most abundant metals in soils, where it occurs as oxides and hydroxides and it cycles through its various oxidation states. It is one out of three toxic essential trace elements, which means that it is not only necessary for humans to survive, but it is also toxic when too high concentrations are present in a human body. Manganese is supplemented to the diet through a variety of foods. These include bread, nuts, cereals and green vegetables (such as peas and runner beans); it's also found in tea, which is probably the biggest source of manganese for many people. However, taking high doses of manganese for long periods of time might cause nerve damage and neurological symptoms such as fatigue and depression. Manganese can also cause Parkinson, lung embolism and bronchitis.

Therefore, it is necessary to establish sensitive and accurate analytical methods for quantitative determination of manganese.

[http://www.lenntech.com/Periodic-chart-elements/Mn-en.htm]
**Abstract**

A sensitive, selective and rapid spectrophotometric method for the determination of trace amounts of manganese has been described. Phenoxazine (PNZ), chlorophenoxazine (CPZ) and trifluoromethylphenoxazine (TMP) were investigated as new class of spectrophotometric reagents for determination of manganese(II) in presence of sulfa drugs as new electrophilic coupling reagent. The reaction was carried out in hydrochloric acid medium. The red color obeyed Beer's law. The color developed was stable for 4h 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 manganese in vegetables and foodstuff samples.

**KEYWORDS:** Spectrophotometry; phenoxazines; sulphanilamide; sulphafoxine; sulphamethoxazine; sulfadazine; manganese(II)

**Hazardous materials (Communicated)**
Chapter IV

Phenoxazines.............. ....... manganese

IV.1. Introduction

Manganese is both micronutrient as well as toxic element for living beings, depending on the concentration level [1]. Manganese is actively absorbed by plants and has an effect on the fertility of soils [2]. The metal plays important role in biological systems. It contributes to the formation of anterior pitutary hormones, vitamin B₁ and C, affecting hematopoiesis, oxidation and proteometabolism in the body. Besides, many enzymes such as dipeptidase, arginase and phosphatase require manganese for proper function. However, an excess of manganese in food or pharmaceuticals will be hazardous to human health. Therefore, it is necessary to establish sensitive and accurate analytical methods for quantitative determination of manganese.

There are several methods available for manganese(VII) determination including atomic absorption spectroscopy (AAS), flow-injection analysis (FIA), spectrofluorimetry [3] and spectrophotometry. In routine analysis, spectrophotometric methods are versatile and economical especially for developing countries. Such oxidants as potassium periodate and ammonium persulfate are commonly used for determination of manganese(VII) by spectrophotometry [4-8]. However, the methods mentioned above are time consuming or less sensitive.

The reagents suggested for determination of manganese include: ethylenebis(triphenylphosphonium)[9]; isophtaldihydroxamic acid [10]; 1,10-phenantroline[11]; 8-hydroquinoline[12]; 4-(2-pyridylazo) resocrinol [13]; 1-(2-pyridylazo)-2-naphtol[14,15]; N—diphenylbenzamidine [16]. Some of the above mentioned extraction methods are characterized by a long procedure, are not selective [11-15], low stability of the complexes obtained [9,10] and low sensitivity [10].

This paper proposes an analytical procedure for determination of manganese(VII) in vegetables and foodstuffs sample by spectrophotometry, using phenoxazines as chromogenic agent in presence of sulfanilamide (SAA), sulfadoxine (SDX), sulfamethoxazole (SMX) or sulfadazine (SDZ). Different experimental conditions, e.g. wavelength, the effect of acid, the amount of chromogenic agent, the effect of coexistence ions and the ranges of applicability of Beer's law on the determination of manganese(VII) have been studied.
IV.2. Experimental

IV.2.1 Apparatus

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

IV.2.2 Reagents

Phenoxazines (Aldrich, India), sulphanilamide (SAA), sulphadoxine (SDX), sulphamethaxazole (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 manganese(VII) (1000 µg ml⁻¹) was prepared by dissolving known amount of potassium permanganate 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) was prepared by dissolving 25 mg 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 diluting quantitatively to 100 ml with distilled water, 2.0 ml of 1M hydrochloric acid was added during the preparation of SDX and SMX. Solutions of diverse ions were prepared by dissolving their corresponding salts. All reagents used were of analytical grade and were used as received.

IV.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/TMP (0.025%, w/v) and different aliquots of standard solutions of manganese(VII) 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 manganese and the calibration graph was constructed. Concentration of manganese 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 manganese with PNZ/CPZ/TMP using SAA/SDX/SMX/SDZ are detailed in the Table IV.1.

IV.3. Results and Discussion

Phenoxazine derivatives exist in neutral form, as monocations, as dications and even as trications depending on the environment [17]. Phenoxazine derivatives are of considerable interest because of their important and impressive number of applications, example; as biological stains [18], as laser dyes [19] and as redox indicators [20]. Phenoxazine derivatives are nervous system depressants particularly with sedative, antiepileptic, tranquillizing activity [21], spasmylytic activity [22], antitubercular activity [23] and anthelmentic activity [24]. In recent years phenoxazine derivatives are reported to be potential chromophoric compounds in host-guest artificial photonic antenna systems [25].

Sulfanilamide (SAA), sulfadoxine (SDX), sulfamethoxazole (SMX) and sulfadazine (SDZ) are the chemicals which contain aromatic primary amino group. 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 [26]. Sulfanilamide (SAA) belongs to short-acting, sulfamethoxazole (SMX) medium-acting and sulfadoxine (SDX) and sulfadazine (SDZ) ultra-long acting sulphanomides.

IV.3.1. Spectral characteristics

The absorption spectrum of the red colored products with manganese 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.

IV.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.
IV.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 great extent. The sequence (i) SAA/SDX/SMX/SDZ+HCl+Mn(VII)+PNZ/CPZ/TMP and (ii) Mn(VII)+HCl+PNZ/CPZ/TMP+SAA/SDX/SMX/SDZ gave less intense and unstable color. While, (iii) SAA/SDX/SMX/SDZ+HCl+PNZ/CPZ/TMP+Mn(VII) gave more intense and stable red color.

IV.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 1.0-5.0 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 minute at room temperature and remained almost stable for about 4 h. Increase in the temperature decreased the intensity of the red color.

IV. 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
Table IV.1. Spectral data for the determination of manganese(VII)

<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>0.2-1.6</td>
<td>0.2-1.4</td>
<td>0.6-2.0</td>
<td>0.4-1.4</td>
</tr>
<tr>
<td>Recommended ion concentration</td>
<td>0.8</td>
<td>0.7</td>
<td>1.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Molar absorptivity</td>
<td>2.40</td>
<td>2.17</td>
<td>1.74</td>
<td>2.21</td>
</tr>
<tr>
<td>(L mol⁻¹ cm⁻¹) x 10⁵</td>
<td>2.28</td>
<td>2.53</td>
<td>3.15</td>
<td>2.26</td>
</tr>
<tr>
<td>Sandell's sensitivity (µg ml⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(x 10⁻⁵)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression equation *</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope (a)</td>
<td>0.234</td>
<td>0.313</td>
<td>0.280</td>
<td>0.206</td>
</tr>
<tr>
<td>Intercept (b)</td>
<td>0.034</td>
<td>0.062</td>
<td>0.050</td>
<td>0.2066</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.970</td>
<td>0.993</td>
<td>1.004</td>
<td>0.997</td>
</tr>
<tr>
<td>R.S.D. **% (n=5)</td>
<td>1.10</td>
<td>0.79</td>
<td>1.02</td>
<td>1.51</td>
</tr>
</tbody>
</table>

* y = ax + b where x is the concentration of Mn(VII) in µg ml⁻¹
** Relative Standard Deviation,
 n – number of the replicates
sensitivity, slope, intercept, correlation coefficient are presented in Table VI.1, which conclusively proves that the reagents are sensitive.

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

**IV.3.6. Interferences**

In order to establish the analytical potential of proposed method, the effect of some possible interfering ions which often accompany manganese(VII) 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 manganese(VII) alone. Metals such as Iron (III), vanadium(V) chromium(VII) and cerium(IV), non metals such as bromate, iodate and periodate were found to be interfered severely and caused low recovery of manganese(VII). However, using appropriate masking agents could eliminate the interference from these ions. Masking agents such as EDTA, tartrate and citrate were not interfered in the determination of manganese(VII). 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 manganese(VII).

**IV. 3.7. Applications**

**IV.3.7.1. Analysis of vegetable samples**

Prior to the determination, the vegetable samples were pretreated in the following way. An edible portion of vegetable was firstly washed clean with tap water and then rewashed with deionized water. After removing deionized water on the surface of vegetables, the sample was cut into small pieces and dried at 65°C in oven. Then the sample was ground and passed through a 60-mesh sieve. The
Table IV.2. Determination of manganese(VII) in vegetable samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Proposed method</th>
<th>Reported method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mn added (µg/ml)</td>
<td>Mn recovered (µg/ml)</td>
</tr>
<tr>
<td>Potato</td>
<td>0.500</td>
<td>0.510</td>
</tr>
<tr>
<td>Cucumber</td>
<td>0.500</td>
<td>0.490</td>
</tr>
<tr>
<td>Lettuce</td>
<td>0.500</td>
<td>0.499</td>
</tr>
</tbody>
</table>

Table IV.3. Determination of manganese(VII) in foodstuff samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Proposed method</th>
<th>Reported method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mn added (µg/ml)</td>
<td>Mn recovered (µg/ml)</td>
</tr>
<tr>
<td>Rice</td>
<td>0.500</td>
<td>0.490</td>
</tr>
<tr>
<td>Wheat</td>
<td>0.500</td>
<td>0.516</td>
</tr>
<tr>
<td>Tea</td>
<td>0.500</td>
<td>0.510</td>
</tr>
<tr>
<td>Horsebean</td>
<td>0.500</td>
<td>0.490</td>
</tr>
</tbody>
</table>

*Average of five determination ± relative standard deviation

*Tabulated t-value at 95% confidence level is 2.18

*Tabulated F-value at 95% confidence level is 4.28
powdered sample (0.5 g) was accurately weighed and placed in a 100 ml beaker followed by the addition of nitric acid and excess of hydrochloric acid (1:3), 10 ml of concentrated nitric acid (1:1). The mixture was digested by heating and evaporated carefully nearly to dryness, a little of water was added and evaporated again. Finally, the residue was dissolved in water and the pH value of the solution was adjusted to 4. The solution was brought to 50 ml in a calibrated flask with water. An aliquot of this solution was taken for analysis by the proposed method. The results are presented in Table IV.2.

IV.3.7.2 Analysis of foodstuff samples

All foodstuff samples were dry. Each sample was first ground in a mortar. Then, 1.0 g of sample (0.1g tea) was weighed accurately and placed in a porcelain crucible. After charred on an electric furnace, the sample was ashed at 55°C in a muffle furnace. Then, 2 ml of HCl and 10 ml of water were added into the ash. The sample was heated below the boiling point for a moment. The solution was cooled and diluted to 100 ml water in a calibrated flask and mixed well. At last, a suitable amount of the sample solution and the stock solution of manganese(VII) were added into 25 ml standard flask for the determination of manganese(VII) by the recommended procedure.

IV.4. Conclusion

The proposed methods have distinct advantages of simplicity, sensitivity, selectivity and reproducibility. Besides, it is superior to other reported method for the determination of trace amounts of manganese(VII), as the procedure is subject to noninterference from other ions. Furthermore, the use of phenoxazines as electrophilic reagents in the determination of essential metals will open up new areas of research. A value-addition to this method can be achieved, if the procedure is combined with on-line are at-line system and this is currently under investigation. Statistical analysis of the results revealed that the proposed method yield accurate and reproducible values in the determination of manganese(VII) in various vegetable and foodstuff samples. Applications of the proposed method in the determination of manganese(VII) in a variety of real samples have demonstrated their practical utility.
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References

    49.
    Commun. 2 (1990) 249.
    Chemical Society, Washington, DC 1948.