Spectrophotometric determination of Fe(III) via complexation with piroxicam in synthetic mixture and soil samples

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Received 08 September 2009; revised 03 December 2009; accepted 11 December 2009

A simple and selective spectrophotometric method has been described for determination of Fe(III) in synthetic mixture and soil samples. Method is based on chelation of ferric ion with piroxicam to produce a coloured metal complex, which absorbs maximally at 495 nm. Beer’s law is obeyed (conc., 2.79-25.13 mg ml−1) with apparent molar absorptivity (5.88 × 103 l mol−1 cm−1) and Sandell’s sensitivity (0.019 mg/cm2/0.001 absorbance unit). Proposed method was successfully applied in determination of Fe(III) in synthetic mixture and soil samples.

Keywords: Ferric sulphate, Metal chelate, Piroxicam, Spectrophotometry, Validation

Introduction
Iron (Fe), one of the most important constituent of biological systems as well as of metallurgical industry, reacts with di-antipyrene methane to form a red coloured complex, which absorbs maximally at 520 nm1. Trace amount of Fe (III) was determined by complexation with potassium thiocyanate and methyl violet in presence of polyvinyl alcohol2. Colour reaction3 between Fe(III) and p-diethyl-amine phenyl fluorine (conc., 0.056-6.7 µg / 25 ml) at pH 9.0 was utilized for determination of Fe(III). A number of kinetic spectrophotometric methods have been utilized for Fe (III) determination using methyl thymol blue at pH 3.54, azocarmine B and potassium periodate5, chlorophosphonazo and potassium bromate6, methyl orange and potassium periodate7, [4,4’-bis(dimethyl-amino) diphenyl-methane], potassium bromate and potassium iodide8, orange G and potassium periodate9, methylene blue and hydrogen peroxide10. Various reagents (4-aminoantipyrene11, 2-hydroxy-5-methyl benzophenone oxime12, 2-hydroxy-4-n-butoxy propiophenone oxime13, 3-hydroxy-3-phenyl-1-m-chlorophenyl triazene14, thioglycolic acid15, 1,2-dihydroxy-3,4-diketocyclo butene16, diformyl hydrazine17, and 1,3- diphenyl -4-carboethoxy pyrazole-5-one18) have been used for determination of Fe(III). Other spectrophotometric methods for determination of Fe(III) have been reviewed19. Derivative spectrophotometric procedures have also been reported for determination of iron20,21.

This study presents a simple and rapid spectrophotometric method via complexation with piroxicam for determination of Fe(III) in synthetic mixture and soil samples.

Experimental
Materials
All absorbance measurements were made on a Spectronic 20D+ spectrophotometer (Milton Roy Company, USA) with 1 cm matched glass cells. Absorption spectra were recorded on a Shimadzu UV-visible spectrophotometer (UV-160 A, Shimadzu Corporation, Kyoto, Japan) with matched quartz cells. Soil samples were collected from Department of Chemistry, AMU, Aligarh, India. All reagents used were of analytical grade. 0.005 M Ferric sulphate (CAS, 10028-22-5; MW, 399.88; Fluka Chemie AG, Switzerland) solution was freshly prepared in distilled water. 6.038 × 10−3 M (0.20%)
Piroxicam (CAS: 36322-90-4; M.W.: 331.35, Sigma Chemical Company, St. Louis, USA) solution was prepared in methanol. The solution was stable up to 3 days.

**Proposed Method (PM) for Determination of Fe(III)**

Into a series of standard volumetric flasks (10 ml), aliquots (0.05-0.45 ml) of $5 \times 10^{-3}$ M standard ferric sulphate solution were pipetted. Then to each volumetric flask, $6.038 \times 10^{-3}$ M piroxicam (2.6 ml) was added and diluted up to the mark with ethanol. Contents of each flask were mixed well at RT (25 ± 1ºC) and absorbance was measured at 495 nm against reagent blank prepared similarly except Fe (III) within stability period (24 h). Concentration of Fe (III) was calculated either from a calibration curve or regression equation.

**Determination of Fe(III) in Synthetic Mixture**

Synthetic mixture of iron (III) was prepared by taking 200 mg of ferric sulphate with 13.9 mg FeSO$_4$.7H$_2$O, 192.3 mg 3CdSO$_4$.8H$_2$O, 90.0 mg Pb(NO$_3$)$_2$, 23.01 mg ZnSO$_4$.7H$_2$O, 222.18 mg MgSO$_4$.7H$_2$O and 84.5 mg MnSO$_4$.H$_2$O in 100 ml standard volumetric flask and diluted up to the mark with distilled water. Fe (III) was determined by PM.

**Determination of Fe(III) in Soil Sample**

Air-dried finely powdered soil sample (2 g) of AMU locality was shaken with 0.5 M HCl (15 ml) for 24 h to extract iron$^{22}$. After filtration, volume of filtrate was made up to 25 ml with distilled water. Of this solution, 15 ml was percolated through column packed with Amberlite IR 400 (Cl-form) because Fe (III) was found to be completely adsorbed on resin in 4 M HCl. Column was washed with 2.5 M HCl to remove unadsorbed species and non targeted metal ions. Fe (III) was eluted with 0.05 M HCl (flow rate, 2 ml /min). Effluent was evaporated and residue was dissolved in 5 ml of distilled water. pH of solution was adjusted to 2.6 by addition of ammonia solution and final volume of solution was maintained to 15 ml. Fe (III) was estimated by both PM and reference method (RM)$^{11}$.

**Procedure for Reference Method (RM)$^{11}$**

Appropriate volumes of standard solution of Fe (III) corresponding to 0.2-4.8 mg ml$^{-1}$ were transferred to a series of 25 ml volumetric flasks. To each flask, 1.0 ml of 1 N HCl, 1.0 ml of 0.1% 4-aminonitrophenyrene and 2.0 ml of 0.1% iminodibenzyl were added. The contents were mixed thoroughly and diluted to volume with ethanol. Absorbance of resultant blue coloured complex was measured at 620 nm against corresponding reagent blank.

**Validation**

PM was validated for specificity and selectivity, accuracy and precision, robustness, linearity and evalu-
Stoichiometry of reaction between Fe (III) and piroxicam was evaluated by limiting logarithmic method\textsuperscript{24}, wherein two sets of experiments were performed. In first set, concentration of Fe (III) was varied keeping a constant concentration of piroxicam, while in second set, concentration of Fe (III) was kept constant and piroxicam concentration was varied. Log absorbance versus log [ferric sulphate] or [piroxicam] (Fig. 2) was plotted to evaluate slope of respective line. Slope was found to be unity in first case and 0.5 in second case; thus confirming molar combining ratio of 1:2 between Fe (III) and piroxicam. Hence, one mole of Fe (III) was consumed in first case and 0.5 in second case; thus confirming molar combining ratio of 1:2 between Fe (III) and piroxicam. Hence, one mole of Fe (III) was consumed with 2 moles of piroxicam (Fig. 3). Formation constant of complex was calculated and found to be $6.12 \times 10^{10}$. Chelating reaction of piroxicam with metal ions can be expected through three coordination sites (-OH, –CONH and $\sum N$ functional groups). Therefore, reaction sequence of PM is given as in Fig. 4.

Optimization of Variables
Optimization of variables was assessed by testing reaction time, concentration of piroxicam and solvents.
Complex got stabilized immediately at 25 ± 1°C after mixing analyte and reagent. Complex remained stable for 24 h. For optimizing concentration of piroxicam, absorbance of metal complex was investigated at 22.34 µg ml⁻¹ Fe (III) with piroxicam (conc., 3.018 ×10⁻⁴-1.691 ×10⁻³ M). Highest absorbance was obtained with 1.45 ×10⁻³ M piroxicam and remained constant up to 1.70 ×10⁻³ M piroxicam (Fig. 5). Therefore, 1.57 ×10⁻³ M
Piroxicam was used for the determination process. Effect of solvents (ethanol, acetone, methanol, 1,4-dioxan and dimethylsulphoxide) was investigated on absorbance of colored complex, which showed maximum absorbance in ethanol (Fig. 6), indicating ethanol as best solvent.

**Validation**

Varying concentrations of each ion with fixed concentration of Fe (III) were taken and absorbance was recorded to know concentration of Fe (III). Tolerated amounts of each ion, which is concentration value tested that caused less than ±2% absorbance alteration, has been found as follows: Fe$^{3+}$, 0.50; Cd$^{2+}$, 84.31; Pb$^{2+}$, 62.16; Zn$^{2+}$, 52.31; Mg$^{2+}$, 21.80 and Mn$^{2+}$, 27.47 μg ml$^{-1}$. However, metal ions (Ni$^{2+}$, Al$^{3+}$, Ca$^{2+}$, and Cu$^{2+}$) interfere in determination of Fe$^{3+}$.

Accuracy and precision were evaluated within same day and on five consecutive days. Recovery and RSD (%) were found between 100.04-100.08%; 0.22-0.74% for intra day and 99.93-100.11%; 0.28-0.81% for inter day precision, respectively (Table 1). Robustness of PM was established by deliberately changing concentration of piroxicam as volume of $6.038 \times 10^{-3}$ M piroxicam, 2.6 ml (± 0.2 ml). Under optimized conditions, Fe (III) solution [25.13 mg ml$^{-1}$ Fe (III)] was analyzed and mean recovery (100.04%) and RSD (0.22%) indicated robustness of PM.

Calibration curve was constructed by plotting absorbance against initial concentration of Fe (III) (Fig. 7). Beer’s law was obeyed (conc., 2.79-25.13 μg ml$^{-1}$) with apparent molar absorptivity ($5.88 \times 10^{3}$ l mol$^{-1}$ cm$^{-1}$) and Sandell’s sensitivity (0.019 mg/cm$^2$/0.001 absorbance unit). Calibration data were fitted to the equation, $A = a + bC$, where $A$ is absorbance at 495 nm, $C$ is concentration in mg ml$^{-1}$ $b$ is slope and $a$ is intercept of calibration. Calibration data ($n = 9$) were treated statistically. High value of correlation coefficient (0.9999) indicated excellent linearity (Table 2). Values of $t$ (calculated as, $t = a /$
S a 25) found to be 1.27, which did not exceed theoretical t-value (2.365) at 95% confidence level, indicate that intercept for PM is not significantly different from zero.

Applicability of PM for determination of Fe (III) in synthetic mixture and soil samples has been tested. Results of PM were statistically compared with those of RM 11 using point and interval hypothesis tests. Paired t- and F-values at 95% confidence level were found to be less than tabulated t- (2.036 at v = 8) and F-values (6.39 at v = 4.4) at 95% confidence level 26, thus confirming no significant difference between performance of PM and RM (Table 3). Thus, bias evaluated by interval hypothesis test by means of θ L and θ U were in the range of 0.98-1.02. Performance of PM was also judged by analysis of soils taken from different locations. Concentration of Fe 3+ in soil samples was found as follows: Soil 1—PM, 8.379 & RM, 8.382 mg ml⁻¹; and Soil 2—PM, 8.382 & RM, 8.375 mg ml⁻¹. Thus, PM is suitable for routine

### Table 1—Test of accuracy and precision of proposed method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Intra day assay</th>
<th>Inter day assay</th>
</tr>
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<tbody>
<tr>
<td>Concentration taken, µg ml⁻¹</td>
<td>5.59</td>
<td>13.96</td>
</tr>
<tr>
<td>Concentration found, µg ml⁻¹</td>
<td>5.59</td>
<td>13.97</td>
</tr>
<tr>
<td>Standard deviation, µg ml⁻¹</td>
<td>0.041</td>
<td>0.043</td>
</tr>
<tr>
<td>Recovery, %</td>
<td>100.07</td>
<td>100.08</td>
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<tr>
<td>RSD, %</td>
<td>0.74</td>
<td>0.30</td>
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</table>

*N* Mean for 5 independent determinations

### Table 2—Optical and regression characteristics of proposed method

<table>
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<tr>
<th>Parameters</th>
<th>Proposed method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength, nm</td>
<td>495</td>
</tr>
<tr>
<td>Beer’s law limit, µg ml⁻¹</td>
<td>2.79 – 25.13</td>
</tr>
<tr>
<td>Molar absorptivity, 1 mol⁻¹cm⁻³</td>
<td>5.88 × 10²</td>
</tr>
<tr>
<td>Sandell’s sensitivity</td>
<td>0.019 µg/cm²/0.001 absorbance unit</td>
</tr>
<tr>
<td>Linear regression equation</td>
<td>A = 5.55 × 10⁻⁴ + 1.52 C</td>
</tr>
<tr>
<td>±tS a</td>
<td>2.814 × 10⁻³</td>
</tr>
<tr>
<td>±tS b</td>
<td>1.791 × 10⁻⁴</td>
</tr>
<tr>
<td>Correlation coefficient (r )</td>
<td>0.9999</td>
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<tr>
<td>Variance (S₀²) of calibration line</td>
<td>2.690 × 10⁻⁶</td>
</tr>
<tr>
<td>Detection limit, µg ml⁻¹</td>
<td>0.10</td>
</tr>
<tr>
<td>Quantitation limit, µg ml⁻¹</td>
<td>0.31</td>
</tr>
</tbody>
</table>

± Sim: θ a and ±t S b are confidence limits for intercept and slope, respectively.

### Table 3—Evaluation of bias: Comparison and applicability of proposed method with reference method for determination of Fe(III) from synthetic mixture sample at 95% confidence level

<table>
<thead>
<tr>
<th>Samples</th>
<th>Proposed method</th>
<th>Reference method</th>
<th>t-value b</th>
<th>F value b</th>
<th>θ L c</th>
<th>θ U c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthetic mixture</td>
<td>Recovery a %</td>
<td>RSD %</td>
<td>Recovery a %</td>
<td>RSD %</td>
<td>0.253</td>
<td>1.200</td>
</tr>
<tr>
<td></td>
<td>100.01</td>
<td>0.91</td>
<td>99.87</td>
<td>0.83</td>
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</table>

*a* Mean for 5 independent analyses; b*Theoretical t (v = 8) and F-values (v = 4, 4) at 95 % confidence level are 2.306 and 6.39, respectively; c*A bias, based on recovery experiments, of ± 2% is acceptable.
analysis of Fe(III) in real samples of soil. Speed of analysis of PM is as good as that of RM.

Conclusions

Proposed spectrophotometric method is found simple, selective and accurate for determination of Fe (III). The method has advantage of using a commonly available solvent (ethanol with the use of one reagent, piroxicam). Proposed method, which has avoided use of acid, buffer solution and heating of reaction mixture, can be used as an alternate method for routine quality control analysis of Fe(III) in soil samples.

Acknowledgements

Authors are grateful to AMU, Aligarh, India and Ministry of ManPower (Higher College of Technology) Muscat, Sultanate of Oman for facilities.

References

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