An Iron Determination Method for Azo Dyes-Contaminated Wastewater

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INTRODUCTION

Iron, in the form of magnetite is one of the most common elements in the earth’s crust, occurring in nearly all types of rocks. Iron in water causes staining of laundry and porcelain, and also a bittersweet astringent taste detectable at concentrations greater than 5 mg l\(^{-1}\) in drinking water. The maximum permissible level of total iron in drinking water in Malaysia is 1 mg l\(^{-1}\) for Standard A (parameter limits discharge into inland waters within the catchment areas) and 5 mg l\(^{-1}\) for Standard B (parameter limits discharge into other inland) [1].

Although a vital element, elevated levels of iron has been found as a pollutant in many water bodies [1–3]. Its removal from waste water has been reported [4]. Its determination in a variety of samples can be carried out colorimetrically or via absorption or emission spectrometry [5]. Generally, a colorimetric determination of compounds is preferred to other methods such as ICP, GC and HPLC due to ease of use and the low technical knowledge required for method development. However, a large number of colorimetric determinations are interfered by the presence of non-target compounds. In the case of colorimetric iron determination in samples from azo dyes effluents, the presence of a mixture of azo dyes would interfere with total iron determination in wastewater.

Existing colorimetric reagent for iron are 1,10-phenanthroline [6] and FerreMo\(^{TM}\) method available from Hach [7]. The 1,10-phenanthroline-ferrous chelate has an orange-red color with a maximum absorption at 508 nm whilst the FerreMo\(^{TM}\) method is read at 590 nm. Both of these methods are strongly interfered by azo dyes with absorption maxima between 470 to 600 nm. Wastewater containing azo dyes are known to be heavily colored and masking or matrix effects from the colored effluents would affect general colorimetric determination.

Previously, we have discovered an azo-dye degrading bacterium [8]. When we tried to analyze the iron content in the polluted samples, we discovered that the intense absorption of the azo dye masked the absorption of the iron reagent even after dilutions of the samples. In order to overcome this problem, we have modified the FerreMo\(^{TM}\) method using 12-molybdophosphate reagent for the determination of total or ferrous iron. This modification of reagent allowed us to monitor iron at 865 nm reducing the masking effect of azo dyes. The development of this method would allow the colorimetric determination of iron in wastewater containing this dye.
MATERIALS AND METHODS

Chemicals and reagents
All chemicals used were of analytical reagent grade and all of the solutions were prepared with deionised water. Direct Blue 71 was purchased from SIGMA. The iron (II) stock solution was prepared as a 200 mg l\(^{-1}\) solution by dissolving 1.404 g of Mohr’s salt (Fe(NH\(_4\))\(_2\)(SO\(_4\))\(_2\).6H\(_2\)O, BDH Laboratory Supplies, Poole, Dorset, UK) in a 100 ml volumetric flask containing 20 ml of concentrated H\(_2\)SO\(_4\). The volume was then top up to 100 ml using deionised water.

Working standard solutions ranging between 0.25 and 25 mg l\(^{-1}\) of Fe\(^{2+}\) were prepared by appropriate dilution of the stock solution in deionised water. Acetate buffer pH 5.5 was prepared from 0.10 M of acetic acid and 0.10 M of sodium acetate. The colorimetric reagent of 1,10-phenanthroline solution was prepared by dissolving 100 mg of 1,10-phenanthroline monohydrate C\(_{12}\)H\(_8\)N\(_2\)H\(_2\)O (Sigma Chemical Co., St. Louis, USA), in 100 ml deionised water by stirring and heating to 80 °C. 12-phosphorous molybdate (Na\(_3\)PMo\(_{12}\)O\(_{40}\)) or 12–phosphomolybdate (Sigma Chemical Co., St. Louis, USA) was prepared in 50 mM acetate buffer pH 5.0 as a 20 mM stock solution.

Iron determination
From the stock iron solutions, 0.2 to 1.0 mls were added into a 10 ml volumetric flask. One hundred microliter of hydroxylamine (10% stock solution) and one hundred microliter of sodium acetate (20% stock solution) were then added to the flask. Finally, 1.0 ml of 1,10 phenanthroline solution (0.1% stock solution) was added and the final volume adjusted to 10.0 ml with deionized water.

The solution was mixed thoroughly and let stand for 30 minutes at room temperature. The absorbance of the solutions were read at 508 nm against a reference blank prepared by treating deionized water with the specified amounts of all reagents except the standard iron solution [6]. 12–Phosphorous molybdate or 12–phosphomolybdate (12-MP) was prepared in deionized water as a 20 mM stock solution and the pH adjusted to pH 5.0.

The resultant solution buffers strongly at this pH. Into a 10 ml volumetric flask containing 3.75 mls of 12-MP, 0.2 to 1.0 mls of the iron stock solutions were added. The final volume was adjusted to 10.0 mls using deionized water and the solution mixed thoroughly and let stand for 3-5 minutes at room temperature.

The absorbance of the solutions were read at 865 nm against a reference blank prepared by treating deionized water with the specified amounts of all reagents except the standard iron solution. When iron is present as a ferric state, treatment of the iron to reduce it to the ferrous state will be carried out according to the method outlined by APHA Standard Methods for the Examination of Water and Wastewater [6].

In the presence of high concentration of a mixture of azo dyes, the treatment tends to reduce the azo dyes absorbance intensity, perhaps due to chemical degradation. Scanned spectrum of the resultant molybdenum blue was carried out from 600 to 980 nm in steps of 1 nm using a UV-Visible Shimadzu Spectrophotometer with baseline correction performed using the reference blank.

Statistical analysis
Values are means ± SE. All data were analyzed using Graphpad Prism version 3.0 and Graphpad InStat version 3.05. Comparison between groups was performed using a Student’s t-test or a one-way analysis of variance (ANOVA) with post hoc analysis by Tukey’s test [9]. P < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

One of the methods to compare the sensitivity of analytical methods is comparing the Limits of Detection between developed and existing methods. LOD is the lowest analyte concentration likely to be reliably distinguished from the blank measurement, where the blank would by itself be assigned its limit and called Limit of Blank (LOB).

LOD is determined through measuring the LOB and test replicates of a sample known to contain a low concentration of analyte. The standard deviation of the blank is then times three and this value would then be converted to the concentration which is equal to the LOD. Another measurement metric-LOQ or Limit of Quantitation LOQ value is usually assigned as 10-times the standard deviation of the blank for the y-intercept but is rarely used.

The extinction coefficient for the 12-MP method measured at 865 nm was 0.0514 (mg l\(^{-1}\))\(^{-1}\)cm\(^{-1}\). The detection limit was 0.046 mg l\(^{-1}\). The FerreMo\(^\text{TM}\) is more sensitive having a detection limit of 0.01 mg l\(^{-1}\). The 12-MP method was approximately four times less sensitive than the 1,10-phenanthroline method.

The detection limit for the 1,10-phenanthroline method was 0.006 mg l\(^{-1}\). Precision studies showed an inter-assay repeatability coefficient of variance (CV) between 1.5–10.5% and a within-assay coefficient of variance between 1.3–3.8% for the 12-MP method. The optimum pH for color development was pH 5.0 (Figure 1).

The FerreMo\(^\text{TM}\) method is also reported to be sensitive to a narrow pH range from pH 3.0 to 5.0. Acetate buffer at 50 mM was used to buffer the reagent from pH 3 to 5 whilst phosphate at 50 mM was used as a buffer at pH 6.0. The phosphomolybdate reagent is by itself a strong buffer at pH 5.0, giving the same results using acetate and this minimizes the use of an extra reagent in the form of buffer in the determination procedure.
The optimum concentration for saturation concentration of colorimetric reagent was 5 mM (Figure 2) whilst Figure 3 shows that 3 to 5 minutes of incubation was optimum for color development. There was a dramatic increase in response of blue colour formation when 12-MP concentration was increased up to 0.5 mM. A plateau was reached at higher concentrations. In this method, 7.5 mM 12-MP was chosen to ensure complete conversion of molybdate (molybdophosphate) to Mo-blue. If this is not optimised, this will affect the linearity of the standard curve especially at higher concentrations of iron where additional 12-MP would not be reduced due to the lower concentration of iron available.

The maximum wavelength for optimum absorbance was at 860 to 870 nm with a mean of 865 nm as shown in Figure 4. The absorbance increases uniformly throughout the scanned spectrum as the intensity of Mo-blue from the media increases visibly. The absorbance at 710 nm is approximately 30% less than at 865 nm. There is an intense absorption near the ultraviolet region approximately at 450 nm and below. Intense absorption near the ultraviolet region was also reported for reduced silicomolybdate [10].

The spectrum of the iron-reduced phosphomolybdate was similar to the spectrum of ascorbic-reduced phosphomolybdate with a peak at 865 nm and a shoulder at 710 nm suggesting similarity in molybdenum blue species produced [11]. The latter
has been suggested as a reduced form of the 12-molybdophosphate species [12–15]. Both spectra have a maximum peak between 860 nm and 880 nm and a shoulder at 700 nm.

The molybdenum blue produced by the FerreMo™ method is probably a different heteropolytungstate species since it has a maximum absorption at 590 nm. Assaying at 865 nm allows the Direct Blue 71 azo dye interference to be kept to the minimum (Figure 5) since it has negligible absorbance at 800 nm and above. Many dyes have absorption maxima in the visible range from 400 to 700 nm (Table 1) and the use of 865 nm would prevent interference by most azo dyes.

Fig. 5. Visible spectrum of molybdenum blue produced from 4 mg l$^{-1}$ (--) and 12 mg l$^{-1}$ (—) ferrous iron in the presence of 42.5 µM Direct Blue 71.

Table 2. Accuracy and precision of the proposed method.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Mean (n=4)</th>
<th>standard deviation</th>
</tr>
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<tbody>
<tr>
<td>1,10 phenanthroline</td>
<td>5.70</td>
<td>0.07</td>
</tr>
<tr>
<td>12-MP</td>
<td>5.71</td>
<td>0.06</td>
</tr>
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</table>

The results in Table 2 show the accuracy and precision of the proposed method in detecting tap water spiked with ferrous iron to a final concentration of 5.7 mg l$^{-1}$. The critical value of $|t|$ for six degrees of freedom (two-tailed) at $p=0.01$ was 3.71 whilst the experimental value of $|t|$ was 0.39 suggesting that the difference in means of the proposed and the 1,10-phenanthroline method is not significant. We have improved on an iron determination method in samples containing azo dyes employing the 12-phosphomolybdate reagent. Although the sensitivity of the proposed method is several times less than the existing 1-10, phenanthroline method, allows the determination of ferrous iron in the presence of azo dyes without complicated treatment to remove the dye from samples.

Table 1. Maximum absorbance of dyes.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Maximum Wavelength (nm)</th>
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<tbody>
<tr>
<td>Congo Red (C.I. 22120)</td>
<td>498</td>
</tr>
<tr>
<td>Cresol Red (C.I. 1733-12-6)</td>
<td>570</td>
</tr>
<tr>
<td>Crocein Orange G (C.I. 15970)</td>
<td>482</td>
</tr>
<tr>
<td>Evans Blue (C.I. 23860)</td>
<td>594</td>
</tr>
<tr>
<td>Fast Green FCF (C.I. 42053)</td>
<td>620</td>
</tr>
<tr>
<td>Fuchsin Basic (C.I. 42510)</td>
<td>625</td>
</tr>
<tr>
<td>Crystal Violet (C.I. 42555)</td>
<td>590</td>
</tr>
<tr>
<td>Metaflor Yellow (C.I. 13065)</td>
<td>414</td>
</tr>
<tr>
<td>Methyl Green (C.I. 42590)</td>
<td>635</td>
</tr>
<tr>
<td>Methyl Orange (C.I. 13025)</td>
<td>505</td>
</tr>
<tr>
<td>Methyl Red (C.I. 13020), Methylene Blue (C.I. 52015)</td>
<td>493, 590</td>
</tr>
<tr>
<td>Naphthol Blue Black (C.I. 20470), Nigrosin (C.I. 50415)</td>
<td>618, 570</td>
</tr>
<tr>
<td>Orange G (C.I. 16230), Orange II sodium salt (C.I. 15510), Ponceau 2R (C.I. 16150), Ponceau S (C.I. 27195), Remazol Black B (C.I. 20505), Rhodamine B (C.I. 45170), Safranin O (C.I. 50240), Direct Blue 71 (C.I. 34140), Sudan Black B (C.I. 26150), Tartrazine (C.I. 19140), Toluidine Blue (C.I. 52040), Trypan Blue (C.I. 23850),</td>
<td>388, 352, 597, 554, 530, 586, 600, 427, 626, 607</td>
</tr>
</tbody>
</table>

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REFERENCES


