tive destruction of bilirubin (14), or eliminating peroxidase itself by reacting directly with a titanium(IV) complex (15). These alternatives must be investigated further, to provide the clinical laboratory with analytical procedures that are free from the chemical and spectral interferences of bilirubin.

We thank Gregory Buffone, Ching Ou, Thomas Spillman, and Loretta Chow for their assistance in this work. We also thank Boehringer Mannheim Diagnostics, Eastman Kodak, Roche Diagnostics, and Behring Diagnostics for generously supplying the test reagents used in this study.

References


Rapid Determination of Iron in Urine, in the Presence of Deferoxamine, by Inductively Coupled Plasma Emission Spectrometry

Paul Leflon and Roger Plaquet

Using a spectrometer with an argon plasma source coupled to a high-frequency magnetic field, we developed a direct method for determining iron in urine of patients being treated with deferoxamine. The detection limit for iron was 75 nmol/L; added iron was satisfactorily recovered; and we observed no interference from deferoxamine at its most commonly used concentrations. Values for between-run and within-run precision (CV) was <5%. Correlation of results with those obtained with a colorimetric method involving bathophenanthroline was good (r = 0.96).

Iron is usually determined in the urine of hemochromatosis patients who are being treated with deferoxamine, to monitor the elimination of iron from their bodies. Van Stekelenburg et al. (1) established that colorimetric methods based on 2,4,5-tripryridyl-s-triazine (TPTZ) or bathophenanthroline are not suitable for use with such patients because of interference with the reagents by the deferoxamine medication. For this reason they replaced it by a ferrozine method, but it requires a 2-h pre-incubation at 37° C in the presence of citric acid in combination with ascorbic acid and thiourea.

Alternatively, we describe here the first proposal of a direct emission-spectrometric method in argon plasma, involving an instrument routinely in use in our laboratory for determining aluminum in biological fluids from hemodialyzed patients.

We optimized the conditions of iron measurement and determined the lack of interference by added deferoxamine, as well as the repeatability and reproducibility of the method and its correlation with a colorimetric technique when no deferoxamine is present.

Materials and Methods

Apparatus. We used a model J.Y. 38 P spectroanalyzer with a Czerny Turner 1-m (focal length) monochromator, which includes a holographic grating ruled at 2400 grooves per millimeter, and a "Plasmatheur" source inductively coupled to a high-frequency (27.12 MHz) magnetic field (1.5 kW maximum power). The sample solution was introduced into the argon plasma by use of a pneumatic nebulizer. (The whole equipment was supplied by Jobin & Yvon, Instrument S.A., 91163 Longjumeau Cedex, France.)

Reagents: Deferoxamine ("Desferal"; Ciba, Basel, Switzerland); argon (argon "U"; Compagnie Francaise des produits Oxygene, Rouen, France); standard iron solution ("Titanol"; Merck, Darmstadt, F.R.G.)

Prepare the iron stock solution by diluting a volume of

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iron standard solution containing 17.9 mmol (1 g) to 1000 mL with 5 mmol/L HCl. Prepare 179 μmol/L iron working solution by further diluting the stock solution with de-ionized water.

**Working conditions.** Power: 1 kW; cooling argon flow: 13 L/min; nebulization argon flow at 180 kPa; 0.4 L/min; sample flow: about 3 mL/min; pre-integration time: 0.5 s; and integration time: 20 s. Measure sample absorbance at 259.94 nm, the nonspecific emission blank at 259.89 nm.

**Procedures.** In glass bottles that have been previously washed with dilute HCl, then rinsed with de-ionized water and dried, collect urine specimens, either with no preservative or with sodium mercurithiolate ("Mercaptoyl"; Houdé, Paris, France). We observed no interference by this preservative in iron determination.

Dilute four aliquots of a urine sample 10-fold with dilute (11 mL/L) HCl, adding respectively to the first and the second ones 1.79 and 3.58 μmol of iron per liter. For the four dilutions, measure the emission signal at 259.94 nm. Rerun the entire procedure, replacing urine with de-ionized water, to determine reagent blanks. Finally, subtract nonspecific emission, measured at 259.89 nm, for diluted urine samples and reagent blanks.

**Results and Discussion.**

Using the above working conditions, we could detect as little as 75 nmol of iron per liter of urine, this being calculated as suggested elsewhere (2, 3) for other elements; this is the concentration corresponding to twice the standard deviation of background noise. As summarized in Table 1, iron added to give concentrations in the range 3.58 to 53.72 μmol/L was satisfactorily accounted for.

The absence of any interference from deferoxamine at 4 g/L in urine was demonstrated by calculating the regression equation between the signal increase with (y) and without (x) deferoxamine for eight different iron additions in the concentration range from 0.179 to 89.5 μmol/L. This gave y = 1.019x - 10.5 arb. units (S_yx = 34.4, S_xy = 33.7 arb. units; correlation coefficient = 0.9999, p > 0.05, not significant).

The within-run CV for iron determined 10 times in the same sample was 3.27% for a mean (± SD) iron concentration of 3.06 (0.10) μmol/L, and 0.66% for 16.65 (0.11) μmol/L.

The between-run CV, determined by repeated measurements 10 times during four months of urines stored at -20 °C was 4.89% at an iron concentration of 1.84 (0.09) μmol/L and 2.64% at 14.77 (0.39) μmol/L.

Comparison of results by this method (y) with results of the colorimetric method (x) involving benzophenanthroline (4) for urines containing no deferoxamine gave the following results: n = 42, r = 0.96, y = 1.014x - 0.13, y = 13.8 μmol/L, x = 13.86 μmol/L, difference between x and y = 0.06 μmol/L, p > 0.05 (not significant).

We conclude that this emission spectrometric method is not sensitive to deferoxamine and requires no special treatment or incubation of urine samples.

The results show a good linearity with iron concentration in the range of values observed during deferoxamine treatment of patients. Iron added is satisfactorily accounted for, and the correlation with a colorimetric method in the absence of deferoxamine is good. Unlike atomic absorption spectrometry, which requires a specific cathodic lamp, no special accessory is necessary for our method. The large range of measurable concentrations obviates the need for multiple dilutions of urine sample, thus making our procedure simple and rapid.

Lastly, it should be noticed that emission spectrometry in argon plasma can easily be used for iron determination in other fluids, as well in dialysates from hemodialyzed patients as in serum and plasma. But in this case great care must be taken to avoid absolutely any trace of hemolysis because of interference by hemoglobin iron.

We thank Annick Pronnier for helpful technical assistance.

**Table 1. Analytical Recovery of Iron Added to Urine**

<table>
<thead>
<tr>
<th>Iron added μmol/L</th>
<th>Iron measured μmol/L</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.58</td>
<td>3.67</td>
<td>102.5</td>
</tr>
<tr>
<td>10.74</td>
<td>10.79</td>
<td>100.5</td>
</tr>
<tr>
<td>21.49</td>
<td>21.81</td>
<td>101.5</td>
</tr>
<tr>
<td>53.72</td>
<td>54.79</td>
<td>102</td>
</tr>
</tbody>
</table>

*Iron content, 1.12 μmol/L.

**References**


