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A Spectrophotometric Comparison of Several Iron Determination Methods

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A study of five different colorimetric iron determination methods was made. The spectra of the coloured iron complexes were recorded. The values for the absorptivity ** of the complexes, calculated on iron basis, are given. Nitroso-R-salt is regarded as the most sensitive of all five reagents used in this study.

The need for sensitive methods for chemical analyses of biological material is more and more recognized, since it is increasingly difficult to provide highly purified materials in the amounts necessary for such analyses. In the course of the work on the haemoproteins of the Atlantic hagfish the need arose for a sensitive but also specific and technically simple method for the determination of iron. Therefore a study of some widely applied iron-complex forming reagents and their spectral properties was undertaken.

EXPERIMENTAL

The spectra of the coloured iron complexes were recorded with a Cary Model 14 recording spectrophotometer with sample and reference in matched 1 cm cuvettes (Zeiss). Single photometric measurements were made with a Beckman DB spectrophotometer. The pH was measured with a combined glass electrode and the Titrator type TTT 1c (Radiometer, Copenhagen). Standardized volumetric vessels and Linderstrøm-Lang pipettes were used for all volumetric dilutions.

The following reagents for the determination of iron were used: sulfosalicylic acid *p.a.* (Merck), 1,10-phenanthroline hydrochloride *p.a.* (Merck), 7-iodo-8-hydroxyquinoline-5-sulfonic acid (BDH), thioglycolic acid (Hopkins & Williams), nitroso-R-salt (Eastman).

All other chemicals were of analytical grade. A solution of $(NH_4)_2SO_4Fe_2(SO_4)_3$ containing 0.1 mg/ml of iron served as standard. (The iron salt was dissolved in 5 ml conc. H_2SO_4 and the solution made up to 1000 ml with distilled water).

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** The spectrometric nomenclature published by Analytical Chemistry 1964[1] was followed throughout the study.

Experimental procedures

a. *The determination of iron with sulfosalicyclic acid according to Lorber.*[2] 0.4 ml of a 20 % solution of sulfosalicylic acid in water and 0.4 ml 10 % ammonia were added to 2 ml of a standard dilution, and the volume of the mixture was made up to 5 ml with distilled water. After standing overnight at room temperature the spectrum of the solution was recorded.

b. *The determination of iron with o-phenanthroline.* The various procedures described in the literature[3-7] differ only slightly. In this study the method given by Snell and Snell[3] was followed. To 2 ml of standard dilution 0.05 ml of a freshly prepared 10 % solution of hydroxylamine-HCl in water was added and after 10 min 0.05 ml of the *o*-phenanthroline reagent (2 % *o*-phenanthroline in 10 % ethanol[4]) and 1 ml of a 25 % Na-citrate solution, pH 3.5, were added. The volume was made up to 5 ml with distilled water. The mixture was kept overnight at room temperature. The spectrum was recorded and the pH measured.

c. *The determination of iron with 7-iodo-8-hydroxyquinoline-5-sulfonic acid (ferron).* According to Snell and Snell[3] the pH of the reaction mixture has a great influence on colour development. The pH value of standard and sample should be adjusted to within 0.2 pH units between pH 2 and 3. The method adopted in these experiments was the following: to 2 ml of the standard dilution 0.2 ml 33 % H_2O_2 and 2 ml of a 1 M glycine-HCl buffer, pH 2.6, were added, followed by the addition of 0.4 ml of a saturated solution of ferron in 60 % ethanol. The solution was made up to 5 ml with distilled water. The absorbance of the coloured complex was measured at its maximum and the spectrum recorded. The pH of the solution was determined.

d. *The determination of iron with thioglycolic acid.* The method given by Snell and Snell[3] was followed in principle. 0.2 ml of a 5.5 % solution of thioglycolic acid in water, previously brought to pH 10 with 1:1 diluted concentrated ammonia, and 0.5 ml 1:5 diluted concentrated ammonia were added to 2 ml of the diluted standard. The mixture was made up to 5 ml with distilled water. The spectrum of the coloured complex was recorded immediately, and the pH was measured.

e. *The determination of iron with nitroso-R-salt.* The procedure adopted was different from that given by Snell and Snell[3] in that the use of buffers to control pH was preferred to the titration with ammonium hydroxide and HCl in the presence of metanil yellow as indicator. 0.2 ml of 20 % hydroxylamine-HCl were added to 2 ml of a standard dilution. After 15 min 0.4 ml of a 2 M glycine-HCl buffer, pH 2.3, 0.2 ml of a 0.5 % solution of nitroso-R-salt in distilled water, and 2 ml of a 3 M Na-acetate solution were added. The mixture was made up to 5 ml with distilled water. The concentration of nitroso-R-salt was enough for the determination of up to 8 μg/ml Fe^{2+}. If higher concentrations are to be measured, the volumes of the acetate solution and of the reagent solution should be changed in order to get a higher concentration of nitroso-R-salt. The spectrum of the mixture was recorded and the pH determined.

RESULTS AND DISCUSSION

The determination of iron with sulfosalicylic acid according to Lorber[2] is very useful, as it is rather insensitive to pH variations. The spectrum of the sulfosalicylic acid-iron complex shows a maximum at 424 mμ (Fig. 1). The absorptivity of the complex at 424 mμ is in accordance with that given by Theorell *et al.*[8] $0.100 \times 10^3 \cdot cm^2 \cdot g^{-1}$ (Fe^{3+}), though the amount of sulfosalicylic acid used in this study is four times greater than that used by Theorell *et al.* Amounts of 2.5 μg Fe in a final volume of 5 ml can be determined with reasonable accuracy.

The use of *o*-phenanthroline as a complex-forming reagent for the determination of iron is widely known. The spectrum of the Fe^{2+}-*o*-phenanthroline complex shows a maximum around 512 mμ and inflexions at 480 mμ and around

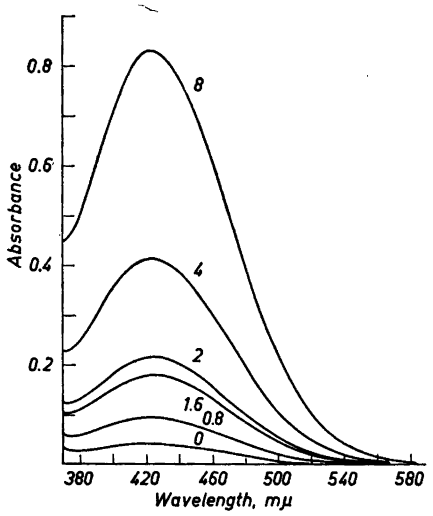

Fig. 1. Spectrum of the iron-sulfosalicylic acid complex. Iron concentration: 0, 0.8, 1.6, 2, 4, 8 μg/ml; reference: water.

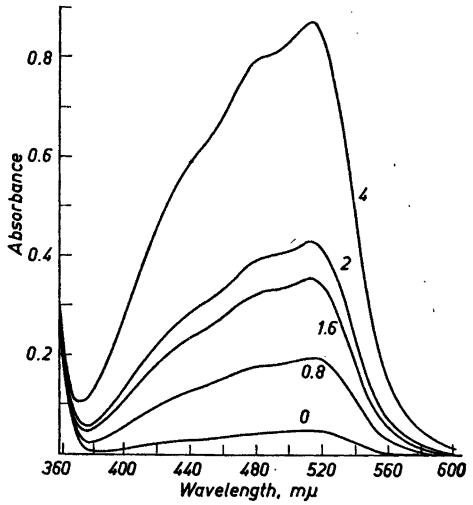

Fig. 2. Spectrum of the Fe^{2+}-*o*-phenanthroline complex. Iron concentration: 0, 0.8, 1.6, 2, 4, μg/ml; reference: water.

420—440 mμ (Fig. 2). The sensitivity of this method appears to be approximately twice that of the preceding one, so that 1 μg of iron can be determined in a final volume of 5 ml. The absorptivity of the iron-*o*-phenanthroline complex at 512 mμ is $0.190 \times 10^3 \cdot cm^2 \cdot g^{-1}$ (Fe^{2+}).

7-Iodo-8-hydroxyquinoline-5-sulfonic acid (ferron) has been introduced by Yoe [9] as a reagent for the colorimetric determination of iron. The maximum of absorbance which can be used for the quantitative measurement of the concentration of the complex lies at 604 mμ. Another maximum at 430—450 mμ can not be used for this purpose, because it lies in the region of increasing absorption of the reagent itself (Fig. 3). The absorptivity at 604 mμ is $0.074 \times 10^3 \cdot cm^2 \cdot g^{-1}$ (Fe^{3+}). In measuring high iron concentrations, *i.e.* over 8 μg/ml sample volume, great care must be taken to provide enough reagent in the final mixture to fulfill the stoichiometric requirements for complex formation. A ratio of 3:1 (reagent to iron) is necessary (Yoe and Hall [10]). For this reason a high concentration of the reagent is very useful. According to Yoe and Hall [10] the solubility of ferron is highest in organic solvent-water mixtures, so for instance in ethanol-water 1:1 it is 0.5696 g/100 ml, in acetone-water 1:1 it is 0.72 g/100 ml solvent.

The determination of iron by thioglycolic acid has been described by Lyons.[11] The spectrum of the thioglycolic acid-iron complex shows a maximum at 535 mμ (Fig. 4), and a second maximum between 420 and 430 mμ. Since at the former wavelength the absorbance of the complex is much higher, the measurements were done at 535 mμ. The absorptivity of the complex at 535 mμ is $0.070 \times 10^3 \cdot cm^2 \cdot g^{-1}$ (Fe^{2+}).

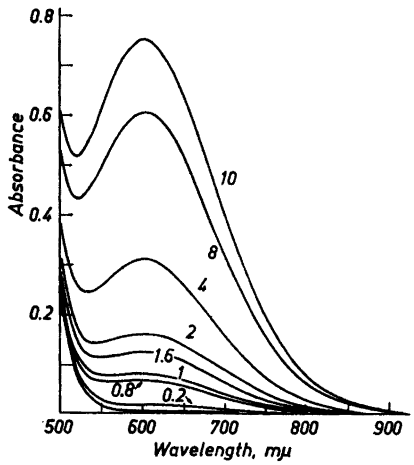

Fig. 3. Spectrum of the Fe^{3+}-ferron complex. Iron concentration: 0, 0.2, 0.8, 1, 1.6, 2, 4, 8, 10 μg/ml; reference: water.

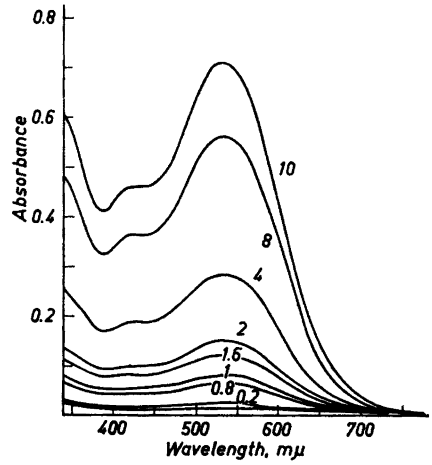

Fig. 4. Spectrum of the iron-thioglycolic acid complex. Iron concentration: 0, 0.2, 0.8, 1, 1.6, 2, 4, 8, 10 μg/ml; reference: water.

Van Klooster [12] described the use of nitroso-R-salt as a sensitive reagent for the determination of cobalt. Its property to form a coloured complex with Fe^{2+} was employed later for the determination of iron. The spectrum of the complex (Fig. 5) shows an absorbance maximum at about 715 mμ and a minimum around 545 mμ. If the stoichiometric ratio of 3:1 (reagent: Fe^{2+}) is fulfilled, both wavelengths can be used for the quantitative determination.

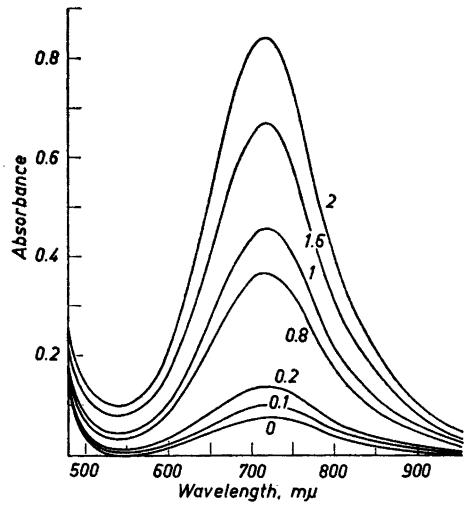

Fig. 5. Spectrum of the Fe^{2+}-nitroso-R-salt complex. Iron concentration: 0, 0.1, 0.2, 0.8, 1, 1.6, 2 μg/ml; reference: water.

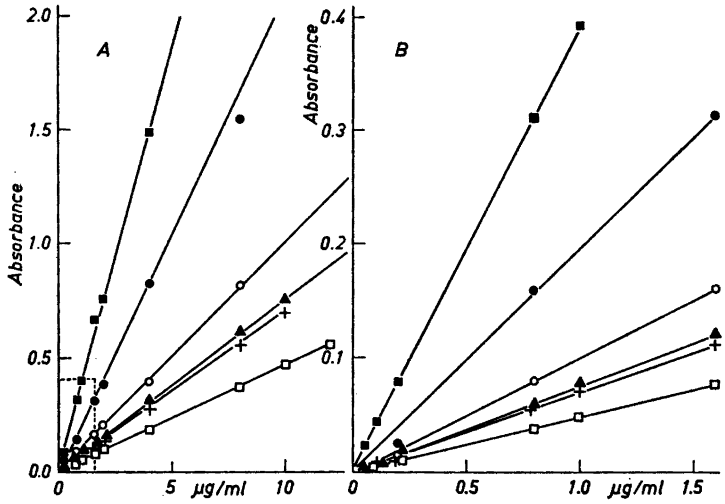

Fig. 6. Plot of absorbance of the different complexes against iron concentration; O iron-sulfosalicylic acid (424 mμ); ● Fe^{2+}-*o*-phenanthroline complex (512 mμ); ▲ Fe^{3+}-ferron complex (604 mμ); + iron-thioglycolic acid complex (535 mμ); Fe^{2+}-nitroso-R-salt complex, ■ maximum (715 mμ), □ minimum (545 mμ). A. showing the whole range of concentrations tested. B. enlarged insert of A.

The absorptivity of the complex at 715 mμ is $0.385 \times 10^3 \cdot cm^2 \cdot g^{-1}$. Variations of about ± 2 % found in different experiments are attributed to differences in pH before and after the addition of the reagent, the time between addition of the reducing agent (here hydroxylamine-HCl) and the reagent, and the final concentration of the reagent. The very high absorbance of the complex at the maximum allows accurate determinations of minute quantities of iron. In that case, however, the experimental procedure should be standardized and rigorously adhered to. The exact adjustment of the pH before and after addition of the reagent (pH 2.3 and pH 5.7 resp.) is of greatest importance. The lowest concentration of reagent compatible with the stoichiometric requirements should also be used. Measurement at the minimum of absorbance provides a means by which higher iron concentrations can be determined without diluting the sample. The absorptivity at the minimum (545 mμ) is $0.047 \times 10^3 \cdot cm^2 \cdot g^{-1}$ (Fe^{2+}).

In Fig. 6 a plot of the absorbance at the respective wavelengths against the iron concentrations of the final reaction mixtures shows the relations between the sensitivities of the described methods. It also shows that Beer's law holds over the concentration range tested.

The comparative study of the described methods has been done without regard to the possible interaction of other coloured metal-complexes or iron complex forming ions. It is known from the literature that there are great differences in sensitivity to the interference of contaminating ions with the formation of the coloured iron-reagent complex. For the evaluation of the

usefulness of an iron determination method this and the optimum conditions for complex formation must be kept in mind. So, for example, the liberation of iron from biological material by acid treatment may render the sample very acid and subsequent pH adjustment very difficult. Obviously a decrease in the lowest determinable amount of iron can be achieved by increasing the sample path length and decreasing the volume of the cell.

The limits for the quantitative determination of iron are higher than those given by some authors for the qualitative detection of traces of iron with the same reagents. The sensitivity of the nitroso-R-salt method compares favourably with that for isonitroso-dimethyl-dihydro-resorcinol described by Shome.[13]

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