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A SPECTROPHOTOMETRIC PROCEDURE FOR IRON[1]

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Prior to our work employing oxine (8-hydroxyquinoline) as a regent for ferric iron, Alexander (1) had found that ferric iron could be extracted from 0.018 M sulfuric acid solutions by a one per cent oxine in chloroform reagent.

Moeler (8) made the 8-hydroxyquinoline deriviates of aluminum, ferric iron, cupric copper, bismuth, cobalt, and nickel by procedures recommended by Berg (2) and found that only the derivatives of aluminum, ferric iron, and cupric copper dissolved at all readily in chloroform.

The only report encountered of the use of oxine as a reagent for ferric iron in biological materials is the work of Lavollay (7) on tissues. In this procedure the iron was precipitated by 8-hydroxyquinoline and the precipitate was dissolved in hot ethanol containing a little sodium hydroxide. The dark green solution that resulted was compared with a standard in a photocolorimeter.

It is interesting to note that 8-quinolinols have been estimated in the urine of experimental animals by complexing the 8-quinolinols with ferric iron. This has been reported by Haskins and Luttermoser (5), by Frensenius (6), and by Grabbe (7).

The purpose of this investigation was to develop a new spectrophotometric procedure for the estimation of blood iron based upon the chloroform extraction of the ferric iron-oxine complex under suitable conditions.

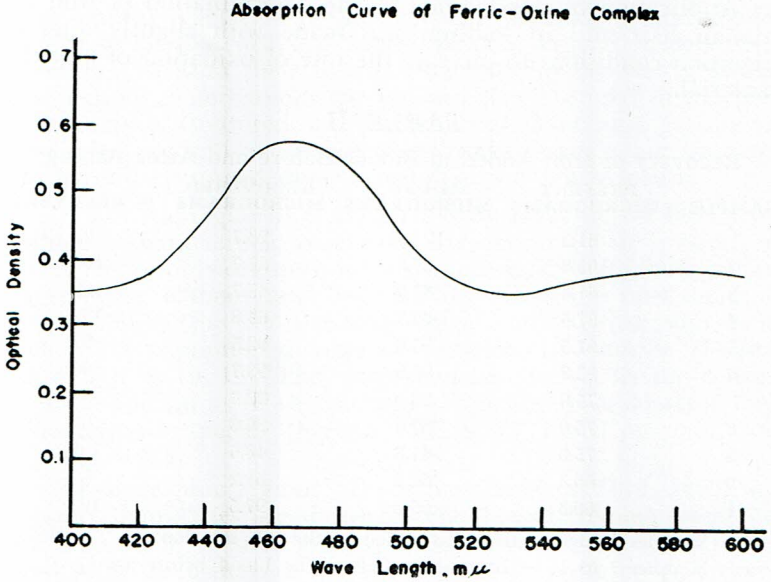
PROCEDURE: One ml. well-mixed oxalated whole blood was transferred accurately to a 25 x 250 mm. Pyrex test tube (or a 100 ml. Kjeldahl flask). To this, 5 ml. of iron-free sulfuric acid and 1½ ml. of 70-72% or 2 ml. of 60% perchloric acid were added. The mixture was digested until colorless (about 10 minutes is required) and then allowed to cool to about room temperature. The solution was transferred quantitatively to a 100 ml. volumetric flask and diluted to volume with distilled water. The resulting solution was mixed well and a 20 ml. aliquot was taken for analysis. After adjusting the pH of the aliquot to 2.5 using ammonium hydroxide, the solution was extracted with four portions of 5 ml. each of freshly prepared 0.01 M 8-hydroxyquinoline in chloroform. The chloroform extracts were combined in a 50 ml. volumetric flask and diluted to volume with chloroform. The intensity of the resulting gray or black color was measured in a Beckman spectrophotometer at 468 millimicrons and a slit width of .403 mm.

1. Taken from the thesis submitted by William K. Easley in partial fulfillment of the requirements for the Ph.D. degree at The Division of Chemistry, Graduate School, Georgetown University, Washington, D. C.

For samples particularly low in iron, it is convenient to dilute the combined chloroform extracts to a total volume of 25 ml.

In order to obtain the proper wave length setting for the spectrophotometer, the absorption curve of the ferric-oxine complex was determined. The plot of optical density versus wave length is shown in Figure one. Examination of this curve indicated a maximum absorption plateau from 466 to 472 millimicrons. The standard curve was obtained from known pure solutions prepared from C.P. iron wire in the usual way and the plot of optical density versus concentration resulted in a straight line indicating that the ferric-oxine complex obeys Beer's law.

Figure One



RESULTS

A series of over one hundred samples were analyzed for total blood iron by both this procedure and the procedure of Kennedy (6). Ten representative samples are shown in Table I. The results obtained agreed with 3.7 per-cent.

TABLE I

Comparison of New Procedure for Total Blood Iron with the Method of Kennedy(6)

SAMPLE	OXINE	KENNEDYa
1	45.0	42.2
2	46.3	44.8
3	23.8	24.5
4	40.0	41.8
5	38.8	38.4
6	46.3	47.1
7	36.9	39.2
8	46.3	47.1
9	45.0	45.8
10	45.0	47.1

a = Values represent milligrams of iron per 100 ml. of whole blood.

The recovery of iron added to samples both prior to and after ashing is shown in Table II and it varied from 94 to 103 percent. The optical densities of duplicate samples agreed in over 80 percent of the analyses within less than three percent. A chloroform solution of the ferric-oxine complex did not show any change in optical density over a period of six weeks indicating excellent stability. The presence of from two to five times the normal concentration of copper in blood samples failed to interfere with the results.

Application of this method to the determination of iron in human hair and in Pablum was made with slightly altered digestion conditions to increase the rate of oxidation of organic material.

TABLE II

Recovery of Iron Added to Bloods Before and After Ashing

SAMPLE	PRESENT MICROGRAMS	ADDED MICROGRAMS	RECOVERED MICROGRAMS	% RECOVERY
1	81.3	23.9	23.7	99
2	103.8	37.8	38.7	103
3	61.3	47.8	48.7	102
4	87.5	62.7	63.8	102
5	87.5	95.6	92.5	97
6	83.8	95.6	93.7	98
7	75.0	23.9	22.5	94
8	75.0	37.8	36.3	96
9	75.0	47.8	48.5	101
10	66.3	62.7	61.2	98
11	66.3	95.6	97.5	102

Samples 1 to 6 — Iron was added to the ash solution.
Samples 7 to 11 — Iron was added to the blood before ashing.

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