SPECTROPHOTOMETRIC DETERMINATION OF IRON

I. USE OF MERCAPTOACETIC ACID

BY RUTH ADELE KOENIG AND C. R. JOHNSON

(From the Department of Chemistry, The University of Texas, Austin)

(Received for publication, September 15, 1941)

It is the purpose of the research reported in the present series of papers to establish accurate spectrophotometric procedures for the determination of iron, more particularly for use in biochemical and nutrition studies, but applicable generally to the analysis of biological materials, foods, pharmaceuticals, and chemical reagents. Several colorimetric methods for the determination of iron are available, but they lack the rapidity and precision of spectrophotometric methods. Colorimetric procedures are not directly applicable to spectrophotometric work, and are apt under spectrophotometric study to show serious flaws and limitations. In fact, the interferences commonly met in analyses of foods and biological materials make it doubtful whether an investigator desiring accurate results in iron determinations should be satisfied until checks are obtained by at least two different methods.

For this reason the present research was designed to adapt for spectrophotometric use a number of the best colorimetric methods for iron, to obtain accurate calibration data over a wide range of iron concentrations, and to establish the validity of these data for determining iron in solutions containing common acids in concentrations that occur in analytical solutions prepared by various wet and dry methods of ashing. This information could be used in testing methods for ashing samples, in studying the effect of interfering substances other than the acids introduced in preparing the analytical solutions, and in devising rapid and accurate methods for determining total iron in foods and biological materials. It also seemed that such work as that outlined was a prerequisite to any successful attack on the problem of developing
trustworthy analytical techniques for determining biologically available iron.

One of the reagents selected for study was mercaptoacetic (thioglycolic) acid. Swank and Mellon (1) have listed applications of this reagent, and describe experiments showing its high degree of freedom from interferences.

**Apparatus and Reagents**

A Coleman model 10-S-30 spectrophotometer was used to measure transmittances. Matched square cuvettes were used to hold reference liquids and test solutions. The dark current adjustment was frequently checked during the measurements, which were made between 29–31°.

15 N nitric acid, 6 N hydrochloric acid, 9 N perchloric acid, 36 N sulfuric acid, trichloroacetic acid, and ammonium hydroxide were purified by distillation methods. 10 N iron-free sodium hydroxide was obtained by electrolysis in platinum at 5 amperes for 30 hours, with a rotating cathode. The reagent (ammonium mercaptoacetate) was made by adding 40 ml. of mercaptoacetic acid to 300 ml. of 4 N ammonium hydroxide and diluting to 500 ml. with distilled water. Three primary standard solutions containing 0.2000 mg. of iron per ml. were used in making all more dilute standard and test solutions. One primary standard was made by dissolving 1.405 gm. of ferrous ammonium sulfate hexahydrate (Mohr's salt) in 200 ml. of water, adding 10 ml. of 36 N sulfuric acid and 20 ml. of saturated bromine water, boiling off excess bromine, and making to a liter. Another was made by dissolving 0.4000 gm. of pure iron wire in 10 ml. of 36 N sulfuric acid, 10 ml. of 6 N hydrochloric acid, and 4 ml. of 15 N nitric acid, heating to remove the excess of volatile acids, and making to 2 liters. A third solution contained 1.727 gm. of recrystallized hydrated ferric ammonium sulfate (mol. wt. 482.2) and 5 ml. of 36 N sulfuric acid per liter. Transmittance readings with three sets of test solutions made from these standards and compared spectrophotometrically at appropriate wave-lengths, with use of mercaptoacetate, α,α'-dipyridyl, and ferron reagents, showed that all three standards had the same iron content, within 1 part in 400. Additional checks were obtained by gravimetric, volumetric, and electrometric methods, but the spectrophotometric check was the most accurate and convenient.
Summary of Calibration Experiments

Calibrations were made by the procedure given below for analyses, except that the test solutions contained known quantities of iron; concentrations of acids, mercaptoacetate reagent, and ammonium hydroxide were varied systematically to find permissible, adequate, or optimum quantities, and the systems were kept under observation for at least 12 hours. Different combinations of primary standards and reagents were used to eliminate constant errors.

The median transmittances obtained in about 400 observations made with 98 test solutions are recorded in Table I. These medians were calculated from the readings taken about 30 minutes after color development. However, transmittances read any time between 5 minutes and 12 hours after color development generally agreed within 0.4 per cent; only a few slightly high values were obtained in the 5 minute readings. Solutions stored in cuvettes showed some tendency to fade. This was not observed in solutions stored in glass-stoppered Pyrex Erlenmeyer flasks for 30 minutes; after longer standing it is advisable to shake the solutions a little and allow air bubbles to disappear before the readings are taken.

The reagent made with mercaptoacetic acid purified by fractional distillation in a vacuum gave results identical with that made from Eastman's practical reagent. When only 5 ml. of mercaptoacetate reagent were used per 50 ml.—about twice the

<table>
<thead>
<tr>
<th>Fe per 50 ml.</th>
<th>Transmittance</th>
<th>Average deviation of single observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg.</td>
<td>Blank reference</td>
<td>Water reference</td>
</tr>
<tr>
<td>0.0500</td>
<td>81.0</td>
<td>80.3</td>
</tr>
<tr>
<td>0.1000</td>
<td>66.2</td>
<td>65.6</td>
</tr>
<tr>
<td>0.200</td>
<td>43.6</td>
<td>43.2</td>
</tr>
<tr>
<td>0.300</td>
<td>29.2</td>
<td>28.8</td>
</tr>
<tr>
<td>0.400</td>
<td>18.9</td>
<td>18.7</td>
</tr>
<tr>
<td>0.500</td>
<td>12.7</td>
<td>12.5</td>
</tr>
<tr>
<td>0.600</td>
<td>(9.0)</td>
<td>(8.5)</td>
</tr>
</tbody>
</table>
amount used in the colorimetric procedure of Leavell and Ellis (2)—Beer's law was not followed at high iron concentrations, and the colors faded badly on standing and could not be restored by shaking. 10 ml. quantities of the reagent were used in obtaining the data in Table I, which show on plotting that Beer's law is followed exactly. Instrumental errors make it undesirable to use transmittances lower than 10 per cent in analyses.

Transmittances for systems without added acid agreed closely enough with those for systems with added acid to show that acids in concentrations up to those specified in the procedure below have no detectable effect on the color. Addition of 0.5 ml. of 30 per cent hydrogen peroxide per 50 ml. did not interfere with the color development, but when 1.0 ml. was added no color appeared.

Study of the data indicated an optimum pH between 9.2 and 9.5, but the excess of 4 N ammonium hydroxide may vary between 1 and 4 ml. without causing transmittance changes exceeding 0.5 per cent. Sodium hydroxide was substituted for ammonium hydroxide in about 5 per cent of the test solutions, and gave transmittances in excellent agreement with the medians in Table I.

**Spectrophotometric Procedure for Total Iron**

The following procedure is based on the experiments described above, and has been further tested by comparison with similar procedures with the reagents α,α'-dipyridyl and ferron, in analyses of some 80 materials, including foods and food concentrates, beverages, urine, feces, and pharmaceuticals.

Procedure—Ash a measured sample and make it up to a suitable volume after hydrolyzing any pyrophosphate present (3, 4). Take for analysis an aliquot containing not more than 0.55 mg. of iron. If a wet ashing method was used, evaporate the aliquot to dryness and destroy organic matter by heating the residue in succession with 0.5 ml. portions of 36 N sulfuric acid and 30 per cent hydrogen peroxide, or by electrical heating in deep fused silica beakers, with precautions to avoid loss of iron. Take up the residue with 5 ml. of 6 N hydrochloric acid and 0.3 ml. of 15 N nitric acid, dilute to 20 ml., and reflux for 30 minutes to hydrolyze pyrophosphate.

In all cases treat the aliquot to remove any interfering inorganic substances known to be present (1, 5) if maximum accuracy is desired. The solution for analysis may contain up to 5 ml. of 6 N
hydrochloric acid, 1 ml. of 36 N sulfuric acid, 5 ml. of 9 N perchloric acid, 2 ml. of 15 N nitric acid, 20 ml. of 0.6 N trichloroacetic acid, or 2 ml. each of 9 N perchloric and 15 N nitric acid.

If necessary, evaporate the solution to a volume which allows for reagent additions. Add a small piece of Congo red paper and neutralize with 4 N ammonium hydroxide. Add 10 ml. of mercaptoacetate reagent, then 2 ml. of 4 N ammonium hydroxide, and make to exactly 50 ml. Filter through a dry Pyrex crucible before diluting to volume if a precipitate forms. Make duplicate transmittance readings on two portions of the clear solution in about 30 minutes, at a wave-length of 535 mµ. Calculate the result of the analysis by substituting the median value of $T$ in the proper equation, as indicated below, or by use of a graph derived from the equation.

If the reagents are free from iron use water as the reference liquid; otherwise use a blank containing the reagents. If a colored aliquot is used directly, prepare as a reference liquid a system containing no mercaptoacetate reagent and the same volumes of colored liquid and ammonium hydroxide as the test solution.

For convenience in calculating results with maximum precision the data in Table I have been reduced by the method of least squares to two equations. In the first,

$$Mg. \text{Fe per 50 ml. test solution} = \frac{-0.731 \log_{10} T + 1.460}{l} \tag{1}$$

$T$ is the percentage transmittance relative to the blank, and $l$ is the thickness of the solution in cm. With water as the reference liquid the constants are respectively $-0.727$ and $1.451$. In the present calibration $l$ was 1.308 cm. The actual value of $l$ should be determined for the particular cuvettes used, with a micrometer and calipers. Under the conditions specified the average deviations of $T$ for various iron concentrations are about half as large as those given in Table I for single observations.

**SUMMARY**

An accurate spectrophotometric method for the determination of iron with mercaptoacetic acid has been developed experimentally.
BIBLIOGRAPHY

SPECTROPHOTOMETRIC DETERMINATION OF IRON: I. USE OF MERCAPTOACETIC ACID
Ruth Adele Koenig and C. R. Johnson

J. Biol. Chem. 1942, 142:233-238.

Access the most updated version of this article at http://www.jbc.org/content/142/1/233.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/142/1/233.citation.full.html#ref-list-1