A 1,10-PHENANTHROLINE METHOD FOR THE DETERMINATION OF IRON IN POWDERED MILK

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Milk contains traces of iron. The amount reported in the literature varies over a very wide range. Stugart (14), Associates of Rogers (2), and Davies (5) summarize the data available on the iron content of milk and state that the amount varies from 0.21 to 56.8 parts per million. Sherman (12) in 1941 reported the iron content of milk as two parts per million. More recently Johnston (7), using Stugart's thiocyanate method (14), analyzed sixty-one bottles of market milk from twenty-five companies in twelve communities. The iron content ranged from 0.114 to 0.650 milligrams per kilogram with a mean of 0.319 mg. per kg. This author also brings out the fact that, during the past ten years, seven out of eight investigations obtained values from 0.34 to 0.96 mg. per kg. Johnston concludes that the best value to adopt for average market milk is 0.3 mg. per kg., and that this value is one-seventh of the value given in previously reported tables of food composition.

The wide variation in the results obtained for the iron content of milk can be traced, no doubt, not only to variable degrees of contamination of the milk but to the variable methods of analysis employed and to the different procedures used in preparing the sample for analysis.

It is to be expected that the equipment used in handling milk at the farm and in the plant will result in a certain amount of metal contamination. It has long been recognized that, in order to prevent or retard the development of off flavors, especially of the oxidized type, the iron content of milk and other dairy products must be kept to a minimum. In recognition of this fact, the Army Quartermaster Corps has placed a maximum of 10 parts per million of iron in their buying standards for powdered whole milk, a standard which in view of the findings of Johnston on the iron content of milk seems very liberal.

There are numerous methods that have been used for the determination of iron in biological materials. Until recently most of these determinations were made either volumetrically, gravimetrically, or by visual means. It is now recognized that the photoelectric spectrophotometer provides a means of quantitative analysis that is of a higher degree of accuracy than is possible by other methods. This is particularly true when there are only traces of the metal present.

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Since the determination of traces of iron in dried milk and milk products is so important, it was thought advisable to study the adaptability of spectrophotometer methods to this problem, with the idea of developing a rapid, accurate test not too complicated in its procedure.

PREPARATION OF THE SAMPLE

The sample may be prepared for analysis by either the wet or dry ashing methods. The wet digestion procedure was successfully used on a few samples but was found to be less convenient than the dry ashing method. When the wet digestion method is used, acid must be added every few minutes during the digestion period; and the samples must be more or less constantly watched throughout the digestion. In some cases a white insoluble precipitate forms which is likely to detract from the accuracy of the analysis. It is also essential that the laboratory be equipped with an effective means of removing gas fumes issuing from the sample while it is being digested.

In the dry ashing procedure it is necessary to eliminate interference from pyrophosphates. This can be done either by fusion with sodium carbonate or by the addition of an acid such as hydrochloric or nitric. When the sample is prepared using sodium carbonate fusion, it is necessary to transfer the sample from the dish to the mortar and, after grinding, transfer it back again to the same dish. The ash is light and feathery, and with the best of care some of the fine ash particles may be lost. Sodium carbonate, even though it is purified before adding, may be a source of contamination. The mortar and pestle may also be a source of contamination. Early in this investigation it was found that results on iron using sodium carbonate fusion were much lower than when the dry ash was hydrolyzed with hydrochloric acid. Further work showed that the length of time of fusing had a definite bearing on the results obtained. In the literature none of the methods using sodium carbonate fusion specified the length of fusion time. It is evident, however, that pyrophosphates are not entirely eliminated by fusion, especially if the fusion time is under two minutes. On standing, these samples become more intense in color, and in some cases after 24 hours the values are still low as compared to the results obtained with a longer fusion time or with acid hydrolysis of the sample. The effect of varying the time of fusion is shown by the data in table 1 secured on a sample of powder to which was added 5 p.p.m. of iron.

When the time of fusion was only one half minute, the percentage transmittance was decreased (a higher p.p.m. value was obtained) as the time interval elapsing between fusing and reading the spectrophotometer was increased up to 24 hours. When the fusion time was one minute, the percentage transmittance was greater after 4 hours than after 10 minutes, but there was no further significant change after 24 hours. When the fusion
time was 2–4 minutes, practically the same values were obtained whether
the readings were made 10 minutes or 24 hours after fusing.

The dry ashing procedure, using hydrochloric acid to dissolve the ash
and to eliminate the interference of pyrophosphates, was found to give
satisfactory results. The ashed sample is hydrolyzed in the dish that is used
for ashing so that there are no transfers necessary except to the volumetric
flask. The method using acid hydrolysis is faster than the sodium carbonate
fusion method. When using the former method, at least twelve to fifteen
determinations can be made in a 4-hour period after the ash is removed from

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<thead>
<tr>
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<th>Fusion time</th>
<th>Transmittance 10 minutes</th>
<th>Transmittance 4 hours</th>
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</tr>
</thead>
<tbody>
<tr>
<td>5210</td>
<td>½</td>
<td>91.0</td>
<td>81.0</td>
<td>75.3</td>
</tr>
<tr>
<td>5210</td>
<td>1</td>
<td>82.0</td>
<td>73.5</td>
<td>72.8</td>
</tr>
<tr>
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<td>2</td>
<td>71.0</td>
<td>70.5</td>
<td>70.8</td>
</tr>
<tr>
<td>5210</td>
<td>4</td>
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</tr>
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1,10-PHENANTHROLINE USED FOR COLOR DEVELOPMENT

For developing the color of the iron, it was decided to use 1,10-phenan-
throline. Sandell (10) states that, for the most accurate determination
of minute quantities of iron, the use of 1,10-phenanthroline is recommended.
Fortune and Mellon (6), in a study of 1,10-phenanthroline using fifty-five
possible interfering ions, found that there were very few ions that seriously
interfered with the production of the quantitative color reaction. Of the
few ions that were found to interfere, the only ones present in milk ash in
significant quantities are pyrophosphates, copper, zinc and nickel.

The pyrophosphate interference can be eliminated by heating for 5
minutes with hydrochloric acid (1+1). Copper can be eliminated by con-
trolling the pH between 2.5 and 4.0. The interference of zinc can be con-
trolled by adding a larger excess of reagent. In this study it was found that
nickel gave no interference when added in quantities up to three parts per
million.

The advantages of the use of 1,10-phenanthroline in the determination
of iron may be summarized as follows: (a) it gives a relatively more intense
color for a given iron concentration (it gives approximately 9 per cent more
color as compared to 2,2'-bipyridyl; (b) it has almost complete freedom from interference by most of the common ions; (c) it has greater working sensitivity; (d) a color is produced that is more desirable for colorimetric comparison; (e) the orange-red complex produced is stable, and no fading has been observed for as long as six months; (f) pH need not be regulated closely; (g) color formations occur in acid solution, eliminating the difficulties usually caused by precipitation of metal hydroxides and hydrated oxides in alkaline solution.

The thiocyanate method according to Sandell (10) is still extensively used for the determination of iron, even though other reagents give better results. He also states that the reaction is a delicate one from the quantitative standpoint, because the color intensity depends upon a number of factors such as the excess of thiocyanate, the kind of acid present and the time of standing. The thiocyanate method is favored when the color reaction must be carried out at a low pH.

HYDROXYLAMINE HYDROCHLORIDE USED AS A REDUCING AGENT

Saywell and Cunningham (11), in their work on fruit juices, found a 10 per cent solution of hydroxylamine hydrochloride to be satisfactory as the reducing agent in the iron determination. Fortune and Mellon (6) tried using hydroxylamine hydrochloride, sodium sulfite, sodium and potassium formates, and formaldehyde as the reducing agents, but found the hydroxylamine hydrochloride solution to be the most satisfactory. Mehlig and Hulett (8) tried to use stannous chloride as the reducing agent, but also found it not to be as satisfactory as hydroxylamine hydrochloride. In the 2,2'-bipyridyl method used by the Quartermaster Corps (10), hydroquinone is used as the reducing agent. Hydroquinone could also be used as the reducing agent with 1,10-phenanthroline but, even though kept cool and in the dark, it will deteriorate in a few weeks.

Further advantages of the use of hydroxylamine hydrochloride are: (a) it does not have to be stored in the dark; (b) it is not necessary to keep it refrigerated; (c) because of its stability, a large quantity can be prepared at one time; (d) it rapidly reduces ferric to ferrous iron; (e) the color intensity of the complex formed between 1,10-phenanthroline and ferrous iron is independent of the acidity in the pH range 2-9. Below pH 2 the color does not develop, develops slowly, or does not fully develop (6, 11).

CONTROL OF pH

To eliminate the interference of copper the pH must be controlled between 2.5 and 4.0 (6). It is preferable to have the pH of the sample solution the same as that of the standards. The method was standardized so that the final pH of the solution and standards were 3.4 to 3.8.
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OUTLINE OF RECOMMENDED PROCEDURE

I. Apparatus Required:
   Platinum dishes (5 cm. diameter and 25–50 ml. capacity).
   Platinum or glass-tipped tongs.
   Photoelectric colorimeter (a Coleman Universal Spectrophotometer was used throughout this work).
   Muffle furnace equipped with a rheostat and pyrometer.
   Pyrex glassware.

II. Reagents and Solutions:
   1. Acetate buffer solution. Reagent grade containing a maximum of 0.0005% iron. The buffer is made up by adding 16.6 grams of sodium acetate and 24 ml. of redistilled C.P. glacial acetic acid and diluting to 200 ml. with redistilled water in a volumetric Pyrex flask.
   2. Hydroxylamine hydrochloride. C.P. 10% solution in water (10 grams hydroxylamine hydrochloride plus 90 grams of redistilled water).
   3. 1,10-phenanthroline monohydrate. Saturated solution in redistilled water (1 gram 1,10-phenanthroline dissolved in 300 grams of water and warmed to effect solution). According to Smith (13) 1,10-phenanthroline is soluble to the extent of 0.016 Moles per liter at 25°C.
   4. Water redistilled from Pyrex.
   5. Concentrated ammonium hydroxide C.P.
   6. Hydrochloric acid C.P. (1 + 1 solution by volume).
   7. Redistilled nitric acid for cleaning glassware (redistilled from Pyrex).

III. Preparation of a Standard Iron Solution:
   The standard iron solution is prepared by dissolving 0.1000 gram analytical grade iron wire in 20 per cent hydrochloric acid and carefully evaporating to dryness on a steam bath. The dried material is dissolved in a minimum amount of hydrochloric acid, transferred quantitatively to a 1,000-ml. Pyrex volumetric flask and diluted to volume. This stock solution will contain 100 micrograms of iron per milliliter. A stronger stock solution can also be prepared by dissolving 1.0 gram analytical grade iron wire and diluting to 1,000 ml. This stock solution will contain 1 milligram of iron per milliliter.
   To produce a working standard from the weaker stock solution, a 100 ml. aliquot is diluted to 1 liter. This gives a standard of 100 micrograms per ml. in solution. For the stronger solution mentioned above, a 10 microgram per ml. solution can be prepared by diluting 10 ml. of the stock solution to 1 liter.

IV. Spectral Transmittance Curves:
   Spectral transmittance curves are made for 20-microgram and 30-microgram iron solutions by adding the required amount of standard solution
and making it up to 10 ml. The iron solutions are made in iron-free Pyrex test tubes. The following are added—in the order indicated—to the 10-ml. iron solution, which is shaken after each addition:

- 2 ml. hydroxylamine hydrochloride
- 5 ml. acetate buffer solution
- 2 ml. 1,10-phenanthroline solution

The transmittancy curves for 20 micrograms and 30 micrograms of iron using a 2-cm. cell are shown in figure 1. The peak of the absorption band is located at 500 millimicrons. Twenty micrograms of iron give a transmittancy of 43.2 per cent while thirty micrograms of iron give a 29.0 per cent transmittancy. In these determinations a reagent blank using 10 ml. of redistilled water plus reagents was set at 100 per cent transmission.

![Figure 1](image)

**Fig. 1.** Spectral transmittance curve for iron using 1,10-phenanthroline and a 2.0-cm. side couvette. 1. 20 micrograms of iron. 2. 30 micrograms of iron.

V. Preparation of Standard Reference Curve:

Aliquots of 0.5, 1.0, 2.0, 3.0 and 4.0 ml. of the standard iron solutions (1 ml. = 10 micrograms) are accurately measured into iron-free Pyrex test tubes and diluted to 10 ml. with redistilled water, and the reagents are added as described in the spectral transmittancy curve. A blank, using 10 ml. of redistilled water, and the reagents are set at 100 per cent trans-
TABLE 2
Preparation of standard curve

<table>
<thead>
<tr>
<th>Ml. standard solution (1 ml. = 10 micrograms)</th>
<th>Fe in 10 ml. solution</th>
<th>Transmittance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p.p.m.</td>
<td>per cent</td>
</tr>
<tr>
<td>0.5</td>
<td>5</td>
<td>82.8</td>
</tr>
<tr>
<td>1.0</td>
<td>10</td>
<td>65.6</td>
</tr>
<tr>
<td>2.0</td>
<td>20</td>
<td>43.4</td>
</tr>
<tr>
<td>3.0</td>
<td>30</td>
<td>29.0</td>
</tr>
<tr>
<td>4.0</td>
<td>40</td>
<td>20.0</td>
</tr>
</tbody>
</table>

mittancy in the preparation of the standard curve. The data obtained for the standard curve using a 2-cm. cell and set at 500 millimicrons are given in table 2. These data were plotted on semi-logarithmic paper plotting per cent transmission against the concentration. The results are given in figure 2. It will be noted that the five points determined for this curve follow a straight line and thus conform closely to Beer's law.

Fig. 2. Standard reference curve for iron using 1,10-phenanthroline, 2.0-cm. side couvette, and wave length set at 500 millimicrons.
VI. Procedure for Iron Determination:

Weigh out accurately 5 grams of powdered milk into a platinum dish or crucible. Place the dish or crucible into a muffle furnace having a temperature of not more than 300° C. When charring is complete (usually not more than an hour), gradually raise the temperature to 500-550° C. and ash until the sample is a greyish white color. With the type of platinum dishes used, it was found that 4 hours’ ashing time after charring was sufficient. Or, if convenient, the sample could be ashed over night (16-18 hours). The ashed samples are removed from the muffle and allowed to cool.

The dish is placed on a clay triangle, and 5 ml. of hydrochloric acid (1 + 1) is added. The sample is then covered with a watch glass and gently boiled for 5 minutes. When the dish and watch glass have cooled, the watch glass is removed, and any droplets formed on it are washed into the dish with redistilled water.

The solution is then transferred, by means of a pipette, a Pyrex funnel or other convenient means, to a 50-ml. volumetric flask. Redistilled water is placed in the dish, which is then heated. The water is added to the 50-ml. volumetric flask. The flask is filled almost to the neck with redistilled water, 1 ml. of concentrated ammonium hydroxide is added and the contents mixed. The flask is then filled to the mark with redistilled water. A 10 ml. aliquot of the prepared ash solution is pipetted into an iron-free Pyrex test tube and the following color reagents are added, the solution being shaken after each addition:

- 2 ml. hydroxylamine hydrochloride solution
- 5 ml. acetate buffer solution
- 2 ml. 1,10-phenanthroline solution

A permanent orange-red complex \([\{(C_{12}H_{8}N_2)_3Fe\}]^{++}\) develops if any iron is present, the intensity being proportional to the iron content.

The iron content of the platinum dish and reagents are determined the same way as the ashed sample and used as a blank and set at 100 per cent transmission in the spectrophotometer. In this determination of iron, the spectrophotometer was set at 500 millimicrons (using a PC-4 filter), and a 2-cm. cell was used as in the preparation of the spectral and standard curves. Since a one-gram aliquot is used in this determination, the p.p.m iron is read off directly from the standard reference curve.

DISCUSSION OF THE METHOD

In this study, flat platinum ashing dishes with a diameter of 5 cm, were used. Vycor dishes were tried on a number of samples but consistent results could not be obtained. The platinum dishes should be handled with platinum- or glass-tipped tongs to prevent contamination. To clean the platinum dishes, hydrochloric acid (1 + 1) should be boiled in them until they are clean and are iron-free.
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It is imperative that all water used be as free from iron as possible. Water redistilled from Pyrex is practically iron-free.

Pyrex glassware should be used throughout the determination. All reagents should be prepared and stored in Pyrex, and need not be refrigerated. The glassware should be cleaned by rinsing with distilled nitric acid, followed by a number of times with distilled water and finally redistilled water.

It was found that a satisfactory blank could be obtained by using reagent grade sodium acetate. A comparison of purified with reagent grade chemicals showed that the amounts of iron present were practically identical.

By ashing under a temperature of 550 °C. and hydrolyzing for five minutes with hydrochloric acid (1+1), no interference from pyrophosphates was encountered. If it is desired to make sure that no pyrophosphates are present, the sample should be read again after one hour.

After the addition of the one milliliter of concentrated ammonium hydroxide to the aliquot in the 50-ml. flask, a white precipitate may form which may be redissolved by the addition of a few drops of hydrochloric acid (1+1).

Loss of iron on ashing and interference from copper. A factor reputed to cause analytical errors is the loss of iron by volatilization during the ashing process. Andrews and Felt (1) have reviewed some of the investigations that deal with this problem, and there seems to be no agreement whether or not there is a loss of iron by volatilization. Andrews and Felt, in the recovery of added iron in the analysis of flour and bread, show that there is no evidence of any loss of iron since the difference between the found and calculated values is within range of the probable error.

The writers have investigated the loss of iron as evidenced by the recovery of iron added to the sample before ashing. The iron solution added was that prepared for the standard reference curve. Copper (copper wire standard) was also added to these samples to note whether there was any interference from copper. The results for the iron recoveries are given in table 3.

When 5 to 15 parts per million of iron and 1 to 3 parts per million of copper were added to powdered milk before ashing, recoveries from 99.14 per cent to 98.20 per cent were obtained. Similar excellent recoveries have

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Fe added p.p.m.</th>
<th>Cu added p.p.m.</th>
<th>Fe calculated p.p.m.</th>
<th>Fe found p.p.m.</th>
<th>Recovery per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1022</td>
<td>0</td>
<td>0</td>
<td>6.7</td>
<td>11.6</td>
<td>99.14</td>
</tr>
<tr>
<td>1022</td>
<td>5</td>
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<td>11.6</td>
<td>98.20</td>
</tr>
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<td>1022</td>
<td>10</td>
<td>2</td>
<td>16.7</td>
<td>16.4</td>
<td>98.61</td>
</tr>
<tr>
<td>1022</td>
<td>15</td>
<td>3</td>
<td>21.7</td>
<td>21.4</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 4

Effect of length of ashing time on iron content of powdered milk

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Ashing time (hrs.)</th>
<th>Iron (p.p.m.) (sample not filtered)</th>
<th>Iron (p.p.m.) (sample filtered)</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3</td>
<td>4.9</td>
<td>4.4</td>
<td>___</td>
</tr>
<tr>
<td>3 + {1 p.p.m. Cu</td>
<td>5 p.p.m. Fe}</td>
<td>3</td>
<td>10.4</td>
<td>9.4</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>___</td>
<td>___</td>
<td>4.4</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>___</td>
<td>___</td>
<td>4.5</td>
</tr>
<tr>
<td>3 + 5 p.p.m. Fe</td>
<td>18</td>
<td>___</td>
<td>___</td>
<td>9.6</td>
</tr>
<tr>
<td>3 + {1 p.p.m. Cu</td>
<td>5 p.p.m. Fe}</td>
<td>18</td>
<td>___</td>
<td>___</td>
</tr>
</tbody>
</table>

been obtained on other samples of milk powder with iron added before ashing. There seems to be no interference from copper in the 1,10-phenanthroline method at the pH used.

Effect of length of ashing time. There was evidence throughout the investigation that, in the preparation of the sample for analysis, it is important to get the sample completely ashed. Incompletely ashed samples usually produced high results as the carbon interfered with the color produced. With the flat type of platinum dish used in this study, it was found that four hours' ashing (after charring is complete) is sufficient to produce a clear sample. Some typical results obtained on 4- and 18-hour ashing and on an incompletely ashed sample are given in table 4.

Effect of length of hydrolysis time on the recovery of iron in powdered milk. In the early study of this method it was found that inconsistent results would be obtained if the hydrolysis time was limited to a few minutes. To note the effect of hydrolysis time, a sample of powdered milk was hydrolyzed from 5 to 20 minutes after ashing for 4 hours (from time of complete charring). The results are given in table 5.

Five minutes' hydrolysis time was found to be sufficient to produce the quantitative color reaction within 10 minutes after the reagents are added. The only objection to longer hydrolysis time is the mechanical losses that may occur. There was no interference from pyrophosphates, as the color did not change after the samples were read again in one hour.

TABLE 5

Effect of length of hydrolysis time on the iron content of a sample of powdered milk

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Hydrolysis time (min.)</th>
<th>Iron, 10 minutes (p.p.m.)</th>
<th>Iron, 1 hour (p.p.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>5</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>5.1</td>
<td>5.0</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>5.0</td>
<td>4.9</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>5.0</td>
<td>5.1</td>
</tr>
</tbody>
</table>
DETERMINATION OF IRON IN POWDERED MILK

TABLE 6
The effect of the order of adding the reagents on the rate and intensity of the color development

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Order of adding reagents</th>
<th>Iron, 10 minutes</th>
<th>Iron, 1 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>5210</td>
<td>RA-B-OP*</td>
<td>4.4</td>
<td>4.4</td>
</tr>
<tr>
<td>5210</td>
<td>B-RA-OP</td>
<td>4.4</td>
<td>4.4</td>
</tr>
<tr>
<td>5210</td>
<td>RA-OP-B</td>
<td>6.9</td>
<td>6.7</td>
</tr>
<tr>
<td>J12</td>
<td>RA-B-OP</td>
<td>6.7</td>
<td>6.7</td>
</tr>
<tr>
<td>J12</td>
<td>B-RA-OP</td>
<td>6.7</td>
<td>6.7</td>
</tr>
</tbody>
</table>

RA = Hydroxylamine hydrochloric reducing agent; B = Sodium acetate buffer; OP = 1,10-phenanthroline.

Effect of order of adding reagents on iron content of powdered milk. Banderer and Schaible (3) made a critical study of the 1,10-phenanthroline method and state that sodium citrate is more satisfactory in adjusting the pH than sodium acetate, and that the order of adding the reagents influences the rate of color development. They used hydroquinone as a reducing agent. Sodium citrate cannot be used when hydroxylamine hydrochloride is used as the reducing agent (4). To note the effect on the rate of color development, the sodium acetate buffer was added in various orders to the sample. The results on two samples of powdered milk are given in table 6.

With the use of hydroxylamine hydrochloride as the reducing agent, it does not seem to matter in what order the sodium acetate is added, as the maximum color is produced within 10 minutes. However, since a standard procedure is desirable, it is recommended that the reagents be added in the following order: (1) reducing agent; (2) sodium acetate buffer; and (3) 1,10-phenanthroline, as this seemed the most logical order of adding the reagents.

Variations in the iron content of a commercial batch of powdered milk. It was noted that, in analyzing samples prepared with the pilot spray drier at the University of Illinois, invariably the first batch of powder made was higher in iron than succeeding batches. These results were checked at a commercial plant where 66 barrels of whole milk powder were made from one batch of milk. Samples were taken out of the first barrel and a number of other barrels throughout the run. Results are given in table 7.

TABLE 7
The iron content of a run of powdered milk

<table>
<thead>
<tr>
<th>Barrel</th>
<th>Iron found p.p.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>4.6</td>
</tr>
<tr>
<td>23rd</td>
<td>3.8</td>
</tr>
<tr>
<td>44th</td>
<td>3.6</td>
</tr>
<tr>
<td>66th</td>
<td>3.3</td>
</tr>
</tbody>
</table>
The first milk through the system produced a powder higher in iron than samples taken from the barrels later in the run. There was a gradual decrease in the iron of this powder as the run continued. This is an important point to keep in mind when samples are taken by regulatory agencies.

SUMMARY

A rapid, accurate method for the determination of iron using hydroxylamine hydrochloride, sodium acetate buffer, and 1,10-phenanthroline is described. The ash is taken up with hydrochloric acid (1+1) and heated for 5 minutes. No interference from pyrophosphates, copper and nickel was observed. In the amounts found in milk, zinc did not interfere with the color development. An excess of 1,10-phenanthroline is added to take care of any zinc complex that may be present.

The reagents used in this iron determination are stable and need not be refrigerated or stored in the dark. The advantages of the use of 1,10-phenanthroline and hydroxylamine hydrochloride are given.

Losses of iron during ashing have not been observed.

The order of addition of the sodium acetate buffer was not found to affect the intensity of the color produced.

The variations of the iron content of a batch of commercial powdered milk are given.

REFERENCES

13. SMITH, G. F. Ortho-Phenanthroline and Substituted Ortho-Phenanthroline Derivatives and Their Application to Analysis. G. Fredrick Smith Chemical Co., Columbus, Ohio. 1944.