In conclusion it should be mentioned that while the deviations which have been reported for animal and plant tissues are small, they are nevertheless appreciable. Deviations of this magnitude are exceedingly difficult to produce in the laboratory; the writer has tried several times himself and has examined many samples prepared by eminent scientists but in no instance has a definite isotope shift been observed. While it is possible to conjecture concerning the mechanism of the isotope effect in nature, the data are still too meager to permit any definite conclusion to be drawn.

The writer is indebted to Mr. N. G. Barbella, of the Bureau of Animal Industry for furnishing many of the samples tested.

## Summary

The abundance ratio of the two principal isotopes of potassium present in animal tissues have been measured with a mass spectrograph. The $K^{39}/K^{41}$ ratio for most organs is close to 14.20, which is the same as that previously obtained for most plants and minerals and for ocean water. A few tissues, such as the lining of the auricle and the lining of the small intestine, appear to possess an abnormally high concentration of $K^{39}$, while bone marrow is high in $K^{41}$. The results with bone marrow are significant in that they indicate a possible relationship between the abundance ratio and the age of the animal, and hence with the development of embryonic cells within the organism.

The atomic weight of potassium has been calculated using the most probable values for the packing fraction and the conversion factor; the value thus obtained for most tissues is 39.094. Since all deviations from this normal value are small, it does not seem probable that the potassium in animal tissue could be distinguished from mineral potassium by radioactive measurements as has been suggested by some investigators.

WASHINGTON, D. C. RECEIVED MARCH 13, 1937

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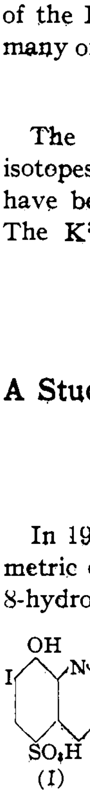
[CONTRIBUTION FROM THE COBB CHEMICAL LABORATORY, UNIVERSITY OF VIRGINIA]

# A Study of 7-Iodo-8-hydroxyquinoline-5-sulfonic Acid as a Reagent for the Colorimetric Determination of Ferric Iron[1]

BY JOHN H. YOE AND ROBERT T. HALL[2]

In 1932 Yoe[3] reported a method for the colorimetric determination of iron by means of 7-iodo-8-hydroxyquinoline-5-sulfonic acid (I).[4] It was found that this compound reacts instantaneously with ferric ions yielding green colored solutions, the color intensity varying with the iron concentration. The reaction is very sensitive and may be used to determine ferric iron in the presence of ferrous, since the latter produces no color with the reagent. Also, the color does not fade on standing, which is an advantage over the thiocyanate method as frequently carried out in acid solution.



(I)

In view of the advantages offered by this method, namely, the stability of the colored compound, the high sensitivity of the reaction, and its ability to distinguish between ferric and ferrous ions, it seemed desirable to investigate it in detail. In fact, the reagent appears to be specific for ferric ions, since no other ion has been found to yield a color reaction with Ferron.[3]

The following studies have been made on the reagent and its reaction with ferric ions: solubility in various solvents; nature of the color reaction; physical and chemical properties of Ferron; influence of various ions; sensitivity; Lambert–Beer law; effect of hydrogen-ion concentration; aging effect and the effect of temperature.

**Reagents and Solutions.**—The general technique and method of investigation has been described in a previous paper by Yoe and Wirsing.[5] 7-Iodo-8-hydroxyquinoline-5-sulfonic acid (Ferron) was first prepared by Claus.[6] The compound used in this investigation was a very pure product obtained from G. D. Searle and Co., Chicago, Ill. A saturated aqueous solution (approximately 0.2%) of Ferron makes a satisfactory reagent solution. For convenience in obtaining various molecular ratios between the Ferron and ferric iron, several other Ferron solutions of known concentration were prepared.

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(1) Original manuscript received April 11, 1936.

(2) Present address, Swann and Company, Birmingham, Ala.

(3) Yoe, *This Journal*, **54**, 4139 (1932).

(4) On account of the lengthy name of this compound we are proposing that it be called "Ferron."

(5) Yoe and Wirsing, This Journal, **54**, 1866 (1932).

(6) Claus, *Friedl.*, **3**, 964 (1892).

The standard iron solution was prepared from ferrous ammonium sulfate, $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$, reagent quality, 0.7020 g. of the salt being weighed out and dissolved in about 100 ml. of distilled water. Five milliliters of concentrated sulfuric acid (iron-free) was then added to the solution to prevent hydrolysis. Five to ten milliliters of saturated bromine water was added and the solution boiled until the iron was oxidized and the excess bromine expelled. The solution was then cooled and diluted to a liter in a volumetric flask. Its iron content was checked volumetrically by reducing (with the Jones reductor) the ferric sulfate solution and titrating immediately with a potassium permanganate solution which had been standardized against sodium oxalate obtained from the National Bureau of Standards. The average of three determinations in close agreement gave the iron concentration as 0.1005 g. per liter. This solution was used as a stock solution from which more dilute ones were conveniently prepared as needed.

Solutions of the salts used in studying the effects of various ions were prepared so that 1 ml. would contain 2 mg. of the desired ion, assuming complete dissociation. Chlorides, nitrates and sulfates were employed and, when necessary, they were recrystallized from conductivity water until iron-free.

The buffer solutions were prepared from various standard solutions according to the directions of Clark.[7]

**Apparatus.**—For the solubility measurements, a large thermostat equipped with an immersed, rotating wheel was used. Its temperature was maintained constant at 25 to ±0.1°. Standard types of apparatus and methods were employed in making the *p*H determinations. In making color comparisons, a daylight lamp and Duboscq colorimeter (Bausch and Lomb) were always used in fixed positions in a dark room. Also a set of twelve carefully matched 50-ml. Nessler tubes (210-mm.) was used. These were placed in a shielded Nessler tube rack.

All weights, pipets and volumetric flasks were calibrated. The reagents were measured out with pipets which were labelled so that a given pipet was always used for one solution only.

**Composition of Ferron.**—An ultimate analysis was made of the 7-iodo-8-hydroxyquinoline-5-sulfonic acid. using standard methods. The results of the analysis are summarized in Table I.

TABLE I

| Constituent | Found | Calcd. |
|---|---|---|
| Carbon | 30.68 | 30.76 |
| Hydrogen | 1.62 | 1.72 |
| Oxygen[a] | 18.43 | 18.27 |
| Nitrogen | 4.03 | 3.99 |
| Sulfur[b] | 9.04 | 9.11 |
| Iodine[b] | 36.06 | 36.15 |

[a] Obtained by difference.

[b] Average of three analyses.

## Experimental

**Solubility of Ferron.**—The solubility of 7-iodo-8-hydroxyquinoline-5-sulfonic acid in several solvents and mixtures of solvents was determined at 25°. Into clean 100-ml. glass-stoppered Pyrex bottles was introduced 50 ml. of the solvent and sufficient of the Ferron so that after thorough agitation a few tenths of a gram of the solid settled out. The bottles were tightly stoppered, firmly attached to the thermostat wheel, rotated for twenty hours and then allowed to stand for sixteen hours. Without removing the bottles from the thermostat, 25 ml. of the clear supernatant liquid was withdrawn and introduced into dried weighed crucibles. The solutions were evaporated to dryness in vacuum desiccators and the crucibles plus their contents then weighed. The data are recorded in Table II.

TABLE II

SOLUBILITY OF FERRON AT 25 ±0.1°

| Solvent | Solubility, g./100 ml. soln. |
|---|---|
| Acetone | 0.0080 |
| Glacial acetic acid | .0192 |
| Alcohol (absolute) | .0304 |
| Water | .2220 |
| Water–alcohol (1:1 by vol.) | .3500 |
| Water–alcohol (3:2 by vol.) | .5696 |
| Water–acetone (1:1 by vol.) | .7200 |

It is interesting to note that the mixtures have a greater solvent action than the individual solvents. The presence of the sulfonic acid group in the compound probably causes it to have a greater solubility in water than in an organic solvent. If the compound did not contain the sulfonic acid group, it would be expected to be more soluble in an organic solvent than in water. Therefore it might be predicted that the maximum solubility would be attained in a mixture of water and an organic solvent. Such was found to be the case.

**Physical and Chemical Properties of Ferron.**—7-Iodo-8-hydroxyquinoline-5-sulfonic acid is a bright yellow crystalline solid. On slow heating it changes from a yellow color to a dark brown at 225°, and at 250° turns completely black, decomposition occurring without melting. On rapid heating it melts sharply at 285°, at the same time undergoing decomposition as is indicated by an evolution of iodine vapor. Boiling its aqueous solution causes a slow decomposition with the evolution of a trace of iodine, easily detected by its odor. Also, the aqueous solution on standing for several weeks takes on a slightly darker shade of yellow due to the presence of a little iodine liberated by the compound. However, such solutions are quite satisfactory for colorimetric work.

**Nature of the Reaction.**—The exact reaction that takes place between 7-iodo-8-hydroxy-

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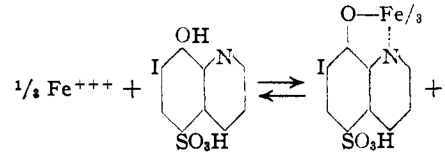
(7) Clark, "The Determination of Hydrogen Ions," 3d ed., Williams and Wilkins Co., Baltimore, Md., 1928.

quinoline-5-sulfonic acid and ferric ions is not known with certainty. In the first part of our work aqueous solutions of the reagent were prepared in such concentrations that when added to known amounts of ferric iron, there existed a definite molecular ratio between the iron and the reagent. By changing these molecular ratios both the color and the intensity could be varied. It was reasoned that if the molecular ratio in which the ferric ions and the reagent combine to give the optimum color formation could be determined, this might give some information as to the probable reaction. With this in view a number of solutions were prepared for observation in Nessler tubes and in the colorimeter. The molecular ratios of the reagent to ferric iron in these solutions were varied widely and covered the range from 0.5 mole of reagent : 1 mole of ferric iron to 6 moles of reagent : 1 mole of ferric iron. The reagent solutions were prepared in varying concentrations so that the same volume of reagent was added to the iron solution in each case, although different molecular ratios existed after final dilution. The amount of iron was constant throughout.

The color studies indicated that 1 mole of ferric iron requires at least 1 mole of reagent but not more than 3 moles. Color studies were made on the three molecular ratios, 1:1, 2:1 and 3:1. The evidence in all cases pointed to a 3:1 molecular reaction between Ferron and ferric iron, *i. e.*, one equivalent weight of ferric iron requires one mole of the reagent. The reaction may be expressed by



This reaction is in accord with the work of Berg[8] on the complex metal salts of *o*-hydroxyquinoline. Berg postulated that the hydrogen of the hydroxyl group, due to the position of this group relative to the quinoline nucleus, had its activity enhanced and that the compound reacting with metals formed an inner complex salt. However, it should be noted that our conclusions concerning this reaction are based purely on color formation and of course cannot be considered as a rigid proof. Attempts to obtain the colored compound in a crystalline form were unsuccessful. Slow evaporation at room temperature always yielded a dark green amorphous mass. In any case, in the preparation of a standard series, at least enough reagent should be added to use up all the iron on the basis of a 3:1 reaction, *i. e.*, 3 moles of Ferron to 1 gram-atom of iron.

**Influence of Various Ions.**—In the preliminary investigation Yoe[3] found that ferric ions are the only ions which give a color reaction with 7-iodo-8-hydroxyquinoline-5-sulfonic acid. He also pointed out that salts which hydrolyze easily, or yield colored ions, interfere with the color reaction. Therefore a quantitative study of the effect of various ions likely to be encountered in analyses was made. Aqueous solutions containing known amounts of ferric iron plus various other ions were prepared. The amount of iron in these solutions was then determined by comparison with standards. The procedure was as follows: measured quantities of the metallic salts were placed in Nessler tubes and to each tube was added a known amount of ferric iron. The acidity was adjusted to a *p*H of about 2.5 by adding hydrochloric acid–potassium acid phthalate buffer solution. The reagent was then added, the solutions diluted to the mark, thoroughly mixed and compared with a series of standards. The results are shown in Tables III and IV.

TABLE III

CONCENTRATION OF IONS IN PARTS PER MILLION

| Soln. | Metal ions | Metal ions | $Fe^{+++}$ Present | $Fe^{+++}$ Found |
|---|---|---|---|---|
| 1 | $Al^{+++}$ 7.0 | | 1.0 | No match |
| 2 | $Al^{+++}$ 2.5 | | 0.5 | 0.5 |
| 3 | $Al^{+++}$ 5.0 | | .5 | No match |
| 4 | $Al^{+++}$ 5.0 | | 1.0 | 1.0 |
| 5 | $Al^{+++}$ 10.0 | | 1.0 | No match |
| 6 | $Al^{+++}$ 30.0 | | 6.0 | 6.0 |
| 7 | $Co^{++}$ 1.6 | | 0.5 | No match |
| 8 | $Co^{++}$ 2.0 | | 1.0 | 1.0 |
| 9 | $Co^{++}$ 0.8 | $Ni^{++}$ 0.8 | 0.5 | 0.5 |
| 10 | $Fe^{++}$ 2.0 | | 2.0 | 2.0 |
| 11 | $Fe^{++}$ 1.0 | | 0.4 | 0.4 |
| 12 | $Co^{++}$ 4.0 | $Ni^{++}$ 4.0 | 4.0 | 4.0 |
| 13 | $Co^{++}$ 6.0 | $Ni^{++}$ 6.0 | 6.0 | 6.0 |
| 14 | $Co^{++}$ 1.0 | $Ni^{++}$ 1.0 | 0.5 | No match |
| 15 | | $Cr^{+++}$ 1.0 | .5 | 0.5 |
| 16 | $Al^{+++}$ 10.0 | $Cr^{+++}$ 10.0 | 5.0 | 5.4 |
| 17 | | $Cr^{+++}$ 10.0 | 5.0 | 5.2 |
| 18 | | $Cr^{+++}$ 1.4 | 0.5 | 0.5 |
| 19 | | $Cr^{+++}$ 20.0 | .5 | No match |
| 20 | | $Cr^{+++}$ 3.0 | 5.0 | 5.0 |
| 21 | $Ti^{++++}$ 0.2 | | 0.5 | 0.5 |
| 22 | .3 | | .5 | No match |
| 23 | 20.0 | | 5.0 | No match |

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[8] Berg, *J. prakt. Chem.*, **115**, 178 (1927); *Z. anal. Chem.*, **70**, 341 (1927).

TABLE IV

CONCENTRATION OF IONS IN PARTS PER MILLION

| Soln. | Metal ions | $Fe^{+++}$ Present | $Fe^{+++}$ Found |
|---|---|---|---|
| 1 | $Sn^{++}$ 0.1 | 0.4 | No match |
| 2 | $Sn^{++}$ .1 | 1.0 | No match |
| 3 | $Sn^{++++}$ .8 | 2.0 | 2.0 |
| 4 | $Sn^{++++}$ 2.0 | 2.0 | No match |
| 5 | $Sn^{++++}$ 1.2 | 0.5 | 0.5 |
| 6 | $Sn^{++++}$ 1.2 | 2.0 | 2.0 |
| 7 | $Cu^{++}$ 0.4 | 0.5 | Color bleached |
| 8 | $Cu^{++}$ .3 | 1.0 | Color bleached |
| 9 | $Cr_2O_7^{=}$ .4 | 0.5 | No match |
| 10 | $Cr_2O_7^{=}$ .2 | 2.0 | No match |
| 11 | $NO_2^-$ 1.0 | 2.0 | No match |
| 12 | $NO_2$ 0.2 | 0.5 | No match |
| 13 | $NO_2^-$ .2 | 2.0 | No match |

The data in Table III show that colored ions should not be present except to a limited extent. Aluminum may be present in an amount approximately five times that of ferric iron without causing marked interference. Colbalt and nickel may be present individually in proportions about twice as great as iron. Titanium should not be present in a concentration greater than a few tenths of a part per million. The chromium concentration should not exceed that of the iron. The effect of the above colored ions appears to be additive. It is of particular interest to note that ferrous iron may be present without causing the least difficulty in the determination of the ferric iron.

Table IV records the ions that cause the most difficulty. These ions must not be present in concentrations greater than a few tenths of a part per million. For precise work they should be removed totally. Cupric ions interfere when present to the extent of only 0.2 p. p. m. Salts that hydrolyze easily, such as those of tin and titanium, should be removed before an iron determination is made. It should also be pointed out that fluoride causes a partial bleaching of the color and must be absent. Relatively large amounts of phosphate may prevent the immediate formation of maximum green color intensity but on standing (five minutes to two hours) the maximum intensity develops.

Sulfate, nitrate and chloride ions do not interfere with the color reaction.

**Sensitivity.**—The sensitivity measurements were made according to the method described by Yoe and Wirsing.[5] The results are recorded in Table V.

In Table V column 1 gives the concentration stated as liters containing one gram-atom of iron.

TABLE V

| $V$ | $B$ | $\Delta$ | $S$ | $B'$ | $B/B'$ |
|---|---|---|---|---|---|
| 7,977 | 0.3500 | Too intense for observation | | | |
| 15,954 | .1750 | 0.0125 | 80 | 0.1875 | 0.933 |
| 21,476 | .1300 | .0075 | 133 | .1375 | .945 |
| 31,023 | .0900 | .0050 | 200 | .0950 | .947 |
| 50,950 | .0548 | .0037 | 266 | .0585 | .936 |
| 186,133 | .0150 | .0025 | 400 | .0175 | .857 |
| 310,230 | .0090 | .0020 | 500 | .0110 | .820 |
| 1,116,800 | .0025 | Barely distinguishable from blank | | | |

In the second column $B$ is the total milligrams of iron in 50 ml. of solution. $\Delta$ is the mg. of iron producing a perceptible difference in the color intensity of 50 ml. of solution. $S$ is the sensitivity and is the reciprocal of the figures recorded in column 3. $B' = B + \Delta$.

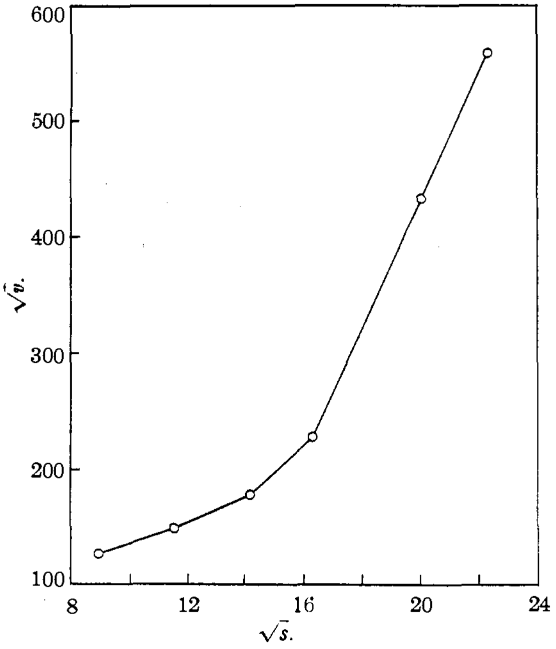

Fig. 1.

Figure 1 shows graphically how the sensitivity varies with the concentration. For convenience in plotting, the square roots of the volume and sensitivity were used. The sensitivity curve for iron is very similar to those obtained by Yoe and Hill[9] for aluminum and by Yoe and Wirsing[5] for nickel. The portion of the curve having the least slope is the range of maximum sensitivity. Except for this portion and at very dilute solutions the sensitivity is a straight line function of the concentration.

Horn[10] has suggested that the ratio $B/B'$ is probably constant throughout colorimetry,

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(9) Yoe and Hill, THIS JOURNAL, **50**, 748 (1928).

(10) Horn, *Am. Chem. J.*, **35**, 253 (1906).

independent of the color examined. The data in Table VI are in agreement with this hypothesis.

TABLE VI

| Ion | Method | Color | $B/B'$ av. | Observer |
|---|---|---|---|---|
| $Al^{+++}$ | "Aluminon" | Red | 0.92 | Yoe and Hill |
| $Al^{+++}$ | Alizarin | Red | .89 | Yoe and Hill |
| $CrO_4^{-}$ | $CrO_4^{-}$ | Yellow | .96 | Horn |
| $Cu^{++}$ | $Cu^{++}$ | Blue | .92 | Horn and Blake |
| $Cu^{++}$ | $Cu(NH_3)_4^{++}$ | Dark blue | .95 | Horn and Blake |
| $Ni^{++}$ | $K_2NiC_4O_4S_4$ | Magenta | .95 | Yoe and Wirsing |
| $Fe^{+++}$ | $C_9H_4NOIHSO_3$ | Green | .93 | Yoe and Hall |

Our experiments showed that the Ferron reagent will determine quantitatively as little as 0.1 mg. of iron in a liter of solution, that is, 1 part in 10 millions.

**Lambert-Beer Law.**—In the case of colored solutions, the absorption of light depends not only on the thickness of the layer traversed, but also on the molecular concentration in the layer. Beer (1852) therefore modified Lambert's law (1760) and expressed it as

$$I = I_0^{-kcl} \qquad (1)$$

in which $k$ is the molecular absorption coefficient; $c$ is the molecular concentration; $I$ is the intensity of the light after passing through the thickness, $l$, of the medium and $I_0$ is the intensity of the incident light upon the medium.

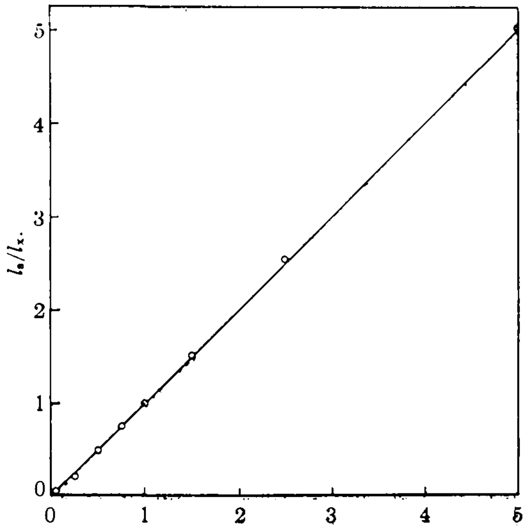

Fig. 2.

As applied to colorimetry, the Lambert-Beer law is generally expressed in the following more convenient form

$$c_x/c_s = l_s/l_x \qquad (2)$$

where $c_s$ and $c_x$ represent the concentrations of the standard and "unknown," respectively, and $l_s$ and $l_x$ are the readings of the heights of the standard and "unknown" solutions, respectively.

According to equation (2) when equal color intensity is obtained from different heights of two solutions, the concentrations are inversely proportional to the heights. In order for this relationship to hold, the color formation in the two solutions must be perfectly formed, or imperfectly formed to the same extent in both solutions. When this is not the case the difficulty must be overcome by the use of a calibration curve which can be prepared experimentally from solutions of known concentrations.

For the study of the Lambert-Beer law colored solutions of various concentrations were prepared from the stock (standard) iron solution and each solution was then matched against the standard colored solution in a B. and L. Duboscq colorimeter. The amount of Ferron added in each case was sufficient to satisfy a 3:1 molecular reaction. The $p$H of the solutions was kept constant at 2.6 by the use of hydrochloric acid-potassium acid phthalate buffer solution. The data are shown graphically in Fig. 2. According to the Lambert-Beer law a straight line would be obtained by plotting the ratio of heights against the inverse ratio of concentrations. An examination of Fig. 2 shows that the law is followed, within the experimental error, over the range of concentration examined.

**Effect of Hydrogen-Ion Concentration.**—The hydrogen-ion concentration has a pronounced influence on the reaction between ferric ions and Ferron. Yoe[3] reported that the reaction is best carried out in a solution acid to methyl orange paper, and that the color was completely destroyed by the addition of either strong acid or alkali. In this work a more extensive study of the effect of hydrogen-ion concentration was made and the optimum $p$H range for the color reaction was determined.

First of all, a study was made of the sensitivity of the reagent solution to hydrogen-ion concentration. It was found that below a $p$H of 1.2 and above a $p$H of 8.3 the reagent solution was completely decolorized. However, over the $p$H range from 2 to 3 there was only a slight change in the color intensity of the reagent solution.

Experiments with the colored iron solutions showed that once the color is formed it could exist with varying intensities over a fairly wide $p$H range (2 to 5 approximately). The color intensity of iron solutions of the same concentration shows no perceptible variation over a *p*H range from 2 to 3, but it was noted in all of this work that above *p*H 3.0 a very small change in hydrogen-ion concentration had a pronounced effect on the color intensity. Since in the *p*H range from 2 to 3 the color intensity is constant and reproducible, in practice, the hydrogen-ion concentration should be adjusted to a *p*H of approximately 2.5. It was found that this could be done with a precision of ±0.2 *p*H by the use of hydrochloric acid–potassium acid phthalate buffer. This buffer does not, in any respect, interfere with the color reaction.

**Aging Effect.**—In many colorimetric methods the color formed has the unfortunate property of fading rapidly on standing, thus making it necessary to make the color comparison at once. Moreover, the standard color solutions must be made up fresh for each determination, unless it be possible to obtain satisfactory permanent standards made from stable colored compounds. In this investigation a thorough study was made of the effect of aging on the green colored solution. Both Nessler tubes and a Bausch and Lomb Duboscq colorimeter were used in this study. It was found that a colored solution which had aged thirty-six days still matched a freshly prepared solution of the same concentration.

**Effect of Temperature.**—The temperatures of various colored solutions of the same iron concentration were fixed at different values, covering a range from 15 to 40° and observations were made on them in Nessler tubes. No color changes or changes in intensity could be detected. Hence, variations that occur within the range of laboratory temperatures have no effect on the color.

**Optimum Experimental Conditions.**—The results of the studies made on the various factors involved enabled the establishment of certain experimental conditions under which the color reaction should be carried out in order to obtain the most satisfactory results. Sufficient reagent solution should be added to react with all the ferric iron present on the basis of a 3:1 molecular ratio. However, for Nessler tube work it was found convenient to add a fixed amount of reagent over a whole range of iron concentration. For iron concentrations less than 1 p. p. m., prepare a solution free from any interfering ions, adjust the acidity to a *p*H of 2.5 by means of hydrochloric acid–potassium acid phthalate buffer and add 5 drops (0.25 ml.) of the Ferron reagent. The color develops at once. Dilute to the mark with distilled water and mix thoroughly.

For iron concentrations from 1.0 p.p.m. to 2.0 p. p. m., 1 ml. of the reagent solution is added and for concentrations above 2.0 p. p. m., add 2 ml. of reagent. Due to the great intensity of the green color formed, precise matching is impossible for concentrations greater than 4.0 p. p. m. of $Fe^{+++}$. In other words, with 50-ml. Nessler tubes (tall form), the range is from 0.005 to 0.2 mg. of ferric iron. Matching is much more easily made over the range from 0.0075 mg. iron per ml. to 0.05 mg. per ml.

In the analysis of unknowns it sometimes happens that a very large excess of reagent is used in preparing both the standard and the unknown. A Corning HR yellow filter No. 351 will be helpful in eliminating the effect of excess reagent.

It must be borne in mind that the use of a buffer solution alone to adjust the *p*H is governed by some practical considerations, the most important of which is that the amount of buffer solution used has a limited capacity to prevent appreciable changes in hydrogen-ion concentration. It is possible that an unknown might be so strongly acidic or basic that the buffer could not hold the *p*H within the required range. For this reason the sample solution should always be tested with an indicator paper (Congo red or methyl orange) and the *p*H approximately adjusted, if too acid or too alkaline.

## Determination of Iron in Various Materials

The colorimetric method described in the preceding section has been used for the determination of iron in a large number of the National Bureau of Standards samples. The samples analyzed varied widely in their chemical nature and included such materials as bauxite, feldspar, glass sands, glasses, clays, limestones and alloys. Hence, it was possible to try out the method over a wide range of conditions.

The colorimetric part of these analyses was done by means of the standard series method using 50-ml. Nessler tubes (tall form). The sensitivity data showed that the concentration range from 1 to 2 p. p. m. of $Fe^{+++}$ was within the range of greatest sensitivity. A difference in iron content of 5 $\gamma$ could be detected. If the magnitude of the amount of iron in the sample was known, then the weight of sample to be taken for analysis could be calculated, so that the concentration of the solution finally analyzed fell within the range of maximum sensitivity. When the approximate iron content was unknown (to the analyst), a rough preliminary analysis was made to determine the correct size of sample to be taken. All samples were issued to one of us (R. T. H.) as unknowns.

In general, the procedures used in the analyses of the various samples followed closely those given in the National Bureau of Standards Certificates of Analyses. In many cases it was possible to simplify these considerably, since in this work only one constituent was sought. For convenience, the materials analyzed may be classified into three groups: glass sand and glass, rocks and alloys.

### Methods of Analysis

After investigating several procedures, the following was found to be the most satisfactory for glass sand and glass.

**Glass Sand and Glass.**—A sample of about 0.25 g. was weighed out after the material had first been dried at 110°. The weight of sample to be taken was governed by certain considerations already pointed out. The sample was then fused to a clear melt in a platinum crucible, using sodium carbonate as a flux. The fusion, after cooling, was disintegrated and treated by the perchloric acid method[11] for the dehydration of silicic acid. The silica was filtered off, washed thoroughly with dilute hydrochloric acid and finally with water. The filtrate was received in a porcelain casserole and its acidity adjusted by the addition of concentrated ammonium hydroxide until it was just acid to Congo red paper. The solution was evaporated on the steam-bath to such volume that it could be introduced into a Nessler tube along with 10 ml. of buffer solution (*p*H 2.6) and 1 ml. of the Ferron reagent. This Nessler was then compared with a standard series, and a match obtained. In some cases, instead of evaporating the solution to a small volume, it was found convenient to dilute it to a known volume in a volumetric flask and then remove an aliquot for analysis. In all these analyses the *p*H of the standard and of the unknown was determined. In every case the *p*H was within the range of 2.6 ± 0.2, and usually did not vary more than 0.1 of a *p*H unit.

When aluminum was present in amounts relatively large in comparison with the amount of iron, as was the case in a few of the samples, it interfered to some extent with the determination. This difficulty was overcome either by adding a little aluminum salt to the standard in cases where the aluminum content was known or by separating the iron from the aluminum (by means of sodium hydroxide) in the manner given by Hillebrand and Lundell.[11] In some instances it was necessary also to remove the titanium. The iron was separated from both the aluminum and the titanium according to the National Bureau of Standards method.[12]

**Rocks and Clays.**—Included in this group were the following: bauxite, plastic clay, fluorspar, feldspar, dolomite and argillaceous limestone. The procedures used for these samples were for the most part based on the methods of the National Bureau of Standards. Aluminum and titanium were present throughout this whole group and in most instances it was necessary to remove these constituents in order to make a satisfactory determination of the iron. The methods used on bauxite and plastic clay were essentially the same as the one given by Lundell and Hoffman.[13] The fluorspar, feldspar, and limestones were analyzed according to standard methods and the iron determined colorimetrically by Ferron.

**Alloys.**—The iron content of the following alloys was determined colorimetrically by means of the Ferron reagent: sheet brass, cast bronze and zinc base die-casting alloy. The elements copper, tin and antimony were present in varying amounts in all of these materials and nickel was present in two of them. It had previously been found that all of these elements interfere with the Ferron method for iron. The separation of these elements from the relatively small amount of iron present in the samples was extremely difficult in some cases. The sheet brass and cast bronze were analyzed according to a procedure developed by Lundell and Scherrer.[14] Of the constituents in the zinc base die-casting alloy, only the copper and aluminum were present in amounts sufficient to make their elimination necessary.

The results of all analyses are summarized in Table VII.

TABLE VII

| | | Total iron as $Fe_2O_3$ | |
|---|---|---|---|
| Material | B. of S. No. | B. of S. Av. | Found |
| Bauxite | 69 | 5.66 | 5.72 |
| Clay, plastic | 98 | 2.05 | 2.00 |
| Dolomite | 88 | 0.084 | 0.092 |
| Dolomite | 88 | .084 | .095 |
| Feldspar | 70 | .03 | .037 |
| Feldspar | 70 | .03 | .04 |
| Fluorspar | 79 | .15 | .14 |
| Glass, boron | 93 | .076 | .077 |
| Glass, lead barium | 89 | .049 | .052 |
| Glass, opal | 91 | .081[a] | .075 |
| Glass, soda lime | 80 | .065 | .062 |
| Glass sand | 81 | .073[b] | .067 |
| Limestone, argillaceous | 1A | 1.63 | 1.64 |
| | | Total iron as Fe | |
| Cast bronze | 52 | 0.12 | 0.08 |
| Sheet brass | 37B | .21 | .18 |
| Sheet brass | 37B | .21 | .18 |
| Zinc base die-casting alloy | 94 | .048 | .037 |
| Zinc base die-casting alloy | 94 | .048 | .040 |

[a] Colorimetric method gave value of 0.070. [b] Colorimetric KCNS method gave values of 0.067 and 0.068, respectively, by two analysts.

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(11) Hillebrand and Lundell, "Applied Inorganic Analysis," John Wiley and Sons, Inc., New York, 1929.

(12) National Bureau of Standards Certificate of Analysis for Standard Sample No. 69.

(13) Lundell and Hoffman, *Bur. Standards J. Research*, **1**, 91 (1928).

(14) Lundell and Scherer, *Ind. Eng. Chem.*, **14**, 426 (1922).

An examination of the table shows that with one or two exceptions the results are in good agreement with those reported by the National Bureau of Standards. The low percentages obtained on the alloys undoubtedly were due to the experimental difficulties of separating small amounts of iron from relatively large amounts of elements such as tin and antimony. These low results can hardly be attributed to any inherent weakness of the colorimetric method since no tendency of this sort is apparent in the other results obtained. Also, it is of interest to note that in the two cases where the Bureau reports analyses by a colorimetric method, the results obtained by the Ferron method checked the latter closer than the average value reported by the Bureau.

## Summary

A study has been made of 7-iodo-8-hydroxyquinoline-5-sulfonic acid and its reaction with ferric ions. The solubility of the reagent has been determined in water, alcohol, acetic acid and in mixtures of these solvents. The color reaction has been investigated and a probable mechanism for it has been postulated. Cupric ions and salts that hydrolyze easily, or yield colored ions, interfere with the reaction and should not be present except in very low concentration. The sensitivity of the reaction has been determined. The reagent will measure quantitatively one part of iron in ten million parts of solution, when the observation is made in 50-ml. Nessler tubes (tall form). The color has been found to obey the Lambert–Beer law over the range of concentration examined. The effect of hydrogen-ion concentration has been studied and the optimum *p*H range for the color reaction determined. Aging and temperature have been found to have no effect on the color reaction. The optimum experimental conditions for the determination of iron have been established.

The iron content of a wide variety of materials has been determined colorimetrically, using 7-iodo-8-hydroxyquinoline-5-sulfonic acid. With one or two exceptions, the results obtained are in good agreement with the values reported by the National Bureau of Standards.

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[CONTRIBUTION FROM THE JOHN HARRISON LABORATORY OF CHEMISTRY OF THE UNIVERSITY OF PENNSYLVANIA]

# Condensations of Aromatic Amines with Formaldehyde in Media Containing Acid. V. Substituted Dihydroquinazolines from *p*-Chloroaniline and *p*-Bromoaniline

BY E. C. WAGNER AND ABNER EISNER[1]

The condensations of *p*-substituted amines with formaldehyde in aqueous acid solution have been studied for *p*-toluidine by Maffei and by the writers,[2] for *p*-phenetidine and *p*-nitroaniline by Maffei,[3] and by Cairncross and Bogert for *p*-aminobenzoic acid and ester[4] and for *p*-bromoaniline.[5] The reported products included the corresponding 3,6-disubstituted-3,4-dihydroquinazolines except in the case of *p*-bromoaniline, from which Cairncross and Bogert[5] obtained as the main product (19%) a base of m. p. 257° identified as 3-(*p*-bromophenyl)-6-bromo-3,4-dihydroquinazolone-4, and smaller amounts of two other bases (m. p. 204° and 193–196°), neither examined further. At the time these results were published the writers had practically completed a study of the condensation of *p*-chloroaniline with formaldehyde in dilute hydrochloric acid solution. The principal product was the expected substituted quinazoline. These contrasting results with two compounds so similar led to extension of the work to include *p*-bromoaniline. Condensation of this amine with formaldehyde in dilute hydrochloric acid solution at or below room temperature gave as the main product (26%) a base of m. p. 205.8° (corr.), found to be 3-(*p*-bromophenyl)-6-bromo-3,4-dihydroquinazoline. No base of m. p. 257° was isolated. Comparison of the two sets of conditions which yielded these unlike results shows that Cairncross and Bogert used less acid, more water, and considerably higher temperature (70°); the separation and purification of the several products involved a laborious and lengthy procedure. In the writers' experiments the isolation of the quinazoline offered no difficulty.

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[1] Present location: U. S. Bureau of Mines, Pittsburgh, Pa.

[2] (a) Lepetit and Maimeri, *Atti accad. Lincei*, [5] **26**, 558 (1917); (b) Lepetit, Maffei and Maimeri, *Gazz. chim. ital.*, **57**, 867 (1927); (c) Maffei, *ibid.*, **58**, 261 (1928); (d) Eisner and Wagner, THIS JOURNAL, **56**, 1938 (1934).

[3] Maffei, *Gazz. chim. ital.*, **59**, 3 (1929).

[4] Cairncross and Bogert, *Collection Czechoslov. Chem. Commun.*, **7**, 58 (1936).

[5] Cairncross and Bogert, *ibid.*, **7**, 548 (1936).