[CONTRIBUTION FROM THE DEPARTMENT OF MEDICAL RESEARCH, DETROIT COLLEGE OF MEDICINE AND SURGERY]

THE STARCH–IODIDE REACTION: STABILITY AND PROPORTIONALITY OF COLOR PRODUCED BY SMALL AMOUNTS OF IODINE

BY R. G. TURNER

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Numerous investigators have discouraged the use of the starch–iodide reaction as a basis for the colorimetric estimation of small amounts of iodine. McClendon[1] objects to the titration method because of the ease of oxidation of potassium iodide. Nichols[2] showed the retarding effect of various salts on the sensitivity of the starch reagent. Von Fellenberg[3] attempted to use the starch–iodide reaction for the colorimetric estimation of small amounts of iodine. He states that in salt solutions it is of little value in quantitative iodine determinations, since the intensity is not always proportional to the iodine content. Treadwell and Hall[4] as cited by Nichols have shown that starch paste requires the presence of an alkali iodide to prevent the dissociation of the blue iodide–starch color. Nichols[2] fulfilled the requirements for a stabilized starch solution. He eliminated the use of salt or chloroform as a preservative and in its place used salicylic acid. This reagent is sensitive, stable and with iodine produces a true blue color.

Failing to obtain satisfactory results in estimating minute amounts of iodine by the method of von Fellenberg[5] and Leitch and Henderson,[6] a study was made of the starch–iodide reaction in regard to the proportion of iodine present and the intensity of color produced. This investigation was carried out with minute amounts of iodine ranging from 0.0005 to 0.005 mg. These small amounts were chosen as the purpose in mind was to find a suitable method for the quantitative estimation of iodine in small quantities of blood.

Comparative tests were made on potassium iodide test solutions to determine the relative proportion of iodine present to the intensity of color produced, the stability of the color and effect of temperature on the production of the color.

Experimental

The study was made with aqueous solutions of potassium iodide. A standard solution containing 0.001 mg. of iodine in the form of potassium iodide was prepared in the following manner.

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[1] J. F. McClendon, THIS JOURNAL, **50**, 1093 (1928).

[2] M. Starr Nichols, *Ind. Eng. Chem., Analytical Ed.*, **1**, 215 (1929).

[3] T. von Fellenberg, *Ergebnisse der Physiol.*, **25**, 176 (1926).

[4] Treadwell and Hall, "Analytical Chemistry," John Wiley and Sons, Inc., New York, 1914, Vol. I, p. 299.

[5] T. von Fellenberg, *Biochem. Z.*, **139**, 371 (1923); **152**, 116 (1924).

[6] Isabella Leitch and J. M. Henderson, *Biochem. J.*, **20**, 1003 (1926).

1.3081 g. of recrystallized potassium iodide is weighed out and transferred to a 100-cc. volumetric flask containing 50 cc. of purified water. After dissolving, dilute to 100 cc. (0.1 cc. of this solution is equivalent to 1 mg. of iodine). A weak solution is made by adding 0.1 cc. of the above solution to a liter volumetric flask and diluting to the mark. (1 cc. of this solution is equivalent to 0.001 mg. of iodine.) The accuracy of the weak solution may be checked by titrating 200 cc. with a 0.005 *N* thiosulfate solution which has been previously standardized against a 0.005 *N* solution of potassium iodate.

Take 200 cc. of potassium iodide standard, acidify with 2 *N* sulfuric acid and add saturated bromine water until the solution is colored orange. Boil off excess bromine and cool. Add a few salicylic acid crystals, 2 cc. of starch solution and an excess of potassium iodide crystals. Titrate with 0.005 *N* thiosulfate.

Test solutions were prepared from the standard solution by delivery from a buret graduated to 0.1 cc.

The technique used for developing the color is based on the method of Leitch and Henderson,[6] which involves the oxidation of hydriodic acid to iodic acid and the liberation of the iodine by addition of an excess of potassium iodide. Kendall and Richardson[7] have shown that this reaction quantitatively produces six times the amount of iodine originally present.

$$HIO_3 + 5HI = 3H_2O + 3I_2$$

The oxidation and production of the color for study are carried out in a 15-cc. pyrex test-tube graduated at 1 cc. The test solution is diluted to 2 cc. and acidified with two drops of 2 *N* sulfuric acid. Three drops of freshly prepared saturated bromine water are added. After one-half minute the excess bromine is boiled off over a low micro burner flame and the volume of liquid in the test-tube is evaporated to one-half cc. This must be done cautiously. By holding the tube in a slanting position and continually rotating, no spitting or loss of liquid takes place. While still hot, add 1 drop of a 1% alcoholic salicylic acid solution and place the tube in a beaker of cold water. A standard solution of potassium iodide containing 0.001 mg. of iodine is prepared in a like manner. When cooled to room temperature, add to each tube five drops of starch solution (prepared according to Nichols)[8] and three drops of 1% potassium iodide solution. Dilute with iodine-free water to the 1-cc. mark. A blue color will form immediately. If necessary, the test solution is diluted to match the standard. The colors are compared in a micro-colorimeter. The standard is set at 20 mm. The iodine present in the test solution is calculated from the observed reading by the formula

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[7] E. C. Kendall and F. S. Richardson, *J. Biol. Chem.*, **43**, 149 (1920).

[8] One-half g. of soluble potato starch is added to 2.5 cc. of cold water and mixed to form a thin paste. Pour it gradually with constant stirring into 200 cc. of water. Boil for fifteen minutes, stirring continually. Allow to cool and add 0.25 g. of salicylic acid. Stir until the preservative is dissolved. Fresh starch solution should be prepared every two weeks. If a good grade of starch is used the solution will be clear and filtration unnecessary. Poor grades of starch may produce cloudy solutions and filtrates of a faint pink color.

$$\frac{\text{Reading of standard}}{\text{Reading of test solution}} \times \frac{0.006}{6} \times \frac{\text{dilution of test solution}}{\text{dilution of standard}} = \text{mg. of iodine present}$$

For example, with a reading of 15-mm. dilution of test solution 2 cc. and standard set at 20 mm., the formula is applied as follows

$$\frac{20}{15} \times \frac{0.006}{6} \times \frac{2}{1} = 0.0026 \text{ mg. of iodine}$$

All glassware used must be cleaned with cleaning solution. Traces of organic matter or alkali soaps interfere with the reaction. Iodine-free water is obtained by redistilling distilled water from potassium hydroxide.

The experimental results are shown in the tables.

Table I

Accuracy of Starch–Iodide Colorimetric Method

Reading of standard 0.001 mg. iodine, mm.	Test KI solution iodine, mg.	Obs. reading of test solution, mm.	Iodine found, mg.	Calcd. reading, mm.	Iodine calcd., mg.
20	0.0005	35.4	0.00056	40.0	0.0005
20	.0006	32.7	.00061	33.3	.0006
20	.0007	29.0	.00068	28.5	.0007
20	.0008	24.6	.00081	25.0	.0008
20	.0009	21.8	.00091	22.2	.0009
20	.0010	19.8	.00101	20.0	.0010
20	.0011	18.4	.00108	18.1	.0011
20	.0012	17.0	.00117	16.6	.0012
20	.0013	14.6	.00139	15.3	.0013
20	.0014	13.8	.00144	14.2	.0014
20	.0015	12.9	.00155	13.3	.0015
20	.0016	12.4	.00161	12.5	.0016
20	.0017	11.2	.00178	11.7	.0017
20	.0018	11.0	.00181	11.1	.0018
20	.0019	10.2	.00196	10.5	.0019
20	.0020	10.0	.00200	10.0	.0020

Table II

Comparative Readings on Diluted Test Solutions. Standard (0.001 Mg. of Iodine) Set at 20 Mm.

Test solution, mg. of iodine	Colorimeter reading, mm.	Dilution of test solution, cc.	Iodine found, mg.
0.001	20.2	1	0.00099
.001	21.0	1	.00095
.001	20.0	1	.00100
.002	10.2	1	.00196
.002	19.4	2	.00206
.002	21.0	2	.00190
.003	13.0	2	.00308
.003	20.4	3	.00294
.004	10.5	2	.00380
.004	21.2	4	.00376
.005	21.0	4	.00475

TABLE III

STABILITY OF COLOR PRODUCED BY THIS METHOD. STANDARD (0.001 MG. OF IODINE) SET AT 20 MM.

Test solution (γ) = 0.001 mg.	Dilution, cc.	Readings on colorimeter scale 5 min., mm.	15 min., mm.	30 min., mm.	60 min., mm.	16 hrs., mm.
KI = 1γ Iodine	1	20.9	21.0	20.4	21.1	19.6
KI = 2γ Iodine	1	10.4	10.3	10.5	10.4	13.0
KI = 3γ Iodine	2	13.8	13.5	13.8	13.3	16.7
KI = 1γ Iodine	1	19.9	20.1	20.3	20.0	18.1
KI = 6γ Iodine	3	10.4	10.0	10.6	10.6	9.3

Discussion

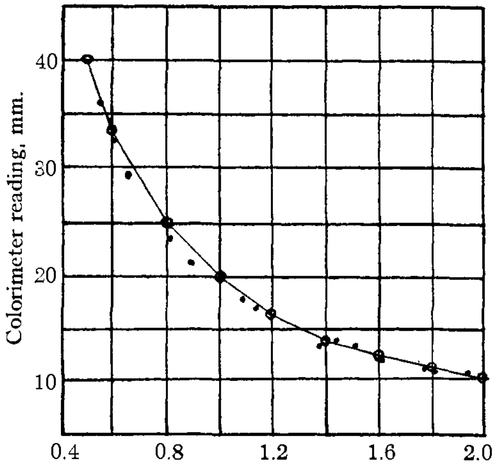
From the observations given it is evident that minute amounts of iodine ranging from 0.0005 to 0.005 mg. produce, under the conditions given, a true blue color which is proportional in intensity to the amount of iodine present. The color produced can easily and clearly be matched in a microcolorimeter with an accuracy of 0.0001 mg. in the colorimetric reading.

The observations in Table I show the relative proportionality of iodine present to the amount of color produced. The test solutions were prepared from the standard potassium iodide solution ranging in iodine content from 0.0005 to 0.002 mg. The iodine found by colorimetric reading did not vary in any case given more than 0.0001 mg. from the actual amount of iodine present. Amounts below 0.0005 mg. cannot be compared against a 0.001 mg. standard unless the standard is diluted to 2 cc. Such small amounts may have a slight pinkish color which interferes with the sharpness of the reading, though approximate estimations can be made.

Table II gives the comparative readings and estimations of iodine on diluted test solutions. The error in the colorimetric estimation reaches 0.0002 mg. in solutions containing over 0.003 mg. of iodine. The same error made in a colorimetric reading on a solution diluted as made on a solution of the same volume as the standard results in a greater error of the calculated value. If the test solution is diluted to 2 cc., then the final error due to colorimetric reading will be twice as great as the same error made if standard and unknown are of equal volume.

The chromogenic color remains stable for one hour or more when compared against a standard prepared at the same time as the test solution (Table III). Readings at a period of sixteen hours after the colors were formed showed a slight increase in the amount of color present. This increase appears to be greater the higher the original iodine content. This may be due to the partial formation of a colloidal suspension or to oxidation of the excess potassium iodide by oxygen of the air. Comparison of the stability with a new standard at each interval of time showed no change in twenty minutes, after which readings became slightly higher (not recorded in table). A slow oxidation of the excess potassium iodide probably takes place but under like conditions this change is proportional for one hour at least, which is sufficient time for colorimetric reading.

Figure 1 shows the calculated colorimetric curve as compared against the observed findings in Table I.



γ Iodine (1γ = 0.001 mg.).

○, Calculated; ●, observed.

Fig. 1.—Color curve.

Figure 2 shows the effect of temperature on color formation. The color production was carried out at temperatures ranging from 0 to 90°. Readings were made while the solutions were at these temperatures. Even at 60° the colorimetric reading varied only 1 mm. At 70° only a slight color was produced, while above that no color was formed. After cooling for one hour, the maximum color developed in those which were negative at the high temperature.

The sensitiveness of this reaction and the stability of the color produced is accomplished no doubt because of the small amounts of iodine estimated and the fact that no interfering salts are present.

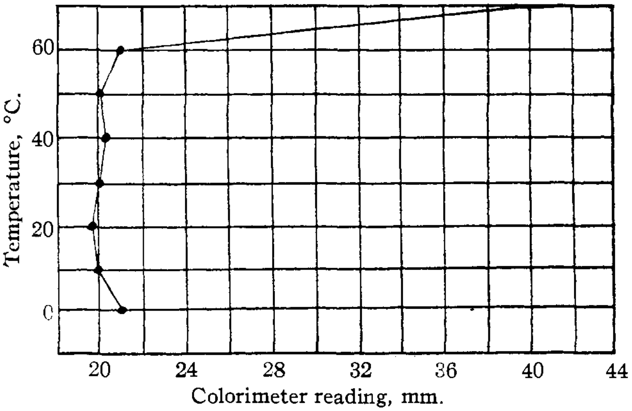


Fig. 2.—Temperature curve.

It is believed that in the estimation of so small quantities of iodine, titration with most extreme cautiousness cannot perform as accurate a quantitative iodine determination as a colorimetric comparison in a microcolorimeter against a 0.001 mg. standard.

Investigation is under way to determine whether amounts of iodine greater than 0.005 mg. may be determined colorimetrically by use of the starch–iodide reaction.

Summary

1. The color produced by starch and iodine in the absence of other salts may be used as a colorimetric measurement of the iodine present in amounts ranging from 0.0005 to 0.005 mg. with an accuracy of 0.0001 mg. in the colorimetric reading.

2. The color produced is proportional to the amount of iodine present.

3. The chromogenic substance is stable for at least one hour when compared against a standard made at the same time.

4. Temperature has no effect on the intensity of the color produced up to 70°, after which the color produced is less.

DETROIT, MICHIGAN

[CONTRIBUTION FROM THE CHEMICAL DIVISION OF THE MEDICAL CLINIC OF THE JOHNS HOPKINS UNIVERSITY]

OXIDATION-REDUCTION POTENTIALS AT CARBON AND TUNGSTEN ELECTRODES

BY LOUIS B. FLEXNER AND E. S. GUZMAN BARRON

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It has been shown[1] that the potentials of cysteine solutions obtained at mercury electrodes are related to mercury-thiol compounds and doubt was cast upon the significance of the potentials obtained at platinum and gold electrodes. It was thought probable that further light would come from investigation of the behavior of cysteine at other electrodes. Of several of these studied, carbon and tungsten appear most promising. This note concerns the behavior of carbon and tungsten electrodes with simple oxidation–reduction systems, the understanding of which must precede investigations on the more complex cysteine systems. Pure graphite was obtained from the Acheson Graphite Company. Tungsten wire came through the courtesy of the General Electrical Company.

The behavior of graphite and tungsten was first studied in two well-poised oxidation–reduction systems of fairly positive E_0. Quinhydrone was first chosen, measurements being made in the presence of air. Table I shows excellent agreements between graphite and gold-plated platinum electrodes in quinhydrone systems in Michaelis' standard acetate buffer, Sørensen's phosphate mixtures, and $N/10$ hydrochloric acid. Purity of carbon is here unimportant, the rods of a carbon arc lamp or the core of a "lead" pencil giving identical results. Tungsten on the other hand gives entirely discrepant values.

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[1] Barron, Flexner and Michaelis, *J. Biol. Chem.*, 81, 743 (1929).