atom is not at the center of gravity of the tetrahedron but is 0.36 of the altitude above the base, the attraction in the axial direction must be 1.37 times greater than in the direction of the bonds to the other immediately adjacent atoms. In a complete structure of indefinite extension, such as a crystal approximates, this value of the axial attraction ratio will be somewhat greater than 1.37.

Summary.

Measurements of the angles of reflection of palladium X-rays from the principal planes of a crystal of carborundum and measurements of the intensities of reflection of the different orders have been made and interpreted. The results show that the silicon atoms and carbon atoms in carborundum are each arranged on face centered rhombohedral lattices, very nearly cubic ($\alpha = 89° 56.6'$) displaced with respect to one another along the hexagonal axis a distance equal to 0.36 of the basal plane (111) spacing.

The elementary tetrahedron of carborundum is, therefore, very similar to that of the diamond, the difference consisting in a slight shortening of the vertical axis (0.15%) and a displacement of the atoms of carbon from the centers of the tetrahedron (0.25 of the altitude in the case of the diamond) to a position slightly less distant from the apex (0.36 of the altitude in the case of carborundum).

From the values of the distances between the atom planes as derived from the X-ray measurements the density of carborundum was calculated to be 3.11 whereas the observed value is 3.123.

SHEFFIELD, ALA.

[CONTRIBUTION FROM THE BUREAU OF PLANT INDUSTRY, U. S. DEPARTMENT OF AGRICULTURE.]

VOLUMETRIC DETERMINATION OF REDUCING SUGARS.

A SIMPLIFICATION OF SCALES' METHOD FOR TITRATING THE REDUCED COPPER WITHOUT REMOVING IT FROM THE RESIDUAL COPPER SOLUTION.

BY W. BLAIR CLARK.[1]

Received August 14, 1918.

One who contributes to the now voluminous literature of reducing sugar determinations must show a good excuse for his temerity. Especially is this so in view of such exhaustive investigations as have been conducted in recent years by Benedict,[2] Kendall[3] and Peters,[4] to cite

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[1] Biochemist, cotton, truck and forage crop disease investigations, U. S. Department of Agriculture.

[2] Stanley R. Benedict, "The Detection and Estimation of Reducing Sugars," *J. Biol. Chem.*, **3**, 101–117 (1907); "A Method for the Estimation of Reducing Sugars," *Ibid.*, **9**, 57–59 (1911).

[3] E. C. Kendall, "A New Method for the Determination of the Reducing Sugars," THIS JOURNAL, **34**, 317–341 (1912).

[4] Amos W. Peters, "Sources of Error and the Electrolytic Standardization of the

these only among the many workers who have addressed themselves to the subject in this country and abroad. The writer would not essay to add to this list did he not feel that the technique which he proposes is accurate and at the same time decidedly simpler than any which has appeared hitherto.

Methods such as Fehling's, Pavy's, and Benedict's, wherein the test solution is added from a buret in just sufficient amount to reduce a given weight of copper, all involve titrating a boiling liquid, and all are also likely to necessitate a wide range in the volume of titrating liquid required to reduce the copper, since irrespective of its concentration there must be added a quantity of reducing sugar corresponding to the weight of copper used. In addition to these inconveniences the end-point is rather difficult to ascertain with certainty in the Fehling method, while the Pavy method involves a somewhat complicated set-up of apparatus for either routine or occasional analyses. The Benedict method is a decided improvement over either of the other types, but still leaves some things to be desired.

In the other group of copper reduction methods, a part only of the copper being reduced and this fraction then determined volumetrically or gravimetrically, the necessity for filtration and washing of the cuprous oxide is added to whatever other processes may be involved. It is true that Maquenne,[1] Lehmann,[2] and others have proposed the titration of the residual copper without removal of the cuprous oxide, but the method has never become popular. Many of these variations also require such quantities of potassium iodide as to make the expense a serious item when numerous analyses are to be made.

F. M. Scales[3] has published a method in which the cuprous oxide is dissolved without removal from its mother liquor by the addition of hydrochloric acid, and the resulting cuprous chloride then treated with an excess of standard iodine solution, the excess being determined by titration with sodium thiosulfate. The oxidation may be more complicated than indicated herein, but from the fact that upon the addition of the iodine a white precipitate is at first formed, which then dissolves as the excess of iodine flows into the mixture, the following reactions would seem to occur:

Conditions of the Iodine Method of Copper Analysis," THIS JOURNAL, **34**, 422–454 (1912); "A Critical Study of Sugar Analysis by Copper Reduction Methods," *Ibid.*, **34**, 928–954 (1912).

[1] Leon Maquenne, "Estimation of Dextrose by Lehman's Method," *Bull. Soc. Chim.*, [iii] **19**, 926–27 (1898); also *J. Chem. Soc. Abstr.*, [ii] 529 (1899).

[2] F. Lehmann, "A Volumetric Method for the Determination of Sugars," *Dissert.*, Marburg; through *Botan. Centr.*, **113**, 142–143 (1910).

[3] F. M. Scales, "The Determination of Reducing Sugars. A Volumetric Method for Determining Cuprous Oxide without Removal from Fehling's Solution," *J. Biol. Chem.*, **23**, 81–98 (1915).

$$\mathit{Cu_2O} + 2\mathrm{HCl} \longrightarrow \mathrm{Cu_2Cl_2} + \mathrm{H_2O}$$
$$\mathrm{Cu_2Cl_2} + \mathrm{I_2} \longrightarrow \mathit{Cu_2I_2} + \mathrm{Cl_2}$$
$$\mathrm{Cu_2Cl_2} + \mathrm{Cl_2} \longrightarrow 2\mathrm{CuCl_2}$$
$$\mathit{Cu_2I_2} + \mathrm{I_2} \longrightarrow 2\mathrm{CuI_2}$$

The cuprous chloride formed in the first reaction is soluble in the mixed sodium tartrate or citrate and chloride solution in which the reaction occurs.

Scales used the ordinary Fehling solutions, carrying out the reduction in a beaker according to directions of Munson and Walker,[1] then transferring the hot mixture to a volumetric flask containing the necessary amount of hydrochloric acid for solution of the cuprous oxide, making up to volume, and withdrawing an aliquot part for treatment in another flask with diluted iodine solution, this being followed by titration of the excess iodine.

When the writer first tried this method he was unable to obtain a satisfactory end-point. The blue color returned almost instantly. It was determined when taking the method up again later that this was due to too great an excess of hydrochloric acid, which reacted with the potassium iodide of the iodine solution to liberate more iodine, a result of not having followed Scales' concentrations exactly. Furthermore, making the solution up to volume, mixing and pipeting out while still hot is undesirable both from theoretical and practical considerations.

Cammidge[2] in adapting the method to clinical use substitutes for the Fehling solution a modified Benedict solution, and dissolves the cuprous oxide before removal of the mixture from the beaker in which the reduction takes place. While working with this modification of the method it was found that the entire process might conveniently be carried out in a single vessel with practical exclusion of the air from the time reduction of the copper begins until after it has been reoxidized by the iodine. As a result the simplified procedure described in later paragraphs has been developed.

The writer has worked out concentrations, volumes and conditions suitable for use up to approximately 60 mg. of reducing sugar. Workers desiring to determine larger quantities may modify and standardize the various factors to suit their conditions. The experimental data here presented should make plain the principles to be followed in making modifications. It is to be noted that there is no table to be referred to in calculating results. Each manipulator standardizes both his conditions

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[1] L. S. Munson and P. H. Walker, "A Unification of Methods for Determining Reducing Sugars," THIS JOURNAL, **28**, 663 (1906); P. H. Walker, *Ibid.*, **29**, 541 (1907); **34**, 202 (1912).

[2] P. J. Cammidge, "An Improved Method for the Estimation of Sugar in the Urine and Blood," *Lancet*, **192**, 613–614 (1917).

and his solutions by means of known solutions of pure dextrose, invert sugar, starch, or whatever reducing substance he may have occasion to handle.

A. The Solutions Used.

(1) **Alkaline Copper Solution.**—As already stated a modified Benedict solution is used. It is made up according to the following formula:

		Grams.
Copper sulfate crystals	($CuSO_4.5H_2O$)	16.0
Sodium citrate crystals	($2Na_3H_5O_7.11H_2O$)	150.0
Sodium carbonate, anhyd	(Na_2CO_3)	130.0
Sodium hydrogen carbonate anhyd	($NaHCO_3$)	10.0

Water to make a final volume of one liter.

The copper may be dissolved separately in 125 to 150 cc. of water; the remaining solid constituents in about 650 cc. water, heating to accelerate solution. When all are in solution pour the copper solution slowly into the other with stirring, make up nearly to volume, cool, complete the volume to one liter and filter. None of the weighings or measurements need be made with extreme accuracy.

The sodium hydrogen carbonate is added to help insure a nonoxidizing atmosphere in the reduction flask. The solution will carry more copper, but the amount given is sufficient to accompany convenient volumes of thiosulfate and iodine solutions in standard concentrations from tenth to fiftieth normal. This solution keeps well, though perhaps not indefinitely, as a small amount of sediment has been found in solutions some months old. Besides better keeping quality this solution has the advantages of greater sensitiveness to reducing sugars and less sensitiveness to sucrose than those in which sodium hydroxide is used, while the copper reduced under standardized conditions is very nearly proportioned to the quantity of reducing sugar present through quite a wide range in concentration of the latter.

(2) **Iodine Solution.**—In the writer's work approximately 0.05 *N* solution has been found the most convenient concentration, although either 0.1 *N* or 0.04 *N* may be used. Dissolve 6.3 g. resublimed iodine in 12 to 15 cc. of potassium iodide solution containing about 9 g. of the iodide. After solution of the iodine the volume is made up to one liter.

(3) **Sodium Thiosulfate Solution.**—0.04 *N* is a convenient strength to use. 10.0 g. thiosulfate crystals per liter make a solution of approximately this concentration. When possible new solutions are made up long enough in advance to stand two or three weeks before being standardized, as there is then little change in strength if evaporation is prevented.[1]

Under the reducing conditions adopted by the writer one cc. 0.04 *N*

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[1] F. P. Treadwell, translated by W. T. Hall, "Analytical Chemistry," 1st Ed. cor. 1909, 2, 506.

solution is equivalent to approximately 1.2 mg. invert sugar. For still greater sensitiveness 0.02 *N* will give a satisfactory end-point, while 0.1 *N* may be used if the quantities of reducing sugar to be determined habitually exceed 50 to 60 mg.

(4) **Starch Solution.**—This may be made of approximately 0.5% concentration from either the commercially prepared "soluble starch" or from ordinary starch by the usual method of cooking. In the writer's routine work no attempt is made to use a preservative, as fresh solutions are made quickly when needed—usually twice or thrice weekly.

(5) **Hydrochloric Acid.**—The concentrated C. P. acid of about 1.19 specific gravity is used.

B. Apparatus Used.

No special apparatus aside from the ordinary laboratory equipment is required. One will have occasion to use 5, 10, 20 and 25 cc. pipets, 10 and 50 or 100 cc. graduated cylinders and a 25 or 50 cc. buret. For routine work this latter should be connected with the thiosulfate bottle for automatic filling.

The reductions are carried out in a 200 or 250 cc. Erlenmeyer flask with wide enough mouth to take a No. 5 or 6 rubber stopper. It is well to select two or three flasks of as nearly the same weight as possible, then determine by several trials their relative heat conducting values. To do this pass a thermometer through one hole of a two- or three-hole stopper of the right size and adjust it so that the bulb just clears the bottom of the flask. Measure 30.0 cc. distilled water into the flask, replace the thermometer and heat over a flame so adjusted as to bring the temperature to 95° in about three minutes. After getting the heat properly adjusted repeat several times with each flask, noting the times to the nearest second. If the flasks do not prove to be substantially equivalent it will be necessary to standardize the conditions separately for each one used. Each reduction flask is fitted with a two- or three-hole stopper, through one hole of which passes a thistle tube or Gooch crucible funnel, of which the tip of the stem has been drawn down so as to allow 50 cc. of water to flow through in from 25 to 30 seconds. In doing this the stem should be left of such length that with 80 to 85 cc. of liquid in the flask and the tip of the funnel dipping just beneath the surface, the body of the funnel will come within one or two centimeters of the stopper.

As a source of heat either a Bunsen burner or an electric hot plate may be used. The latter will be found more convenient if the voltage remains fairly constant; but if this is subject to much variation the fact will be reflected in the results.

On the electric hot plate used by the writer, with a certain 200 cc. Jena flask containing 30.0 cc. of the reaction mixture, and voltage of the heating current normal, boiling begins quite uniformly at 3 minutes and 50 to 52 seconds. Were a Bunsen burner being used it would be adjusted to reach this result in 40 to 45 seconds less time and a total heating period of 5 minutes instead of 6 would be used. In general, whatever the source of heat, one must standardize his conditions with sufficient definiteness to be readily reproducible. This whole subject of heating power has been admirably worked out by Peters,[1] and need not be discussed further here.

C. Details of Procedure.

A solution containing the reducing sugar or sugars to be determined may be prepared in accordance with any of the usual methods described in the literature. Solutions which have been subjected to acid hydrolysis should be nearly neutralized with alkali hydroxide or carbonate.

In case there is reason to suppose that 10.0 cc. of the solution as finally prepared for test contains not more than 30 mg. reducing sugar, measure 20.0 cc. of the copper citrate-carbonate solution and 10.00 cc. of the test solution into one of the reduction flasks, insert the stopper with funnel or thistle tube prepared as previously described, and place over the source of heat, regulated as for comparing the flasks. Boiling should begin in 3 or 4 minutes and should continue for a definite time, say two minutes, so that the total time of heating ranges from 5 to 6 minutes. The exact period of heating is not material, but it is quite essential that both the period of heating and the heating power remain constant for any series of determinations which depend upon the same standardization of the thiosulfate solution.

While the above mixture is heating, measure out about 60 cc. distilled water and 4.6 to 4.8 cc. conc. hydrochloric acid. Just enough acid is required to neutralize the alkali carbonate, dissolve the precipitated cuprous oxide, and render the resulting solution distinctly acid to litmus.

When the reduction mixture has boiled for the proper length of time, remove from the heat and immediately add through the funnel a few drops of the hydrochloric acid.[2] Cool under the tap to approximately room temperature, adding the remainder of the acid, a few drops at a time to avoid loss of any appreciable quantity of it by volatization with the escaping carbon dioxide. When all of the acid has been added shake the flask sufficiently to insure thorough admixture of the contents and complete solution of the cuprous oxide. There should now be a clear, light green solution, provided the test solution was not highly colored. Next, add (also through the funnel) about half of the 60 cc. water previously measured. If the funnel is properly adjusted this should result in covering its tip to a depth of from 1 to 3 mm. Next add an accurately measured

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[1] Amos W. Peters, *Loc. cit.*

[2] Should the color at this point indicate lack of an excess of copper, the test should be discarded and another experiment run, using less of the test solution.

quantity of iodine solution equivalent to 1.00 cc. approximately normal solution.[1]

In adding this let the tip of the pipet rest in the neck of the funnel, then pour a few cc. of the remaining water into the funnel to make a water seal that will prevent air being drawn into the flask with the iodine solution. When the iodine has drained from the pipet remove the latter and rinse down the funnel with the balance of the water. If there is much cuprous chloride present in the solution, the first portions of the iodine will produce a white precipitate of cuprous iodide, which later is oxidized to cupric salt by the excess of iodine and dissolves. Should any of this precipitate remain undissolved it indicates an insufficiency of iodine and more must be added, in measured amount. Mixture of the contents should be accomplished by a gentle rotary motion of the flask rather than by violent agitation. According to the excess of iodine present the color of the solution will now range from a light emerald-green to a dark brown, the former indicating a very small excess of iodine.

The funnel and stopper may next be removed and the solution immediately titrated with 0.04 N sodium thiosulfate[2] to determine the excess of iodine.

Starch solution as indicator need not be added until the solution has acquired a light emerald-green tint, a measured quantity of 2 or 2.5 cc. being then used. As the end-point is approached the deep blue of the starch-iodine complex becomes less and less dense until there is a sudden change from blue to green. Even with 0.02 N thiosulfate a single drop is sufficient to show this change and there should be no difficulty in recognizing it, except possibly by those who do not readily distinguish between blues and greens. The difference between the cc. of thiosulfate used and the volume required by a blank test in which water instead of the substance under examination is used with the copper solution, gives the thiosulfate equivalent to the iodine consumed in oxidizing the copper reduced by the sugar.

The thiosulfate solution may be standardized by carrying out an exactly similar test in which a known quantity of invert sugar (20 to 30 mg.) or dextrose is used. From the data of this test one can calculate the milligrams of invert sugar or dextrose equivalent to 1.00 cc. thiosulfate solution, and use this factor in calculating the results with unknown solutions. The value of the factor varies somewhat for very small

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[1] This may be 10.00 cc. 0.1 N solution, 20.00 cc. 0.05 N solution, or 25.00 cc. 0.04 N solution. In blank tests or when the amount of reduced copper is seen to be small half this quantity of iodine may be used.

[2] For most accurate results the thiosulfate solution should be run in in small quantities of 1.5 to 2 cc. shaking thoroughly after each addition, and running in the next portion while the liquid in the flask is still in motion. See R. Kempf, *Z. angew. Chem.*, **30**, 71–2 (1917); also *C. A.*, 11, 2646 (1917).

amounts of reducing sugar, hence it is advisable to carry out standardizations with approximately the same quantities of reducing sugar as are found in the samples to be analyzed (see Table II).

This standardization must be made each time a new lot of thiosulfate or iodine solution is introduced and also, in routine work, at weekly intervals.

If one prefers to use larger quantities of material the reduction may be carried out in 300 or 500 cc. flasks, using 40 cc. to 75 cc. coppercitrate-carbonate solution and corresponding volumes of the test solutions and iodine. Where the quantities of reducing sugar habitually exceed 45 to 50 mg., 0.1 *N* thiosulfate solution may be used without sacrifice in the relative error. On the other hand if one has material in which the reducing sugar in a convenient aliquot seldom exceeds 15 mg., it will be advisable to use 0.02 *N* thiosulfate with correspondingly less iodine for oxidation of the reduced copper. That which is to be kept in mind when dealing with any of these variations is that within the general limitations outlined each individual may restandardize his conditions to suit the kind and quantity of material under investigation.

When engaged on routine work one operator, using a single reduction flask, can complete about 4 tests per hour; using two flasks he should be able to make 6 or 7 tests per hour. In this case the contents of one flask will be undergoing reoxidation and titration while the other is being reduced. This estimate does not include the time spent in preparing the sugar solutions.

D. Data.

In the following paragraphs and tables data are given regarding certain features of the method which the writer has had occasion to investigate during the course of routine work.

(1) **Agreement of Reduplicate Titrations.**—Table I gives the results of 10 consecutive determinations on a certain invert sugar solution. The tests were carried out as already described, using 20.0 cc. copper citrate-carbonate solution, 10.00 cc. invert sugar solution, and heating for 6 minutes on an electric hot plate.

In Col. 2 is recorded the number of cc. of sodium thiosulfate solution required by the excess of iodine solution. In the third column are the differences between these figures and the blank test, these differences being the cc. of thiosulfate equivalent to the copper reduced by the invert sugar. Col. 4 shows the differences between the numbers in Col. 3 and the arithmetical mean of these numbers, taken to the nearest hundredth cc. In Col. 5 the figures of Col. 4 are converted into milligrams of invert sugar by multiplying by the factor 1.2. Inspection of Col. 5 shows that the greatest deviation from the mean of the 10 reduplications was less than 0.2 mg. of invert sugar, and that this largest deviation occurred in a test which did not closely follow the normal heating course. It will also be observed that 7 of the 10 tests differed from the mean by less than 0.1 mg. of invert sugar. There seems to be no question but that the chief part of the observed variations is due to uncontrollable factors in the reduction process.

TABLE I.

Variations in Reduplicate Titrations when Using the Modified Scales Method for Titrating Reduced Copper.

1. Titration No.	2. $Na_2S_2O_3$ required. Cc.	3. $Na_2S_2O_3$ equiv. to reduced Cu. Cc.	4. Dif. $Na_2S_2O_3$. Cc.	5. Dif. inver. sugar. Mg.
Blank	25.10	..	..	...
1	3.83	21.27	−0.08	−0.096
2	3.63	21.47	+0.12	+0.144
3	3.70	21.40	+0.05	+0.060
4[1]	3.90	21.20	−0.15	−0.180
5	3.71	21.39	+0.04	+0.048
6	3.80	21.30	−0.05	−0.060
7	3.78	21.32	−0.03	−0.036
8	3.80	21.30	−0.05	−0.060
9	3.75	21.35	0.00	0.000
10	3.61	21.49	+0.14	+0.168
		213.49		

(2) **Ratio of Reducing Sugar to Thiosulfate Used.**—It has been stated that the copper reduced in the copper citrate-carbonate solution, and consequently the thiosulfate equivalent to the copper, is very nearly proportional to the reducing sugar present. Variations from strict proportionality become significant only when the quantity of reducing sugar is quite small, less than 7 or 8 mg. in the reduction mixture. This is brought out in Table II and the accompanying graph (Fig. 1).

All the tests shown in Table II except two of the blanks were made on the same day. Starting with (1) (*a*), those lettered (*a*) were run through in order as given, then those lettered (*b*), and finally those lettered (*c*). It was the intention to run 3 tests for each quantity of dextrose taken, but a quite unusual fluctuation of the heating current during a part of the afternoon rendered this impossible; so for the third set of tests those quantities of dextrose were used which had shown the greatest variations between tests (*a*) and (*b*).

In Col. 4 are entered the cc. of sodium thiosulfate solution used in titrating the excess of iodine. Col. 5 gives the differences between these figures and the means for the blank tests.[2] These figures in Col. 5 represent therefore the cc. of thiosulfate solution equivalent to the copper reduced by the dextrose, and consequently are a measure of the

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[1] Owing to fluctuation of the voltage this test did not begin to boil for nearly 4.5 minutes, but the total period of heating was not lengthened.

[2] The blanks for 10.00 cc. and 20.00 cc. iodine were not combined.

dextrose. Col. 6 gives the dextrose factors obtained by dividing the mg. of dextrose shown in Col. 2 by the cc. of thiosulfate in Col. 5. The mean of Col. 6 is obtained by dividing the total of Col. 5 into the total of Col. 2, test (8) (*b*) being omitted.

In Col. 8 are shown the deviations from the correct value of dextrose that would occur if the mean value of Col. 6 were used as the factor by which to multiply the values in Col. 5. The minus sign indicates that the dextrose value so obtained would be too small by the given amount, while the plus sign indicates too large a value. Col. 10 gives the means of these deviations for each group of tests for a given quantity of dextrose.

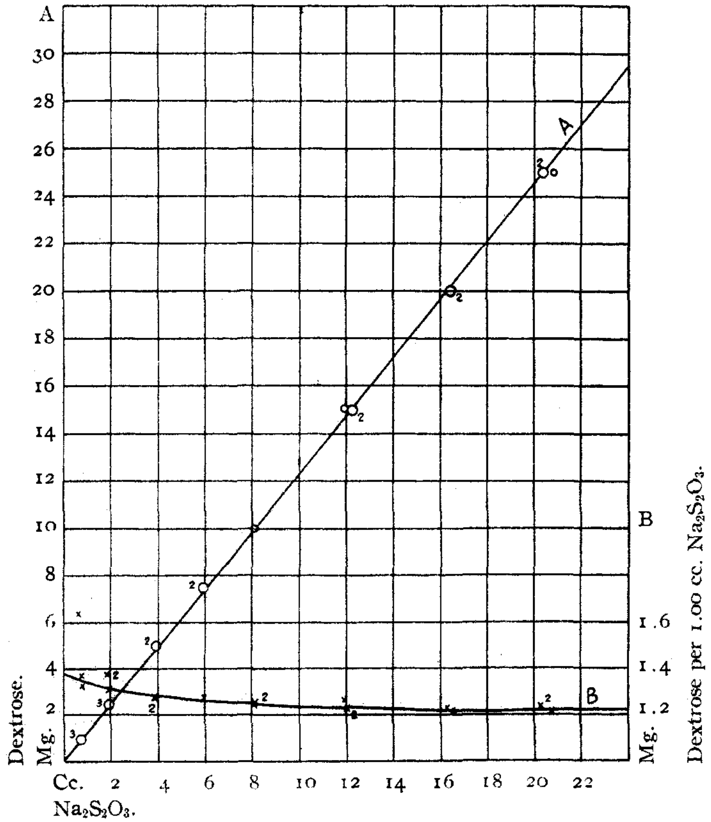

Fig. 1.—Relation between dextrose and sodium thiosulfate equivalent to the iodine required to oxidize the copper reduced by the dextrose: Curve A. Cubic centimeters of a certain approximately 0.05 *N* sodium thiosulfate solution required for the indicated milligrams of dextrose. Curve B. Milligrams of dextrose corresponding to 1.00 cc. of the same sodium thiosulfate solution when required in varying amounts. In both curves the thiosulfate figures represent the difference between the blank test and that of the solution under examination.

TABLE II.

Ratio of Reducing Sugar to Thiosulfate Solution Equivalent to the Copper Reduced.

1.	2.	3.	4.	5.	6. Dextrose factor.	7.	8. Dextrose deviation.	9.	10. Means for columns.	11.
Expt. No.	Dextrose. Mg.	Iodine. Cc.	$Na_2S_2O_3$. Cc.	$Na_2S_2O_3$ equiv. to red. Cu. Cc.	Single tests.	Curve B.	Calculated from mean factor. Mg.	From curve B. Mg.	8.	9.
(1)(*a*)	1.00	10.00	11.90	0.61	1.64	..	−0.24	−0.18	..	...
(*b*)	..	...	11.78	0.73	1.37	1.35	−0.09	−0.01	..	...
(*c*)	..	...	11.76	0.75	1.33	..	−0.07	+0.01	−0.13	−0.060
(2)(*a*)	2.50	10.00	10.70	1.81	1.38	..	−0.24	−0.12	..	...
(*b*)	..	...	10.60	1.91	1.31	1.32	−0.12	+0.01	..	...
(*c*)	..	...	10.70	1.81	1.38	..	−0.24	−0.12	−0.20	−0.077
(3)(*a*)	5.00	10.00	8.60	3.91	1.28	1.28	−0.13	0.00	..	...
(*b*)	..	...	8.60	3.91	1.28	..	−0.13	0.00	−0.13	0.00
(4)(*a*)	7.50	10.00	6.65	5.86	1.28	1.27	−0.20	−0.06	..	...
(*b*)	..	...	6.60	5.91	1.27	..	−0.14	+0.006	−0.17	−0.027
(5)(*a*)	10.00	10.00	4.50	8.01	1.25	1.25	−0.02	+0.01	..	...
(*b*)	..	...	4.48	8.03	1.245	..	+0.005	+0.02	−0.01	+0.015
(6)(*a*)	15.00	10.00	0.37	12.14	1.235	1.24	+0.13	+0.05	..	...
(*b*)	..	...	0.60	11.91	1.26	..	−0.16	−0.23	..	...
(*c*)	..	20.00	13.10	12.15	1.23	..	+0.14	+0.07	+0.04	−0.037
(7)(*a*)	20.00	20.00	8.82	16.43	1.22	1.22	+0.47	+0.04	..	...
(*b*)	..	...	8.90	16.35	1.22	..	+0.35	−0.05	+0.41	−0.003
(8)(*a*)	25.00	20.00	4.90	20.35	1.23	1.22	+0.36	−0.17	..	...
(*b*)[1]	..	...	4.52	20.73	1.21	..	+0.83	+0.29	..	...
(*c*)	..	...	5.00	20.25	1.235	..	+0.23	−0.29	+0.30	−0.057
(9)(*a*)	0.00	10.00	12.50	0.00	...	..	...	...	..	...
(*b*)	..	...	12.52	0.00	...	..	...	...	..	...
(*c*)	..	20.00	25.20	..	...	..	...	...	..	...
(*d*)	..	...	25.30	..	...	..	...	...	..	...
Totals and mean	190.50	...	...	152.83	1.246	..	...	...	..	...

Before discussing the remainder of Table II, attention is directed to the graphs shown in Fig. 1. Graph A is a straight line drawn from the origin through the intersection of the coördinates for 25.00 mg. dextrose and the cc. thiosulfate solution corresponding to this quantity of dextrose, the mean of the two concordant titrations being taken. The points for the remaining titrations have been plotted and it is seen that they all lie on or near the line A. Along that part of the line toward the origin the points lie above, while at the opposite end they lie below the line. On a somewhat larger scale it would be feasible to draw a reversed curve that would be quite smooth and at the same time pass almost exactly through the plotted positions for all consistent titrations. In this way the curve could be used to find directly the quantity of dextrose corresponding to any given volume of thiosulphate. Curve B shows

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[1] Heat was low and time of heating was extended about one minute. This test was omitted in the totals and means of Cols. 2, 5, 6, and 10.

a different method of arriving at the same result. In this curve, as in Graph A, cc. of thiosulfate solution were plotted as abscissas, while the factors of Col. 6, Table II, were plotted as ordinates, the scale being that on the right of the figure, and considerably enlarged over that for Graph A. Curve B was then located from these points and the factors in Col. 7 of Table II were read from it. Column 9 gives the deviations from correct values resulting from the application of these factors to the readings of Col. 5, while Col. 11 gives the mean deviations for the titrations pertaining to the several quantities of dextrose. None of the means reaches a value of 0.0001 g. dextrose, while 5 of the 8 are less than 0.00004 g.

Curve B is typical of a number of different sets of reductions, so that when working with thiosulfate solutions of approximately the same concentration, the curve for a new solution may be prepared by determining one or two points, as for, say, 5.00 mg. and 20.00 mg. dextrose, then drawing through these points a curve substantially parallel to a previous one which has been carefully located by a series of determinations such as for the curve shown in the figure. In case of solutions which have been kept for some time, restandardization curves may be prepared in the same manner.

(3) **Effect of Time of Heating upon the Copper Reduction.**—In practical working the amount of heat applied to the reducing mixture may be varied by changing either the length of the heating period or the intensity and quantity of the heat. To determine the effect of moderate variations, tests were made as shown in Table III. From this table it can be seen that the time of heating is an important factor. It should be noted

TABLE III.

Effect upon Reduction of Copper by Invert Sugar when the Time of Heating Is Varied.[1]

Time of heating. Min.	Vol. of red. mixt.	Thiosulfate solution used for: 10.0 mg. invert sugar. Cc.	20.0 mg. invert sugar. Cc.	25.0 mg. invert sugar. Cc.
4.5	25.0	14.32	8.20	..
5.0	25.0	14.12	7.79	..
5.5	25.0	13.98	7.44	..
6.0	30.0	...	..	9.00
7.0	30.0	...	..	8.93

[1] The several figures given for thiosulfate solution used are the means of three or more titrations. The reductions were made on an electric hot plate operating under quite constant voltage. The heating periods in these and all other tests mentioned in this article were measured with an interval timer graduated in one-quarter minutes, and readily set to time operations with a variation of less than 3 seconds. Incidentally, it may be stated that this has been found to be an exceedingly convenient piece of apparatus to have in a laboratory, as it releases nervous energy for use in more profitable ways.

that the last two items are not comparable with the first three. Besides being carried out with a different volume of the reduction mixture, different solutions of invert sugar were used. As a result of these and other similar tests, of which those given are typical, it was decided to use in the routine work of this laboratory a reduction period of 6 minutes and a volume of 30.0 cc., made up of 20.0 cc. copper citrate-carbonate solution and 10.00 cc. test solution, the last suitably diluted when necessary. No quantitative determination was made of the effect of the volume of the reduction mixture.

(4) **Effect of Sucrose, Alcohol and Formaldehyde upon the Copper Reduction.**—One of the claims made for the copper citrate-carbonate solution is that it is much less sensitive to sucrose, alcohol, and aldehydes than is Fehling's solution. Table IV gives the result of some tests with sucrose.

TABLE IV.

Effect of Sucrose upon Copper Reduction, Using a Reduction Volume of 25.0 cc. and a Heating Period of 4.5 Minutes.

No. of test.	Sucrose taken. Mg.	Thiosulfate used. Cc.
1	0.0	20.60
2	50.0	20.60
3	100.0	20.60
4	250.0	20.27

From these results it is seen that sucrose has no sensible effect unless present in great excess as compared with the reducing sugar to be determined.

In studying the effect of alcohol, there was used as a test solution 10.0 cc. of an alcoholic dilution made by adding to some 95% alcohol its own volume of distilled water. The reduction mixture of 30.0 cc. was heated 6 minutes on the electric hot plate, two closely concordant tests requiring a mean of 26.33 cc. thiosulfate solution. The blank tests with water gave a mean value of 26.45 cc. thiosulfate. Thus the effect of the quantity of alcohol used is hardly appreciable and is in fact within the experimental error.

5.00 cc. portions of a 10% formaldehyde solution which had been standing on the shelf for several months were used in reduction mixtures of 25.0 cc. volume, heated 4.5 minutes. The mean of two concordant tests was 22.6 cc. thiosulfate solution, while the corresponding blank tests with water gave a mean of 24.2 cc. The considerable quantity of formaldehyde present gave a copper reduction equivalent to that of about 1.87 mg. of invert sugar.

(5) **Effect on Copper Reduction of the Czapek-Dox Nutritive Fluid.**—In the laboratory a considerable amount of routine work is being done in determining reducing sugars added to or developed in Czapek-Dox

nutritive fluid and in a modified form of this fluid in which ammonium nitrate is substituted for sodium nitrate. For test purposes fluids of both forms were made up to double strength in all ingredients except carbohydrates, which were omitted entirely. Copper reduction tests were then made, using these fluids and comparing them with blanks in which distilled water was used. The results are shown in Table V.

TABLE V.

Effect on Copper Reduction of the Czapek-Dox and Modified Czapek-Dox Nutritive Fluids. Volume of Reduction Mixtures 30.0 cc. and Heating Period 6 Minutes.

	Thiosulfate solution required with 10.00 cc. iodine.		
Test.	Blank. Cc.	Unmodified solution. Cc.	Modified solution. Cc.
(*a*)	12.75	12.75	12.70
(*b*)	12.72	12.72	12.75
(*c*)	..	12.70	..
Mean	12.735	12.723	12.725

From these figures it is evident that the effect upon the copper citrate-carbonate solution of both the unmodified and the modified forms of this nutritive solution is in no wise different from that of distilled water.

Summary.

The foregoing article may be summarized as follows:

(1) There has been described a quick and simple iodometric method of titrating the copper reduced in copper citrate-carbonate solution in the original reduction flask.

(2) Concentrations of solutions used and a definite method of procedure have been described for quantities of reducing sugars up to 75 mg. and principles pointed out for adapting the process to larger quantities of such sugars.

(3) The accuracy is such that with care the results of duplicate determinations should not differ by more than 0.25 mg. reducing sugar. In routine work the writer finds the difference less than 0.1 mg. in the majority of cases.

(4) The ratio of reducing sugar to copper is nearly constant, the greatest variation occurring in low values. Instead of using tables it is advised that each observer standardize his own procedure and then determine the ratio for that procedure.

(5) Sucrose in quantities not exceeding 100 mg. per 10 cc., 50% ethyl alcohol and two modifications of Czapek's nutritive fluid have no appreciable reducing effect on the copper citrate-carbonate solution; but 10% formaldehyde effected a small reduction of copper.