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COPPER REDUCTION OF DEXTROSE, LEVULOSE, INVERT SUGAR, AND SUCROSE-INVERT-SUGAR MIXTURES IN CITRATE-CARBONATE SOLUTION

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ABSTRACT

The copper reduction values for dextrose, levulose, invert sugar, alone and in the presence of sucrose, have been determined, employing a modified Benedict's copper citrate-carbonate reagent and the iodometric titration method of Shaffer and Hartmann. It has been found that under carefully controlled conditions this method is inferior with respect to precision to the methods of Munson and Walker and of Lane and Eynon, in which caustic alkali is a constituent of the copper reagent. Because of its convenience, it is valuable for rapid work where a precision not greater than 0.5 percent is required.

A table of equivalents for use in routine sugar analysis has been computed.

CONTENTS

	Page
I. Introduction.....	213
II. Preliminary experiments.....	214
1. Effect of varying citrate concentration.....	216
2. Effect of varying carbonate concentration.....	217
3. Relative merits of sodium and potassium salts for preparing reagents.....	218
4. Iodide-iodate solution.....	219
5. Effect of varying boiling time.....	219
6. Determination of blank.....	220
III. Details of method.....	220
1. Reagents.....	220
2. Procedure.....	221
IV. Standardization.....	221
1. Experimental results.....	222

I. INTRODUCTION

Complex copper solutions in which the alkalinity is furnished by alkali carbonates and bicarbonates were proposed during an early period of reducing-sugar analysis, but such solutions have in general proved less reliable with respect to the reproducibility of analytical results than those containing caustic alkali. It has been shown¹ that

*Deceased.

¹ R. F. Jackson and E. J. McDonald, *J. Assn. Official Agri. Chem.* **24**, 767 (1941).

independent operators using Munson and Walker's method for the analysis of pure sugars can attain a precision of about 0.2 percent within the range of 70 to 210 mg of reducing sugar. Lane and Eynon's method² has been found to compare well with Munson and Walker's method. Here the skill of the operator is a very important factor in determining the precision of the method. The authors say that titrations on a given sugar solution will agree well within 0.1 ml. Since their tables include values for a titration of 15 to 50 ml, the careful operator, using 25 to 50 ml for a titration should obtain a precision well within 0.4 percent. In spite of this fact, carbonate solutions have been widely advocated for reducing-sugar analysis because the mildly alkaline solutions attack accompanying "nonreducing" substances less than those containing caustic alkali. The carbonate-bicarbonate solutions have proved particularly serviceable for the determination of small quantities of reducing sugar in the presence of large percentages of sucrose. When caustic alkaline copper solutions are used for analyzing such mixtures, the reducing power of sucrose is relatively large and uncertain. Such analyses are of special importance in European countries, where great significance is attached to the reducing-sugar content of raw beet sugar, and consequently a number of methods have been recently elaborated in which, invariably, mildly alkaline copper solutions have been employed.³ In this country, however, where a somewhat wider variety of sugar products is encountered, the requirements for a reducing-sugar method are more general and a satisfactory method should be applicable to the entire range of ratios of sucrose to reducing sugar.

In modern reducing-sugar analysis the tendency has been to determine the reduced or unreduced copper in the original reaction vessel by iodometric titration, a procedure which is particularly well adapted to the carbonate methods. Thus convenience and rapidity are added to the other advantages. There remains, however, the fact that the carbonate methods have yielded less reproducible results than the caustic alkali methods. In the present investigation an effort has been made to determine the cause of some of these uncertainties and to ascertain the precision that is attainable under carefully controlled conditions. Since the method selected was rapid and convenient, it was found feasible to conduct a considerable number of analyses and to include a wide range of concentrations of sugars and sugar mixtures.

II. PRELIMINARY EXPERIMENTS

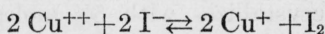
Preliminary to the extended series of measurements described in later paragraphs, a cursory study was made of various complex copper carbonate solutions, and measurements were made of the ratio of reduced copper to the dextrose taken for analysis. Quisumbing and Thomas,⁴ in comparing the influence of different alkalis in alkaline copper solutions, found that the copper reduced by dextrose varied rapidly with change in carbonate concentration. It was also observed in this work that large variations in the ratio occurred with change of

² J. H. Lane and L. Eynon, *J. Soc. Chem. Ind.* **42**, 32T, 143T, 463T (1923).

³ O. Spengler, F. Tödt, and M. Scheuer, *Z. Wirtschaftsgruppe Zucker-ind.*, **86**, 323 (1936); R. Ofner, *Z. Zuckerind. Českoslov. Rep.* **58**, 36 (1933); N. Schoolt, *Chem. Weekblad*, No. 9 (1920); R. F. Jackson and E. J. McDonald, *J. Assn. Official Agri. Chem.* **26**, 462 (1943).

⁴ F. A. Quisumbing and A. W. Thomas, *J. Am. Chem. Soc.* **43**, 1510 (1921).

composition of the copper solution, amounting in extreme cases to about 50 percent. A partial compilation of such data is given in table 1. Evidently any change in composition of the copper reagent produces a relatively large change in the ratio of reduced copper to sugar taken. Many authors have used as a basis of selection those methods which yield the larger ratios of copper to sugar. It appears that the higher ratios of copper are not necessarily advantageous. The actual determination of copper is the most precise step in the whole analysis and the error of determination is not measurably diminished by having a slightly increased amount of it. Far more important considerations are the stability of the copper solution, the reproducibility of the analyses, and the smaller variations of the ratios with changing concentration of sugar. These advantages appeared to be combined in greatest degree in the copper citrate-carbonate reagents proposed by Benedict⁵ and modified by Shaffer and Hartmann.⁶ The latter authors, in a thorough investigation, determined by studying the respective equilibria, the exact conditions required for the quantitative iodometric titration of either cupric or cuprous copper. They found that the reaction



goes quantitatively to the left if the final concentration of copper and of iodide does not exceed 5 millimolar each. Since the cupric ions may be removed by the addition of potassium oxalate, they were able to determine cuprous copper in the presence of cupric copper at higher concentrations. As a result of this study, they proposed a procedure whereby an iodide-iodate solution is added to the cold reaction mixture and the reduced copper determined by thiosulfate titration.

TABLE 1.—Effect of varying composition of copper reagent on ratio of reduced copper to dextrose

Composition of copper reagent per liter		Ratio: mg of copper 100 mg of dex- trose	$\frac{\Delta R^1}{\Delta S}$
CuSO ₄ · 5H ₂ O	Alkali salts		
<i>Grams</i> 17.5	250 K ₂ CO ₃ , 100 KHCO ₃	3.15	-0.0033
25	150 KNaC ₄ H ₄ O ₆ · 4H ₂ O, 53 Na ₂ CO ₃ ... 25 Na ₂ HPO ₄ · 12H ₂ O.	2.653	-.0056
25	75 KNaC ₄ H ₄ O ₆ · 4H ₂ O, 27 Na ₂ CO ₃ ... 13 NaHCO ₃ .	2.572	-.0046
25	81 K ₃ C ₆ H ₅ O ₇ · H ₂ O, 70 K ₂ CO ₃	2.197	-.0009
25	92 K ₃ C ₂ O ₄ · H ₂ O, 3.57 KIO ₃ , 50 KI. 89 Na ₃ C ₆ H ₅ O ₇ · 2H ₂ O, 53 Na ₂ CO ₃	2.084	-.0010

¹ The mean change in the ratio caused by 1-mg increase in concentration of sugar between about 40 and 120 mg of dextrose.

In a limited number of experiments Shaffer and Hartmann showed that this method of copper analysis could be applied to copper reductions carried out in caustic alkaline solution or in citrate-carbonate solution. However, they confined themselves to an exhaustive study of a micromethod for determining dextrose by means of a copper citrate-carbonate solution.

⁵ S. R. Benedict, *J. Biol. Chem.* **5**, 485 (1908).

⁶ P. A. Shaffer and A. F. Hartmann, *J. Biol. Chem.* **45**, 375 (1921).

As a macromethod, which is the subject of the present investigation, Shaffer and Hartmann suggested a modification of Benedict's solution containing potassium citrate, potassium carbonate, and copper sulfate. For convenience, they added potassium iodate, potassium iodide, and potassium oxalate, thereby combining in a single reagent all the chemicals required for the analysis up to the point of acidification and titration. As an alternative and mainly for the purpose of diminishing the cost of the chemicals, they described a similar solution containing sodium citrate and sodium carbonate. In this case it was necessary to prepare separate solutions of iodide-iodate and of potassium oxalate, since the latter would have caused the precipitation of sodium oxalate if added to the copper reagent.

1. EFFECT OF VARYING CITRATE CONCENTRATION

The precision with which the alkaline copper reagent must be prepared was first determined. Both sodium and potassium citrates contain water of crystallization which might conceivably be variable and hence render variable the concentration of dry substance. For reasons which are explained in a later paragraph, the sodium salts have been selected for preparing the reagents.

Trisodium citrate crystallizes with $5\frac{1}{2}$ moles of water, which is theoretically 27.74 percent of the substance. On exposure of a pulverized sample to the air, it was found to lose more than 10 percent of this moisture overnight and more than 30 percent during a period of 3 days. On long standing, it approached a state corresponding to 2 moles of water. This same crystalline phase is reached by drying at 100° C for about 36 hours. With this tendency to effloresce, it was anticipated that the substance purchased from the supply houses might vary in water content. A sample of crystals from a freshly opened bottle contained 22.81 percent of moisture instead of the 27.74 percent required by theory. Other samples approached the theoretical water content more closely, although in variable degree.

Three alkaline copper reagents were prepared, each containing 25 g of copper sulfate crystals and 53 g of anhydrous sodium carbonate in 1 liter. One solution contained 71.435 g (0.20 mole) of trisodium citrate crystals, the second 89.294 g (0.25 mole), and the third 107.153 g (0.30 mole). Each of these solutions was used for the analysis of three 50-ml solutions of dextrose containing, respectively, 140.1, 113.5, and 56.3 mg of sugar, the copper being determined after a 5-minute period of boiling by the procedure described in detail. The results plotted in figure 1 are expressed as the ratio of reduced copper to the weight of sugar. It is at once evident that variations in the concentration of citrate exert a considerable effect and that as the concentration increases the amount of reduced copper diminishes. The differences in each series caused by a change of 0.05 mole (=18 g) in citrate concentration leads to the conclusion that in average such a change causes a difference of 0.04, or about 2 percent of the ratio. Since a precision of 0.1 percent is desired, the citrate concentration must be adjusted within about 0.9 g, that is $(0.002/0.04) \times 18$, or for a weight of 89.3 g (0.25 moles) within 1 percent. It is therefore necessary not only to weigh with this precision but to determine the moisture content of the trisodium citrate crystals.

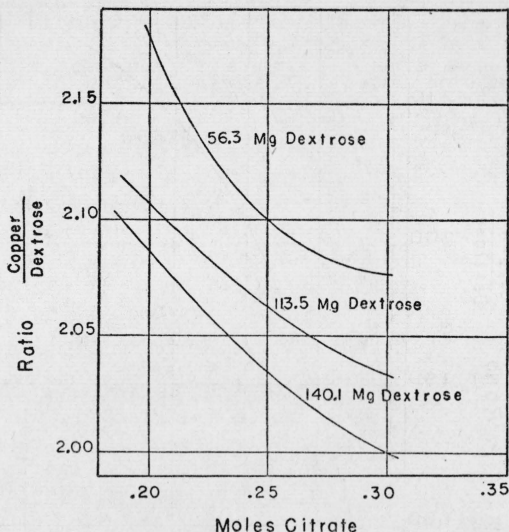


FIGURE 1.—Variation in the copper-dextrose ratio with the amount of citrate present.

To determine moisture in trisodium citrate, weigh rapidly a pulverized sample and dry at 100°C for 24 or 36 hours. Then dry to constant weight at 160° to 170°C , preferably allowing the temperature to rise gradually. The substance tends to decrepitate, and it is therefore advisable to cover the drying vessel loosely until the danger of loss is past.

2. EFFECT OF VARYING CARBONATE CONCENTRATION

Variations in the concentration of sodium carbonate also affect the ratio of reduced copper to sugar. Four alkaline copper reagents were prepared, in which the copper and citrate were held constant but in which the concentration of sodium carbonate was varied. With these solutions, analyses were made of 50-ml samples, each containing 100 mg of dextrose. As shown in figure 2, an increase in sodium carbonate concentration causes an increase in the ratio of reduced copper to sugar. Between 0.46 and 0.65 mole the ratio increases at the rate of 0.0443 for each 0.1 mole, or about 2 percent. For a precision of 0.1 percent, the variation in the ratio from this cause must not exceed 0.002, and therefore the concentration of carbonate must not vary more than $0.002/0.443=0.00475$ mole. If we arbitrarily select 0.5-mole concentration of sodium carbonate for the preparation of the copper reagent, its concentration must not vary by more than ± 0.00475 mole, or about 1 percent. Many samples of anhydrous sodium carbonate from the supply houses proved to be dry well within this tolerance, but others exceeded it slightly. Moisture can be very simply determined by heating a sample in a covered platinum crucible to dull redness, but care must be taken to avoid fusion of the salt. For the analyses described in the later paragraphs, the alkaline copper reagents were prepared with a precision well within the tolerances here indicated.

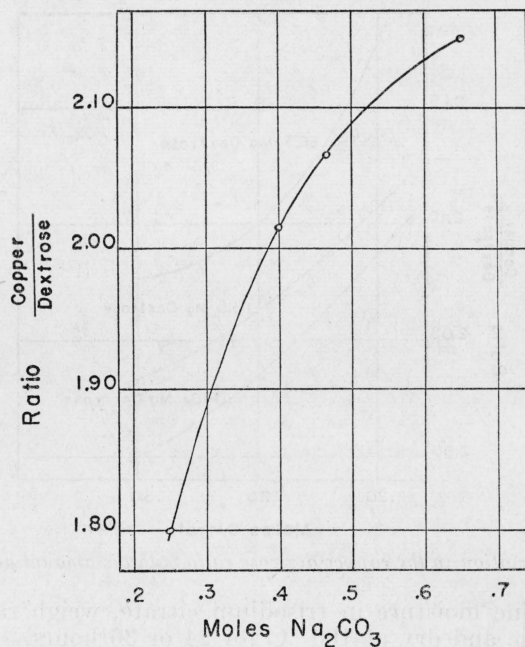


FIGURE 2.—Variation in the copper-dextrose ratio with the amount of Na₂CO₃ present.

3. RELATIVE MERITS OF SODIUM AND POTASSIUM SALTS FOR PREPARING REAGENTS

Both modifications of Shaffer and Hartmann's reagent were investigated. In one the copper reagent was prepared with tripotassium citrate, carbonate, and oxalate, and contained in 1 liter 3.57 g of KIO₃ and 50 g of KI. Fifty milliliters of the copper solution was pipetted accurately and the copper reduced by 50 ml of sugar solution. After the reduction was completed the reaction mixture was cooled and acidified with 20 ml of 5-*N* H₂SO₄. The acidification caused the release of the equivalent of 50 ml of 0.1-*N* I₂, a part of which reoxidized the reduced copper, and the remainder was titrated back with standard thiosulfate. When the ratio of copper to sugar was plotted against sugar, the graph showed the same curvature as that with the sodium salts but with slightly different numerical values.

In the other modification the copper reagent was prepared with sodium salts. It was therefore necessary to prepare in separate solutions saturated potassium oxalate and the standard potassium iodide—potassium iodate solution. After extended experimentation with both of these modifications the latter was chosen and all of the later analyses were conducted with it alone. It appears at first glance that the convenience of combining all the chemicals in a single reagent has been sacrificed. However, the advantages of the second modification mentioned outweigh those of the combined reagent.

The combined reagent always contains the equivalent of 50 ml of 0.1-*N* I₂, regardless of the amount of copper reduced, and for the smaller concentrations of reducing sugar requires a long, tedious back titration. A blank determination must be run with great precision, since its value enters directly into all calculations. The thiosulfate must be standardized and its constancy assured by separate operations. On the other hand, when using the second modification in which the solutions of iodide-iodate and potassium oxalate are added just before titrating, one can gage roughly the quantity of the former to add in order to produce the slight excess necessary. Since potassium iodate can be obtained as a very pure chemical, the iodide-iodate can be prepared as a standard solution merely by weighing out the chemicals. Thus the working solution becomes the fundamental standard, and no additional standardization is required. If made slightly alkaline and if tightly stoppered, the solution keeps indefinitely.

4. IODIDE-IODATE SOLUTION

Shaffer and Hartmann proposed an iodide-iodate solution containing 5.4 g of KIO₃ and 60 g of KI in 1 liter. This solution upon acidification yields an equal volume of 0.1514-*N* I₂. In the present investigation 5.611 g of KIO₃ and 60 g of KI have been used. This solution upon acidification yields an equal volume of 0.1573-*N* I₂. This factor is the reciprocal times 10 of the atomic weight of copper, and 1 ml is exactly equivalent to 10 mg of copper. With the iodide-iodate solution adjusted to 0.1573 *N*, the calculation of reduced copper becomes very simple. The ratio of concentration of the standard iodate solution to that of 0.1-*N* thiosulfate is determined by titration of not less than 10 ml of the former. The back titration of thiosulfate in the analytical determination is then multiplied by this ratio factor and the thiosulfate thus converted to iodate. This is deducted from the measured volume of iodate added, yielding directly after multiplication by 10 the number of milligrams of copper reduced.

It is of course important that the KI shall be pure and free from iodate. Occasionally, potassium iodide becomes discolored after long standing, possibly indicating free iodine. Washing with chloroform, which restores its white color, and allowing it to dry in air purifies such samples. The iodide-iodate solutions were prepared at a known temperature, and when necessary, the measured volumes were corrected for variations of temperature by assuming an expansion coefficient of 0.0002.

5. EFFECT OF VARYING BOILING TIME

As is true of all reducing-sugar methods, the 5-minute boiling arbitrarily specified does not effect a maximum reduction of copper. It does, however, serve adequately for an arbitrarily selected terminal point. Figure 3 shows the rate of increase of precipitation of copper by 100 mg of dextrose for the several periods of boiling time. At the 5-minute point the ratio (2.195) is increasing at the rate of 0.01 per minute. Therefore, an error of 0.1 minute in judging the boiling time results in a difference of less than 1 part per thousand.

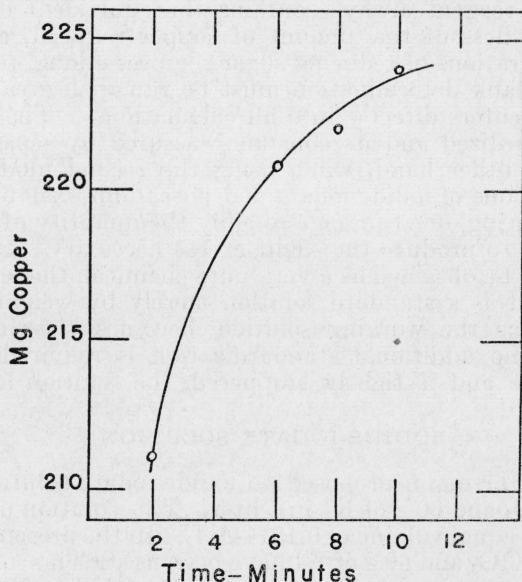


FIGURE 3.—Effect of time of boiling on weight of copper reduced by 100 mg of dextrose.

6. DETERMINATION OF BLANK

Blank determinations were made frequently by boiling the copper reagent with 50 ml of water. The mean value of copper found was 0.6 mg, with a mean deviation of 0.09 mg. Practically, this same value was found when the copper reagent itself was treated as in the analysis but without boiling. In other words, the value of the blank is not caused by autoreduction of the copper reagent, but is conceivably the result of a slight reduction of the copper by the iodine. This view is at variance with the findings of Shaffer and Hartmann, that the reaction given on page 215 runs quantitatively to the left in the presence of potassium oxalate of sufficient concentration, but, nevertheless, it was not possible to obtain the same titration values in the presence of copper and oxalate as in their absence.

These blank values are remarkably reproducible, and therefore all the data for reduced copper in the following tables have been left uncorrected for the blank. These uncorrected values are the more serviceable, since the analyst who makes only occasional analyses seldom makes a blank determination. If he does make a blank determination and finds a value different from 0.6 mg, he has merely to apply the difference as a correction.

III. DETAILS OF METHOD

1. REAGENTS

(a) *Modified Benedict's solution*.—Dissolve the equivalent of 64.52 g of anhydrous trisodium citrate or 89.29 g of crystals containing $5\frac{1}{2}$ moles of water, the moisture content of which has been corrected for, and 53 g of anhydrous sodium carbonate in about 600 ml of water.

Add with vigorous agitation a solution of 25 g of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in about 150 ml of H_2O . Both solutions must be cool before mixing. Make to 1 liter and filter.

(b) *Standard potassium iodate solution.*—Dissolve 5.611 g of pure KIO_3 and 60 g of KI and make to 1 liter.

(c) *Saturated potassium oxalate solution.*

(d) *Sulfuric acid.*—Approximately 5 *N* (250 g of concentrated acid in 1 liter of solution).

(e) *Sodium thiosulfate.*—0.1 *N* (25 g of crystals in 1 liter).

2. PROCEDURE

Transfer 50 ml of solution (a) to a 300-ml Erlenmeyer flask and add 50 ml of a solution containing not more than 150 mg of reducing sugars. Mix with a rotary motion, cover the flask with a small inverted beaker, and place on a plate of asbestos gauze about 4.5 cm above the top of a gas burner.⁷ Heat to boiling in 4 minutes and continue the boiling for exactly 5 minutes. In judging the beginning of the 5-minute period, disregard the early sporadic appearances of boiling and start the final period at the moment when the ebullition suddenly becomes violent. This occurs usually about 20 seconds after the first appearance of bubbles.

At the expiration of the prescribed period of boiling immerse without agitation in a cold-water bath, allowing to remain until cool. Add accurately a volume of standard iodate (b) in excess of that required for oxidation of the cuprous oxide and add approximately 20 ml of saturated potassium oxalate. Add by means of a rapidly delivering pipette 20 ml of 5-*N* H_2SO_4 and agitate with a rotary motion until the cuprous salts are completely dissolved. Examine the mixture by holding the flask above the level of the eye. Any undissolved cuprous oxide or cuprous sulfate can be clearly discerned and must be dissolved by continued agitation. Dilute to about 200 ml and add from a burette standard thiosulfate until the solution starts to turn from green to blue-green. Add starch indicator and complete the titration dropwise to the disappearance of deep-blue starch iodide, the final additions of thiosulfate being in split drops.

Determine the ratio of concentrations of thiosulfate to iodate, and by means of the factor thus obtained convert the thiosulfate volume to its equivalent volume of iodate. Deduct this from the volume of iodate added, and multiply the result by 10 to obtain the number of milligrams of copper reduced. From table 4 obtain the amount of reducing sugar corresponding to the weight of copper found.

IV. STANDARDIZATION

For the purpose of standardization, eight series of analyses were carried out. These included the reducing powers of pure dextrose, levulose, and invert sugar, and of sucrose-invert-sugar mixtures taken in such proportions that all possible ratios of the two were covered. The reducing sugar was varied from various low concentrations to about 150 mg in 50 ml. In each of the five series of analyses of sucrose-invert-sugar mixtures the sucrose was kept at a constant value and the invert sugar was varied.

⁷ Electric heating may be used.

The sucrose and dextrose were National Bureau of Standards Samples 17 and 41, respectively. The sucrose was free from a measurable amount of moisture and the dextrose contained 0.01 percent of moisture, which was corrected for when the sample was weighed. The levulose was prepared by crystallization, once from water and three times from aqueous alcohol. When the crystals were dried for several hours at 60° C to constant weight, a small sample was found to suffer no further loss at 70° to 75° C. The levulose contained less than 0.002 percent of ash.

In preparation for analysis, a 500-ml solution was made up. This contained about 1.5 g of the sugar accurately weighed. Aliquot portions of this solution were taken by means of a series of odd-volume pipettes at roughly 2- to 5-ml intervals. These pipettes by frequent calibration were found capable of delivering the respective volumes of solution with a precision of 0.002 to 0.003 ml. The sucrose solutions were prepared of such concentration that 10 ml contained the weight of sugar desired. For the 10-g sucrose series the dry sugar was weighed directly into the reduction flask and 6 ml of water added to make a total calculated volume of 12.19 ml of solution. Invert-sugar solutions and water were added in such quantity that the total volume became 50 ml.

Invert sugar was prepared in the earlier measurements by inverting sucrose for 35 minutes at 70° C. in the presence of 0.1-N HCl. When cold the acid was neutralized with NaOH. Comparative measurements were made with invert-sugar solutions prepared by weighing equal quantities of dextrose and levulose. As no measurable difference was detected, the later measurements were made with the synthetic invert sugar solely.

When the Erlenmeyer flasks used for the reduction had dried in the air for several days the reaction mixtures boiled without bumping, but if the flasks were used more frequently, bumping occurred. The addition of talc in amounts of 2 mg or less prevented bumping and did not obscure the start of boiling.

1. EXPERIMENTAL RESULTS

The results of the reduction measurements made as described in previous pages are assembled in table 2. For each sugar a formula was devised to express the relation of sugar present to copper reduced. These formulas were prepared by the method of averages.

It is difficult to make an exact estimate of the precision of the method. The curves, figure 4, showing the relation of the copper-sugar ratio to copper, especially in the case of invert sugar, may shift an appreciable amount if the analysis is not carried out under exact conditions. Since the variations in gas pressure seemed to be sufficient to cause such a shift in the curves, a series of analyses was made over a period of time in which the heating was done electrically. The voltage of the current was stabilized by a voltage regulator and adjusted by a Variac. The flask was placed in a conical heater in such a way that the surface of the liquid was just below the top of the heater. Table 3 gives the results of this series of analyses.

TABLE 2.—Milligrams of copper reduced by dextrose, levulose, invert sugar and sucrose—invert-sugar mixtures

Sugar taken	Copper	Sugar by formula ¹	Error	Sugar taken	Copper	Sugar by formula	Error	Sugar taken	Copper	Sugar by formula	Error
DEXTROSE				INVERT SUGAR				INVERT SUGAR +0.3 g OF SUCROSE			
mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg
145.5	295.9	145.1	-0.4	149.5	307.1	149.5	0	148.4	306.4	148.1	-0.3
132.3	272.4	132.7	+0.4	148.2	304.0	147.8	-0.3	141.1	292.7	141.1	0
117.9	243.8	117.9	0	141.0	290.6	140.9	-0.1	133.0	277.4	133.2	+0.2
98.6	205.9	98.7	+0.1	140.2	289.4	140.2	0	117.7	246.9	117.8	+0.1
87.3	183.0	87.2	-0.1	135.8	280.8	135.8	0	98.3	208.3	98.4	+0.1
80.3	169.3	80.4	+0.1	134.7	279.2	135.0	+0.3	78.8	168.1	78.8	0
72.9	154.5	73.2	+0.3	127.4	264.8	127.5	+0.1	59.0	126.7	58.9	-0.1
65.1	137.4	64.8	-0.3	120.1	250.7	120.4	+0.3	49.1	105.9	49.0	-0.1
58.3	128.8	58.3	0	113.6	238.1	114.0	-0.4	39.3	84.9	39.2	-0.1
54.4	115.4	54.3	-0.1	100.4	210.8	100.3	-0.1	27.7	60.3	27.8	+0.1
48.1	102.9	48.3	+0.2	95.0	199.9	94.8	-0.2	19.6	42.3	19.6	0
40.2	85.7	40.1	-0.1	84.6	179.7	84.9	+0.3	11.8	25.2	11.8	0
32.2	69.3	32.4	+0.2	74.8	159.3	74.9	+0.1	INVERT SUGAR +1 g OF SUCROSE			
29.1	61.8	28.9	-0.2	74.2	157.8	74.4	+0.2	148.0	307.9	147.9	-0.1
24.1	51.3	24.1	0	59.4	126.4	59.3	-0.1	140.7	292.3	139.9	-0.8
16.1	33.5	15.9	-0.2	56.5	120.8	56.6	+0.1	132.6	278.7	132.9	+0.3
LEVULOSE				INVERT SUGAR +0.1 g OF SUCROSE				INVERT SUGAR +3 g OF SUCROSE			
146.7	305.8	146.9	+0.2	56.2	119.7	56.1	-0.1	148.4	308.4	147.5	-0.9
133.3	270.1	133.3	0	59.9	127.4	59.8	-0.1	140.8	296.8	141.5	+0.7
118.9	250.4	118.9	0	44.9	95.8	44.8	-0.1	133.0	281.4	133.4	+0.4
108.7	229.4	108.5	-0.2	42.4	90.5	42.4	0	117.7	250.6	117.4	-0.3
99.4	211.0	99.4	0	37.4	79.4	37.2	-0.2	98.3	213.6	98.6	+0.3
88.0	187.3	87.9	-0.1	44.5	95.1	44.5	0	78.8	173.6	78.8	0
73.4	157.5	73.5	+0.1	42.1	89.8	42.0	-0.1	58.8	128.6	59.0	+0.2
65.8	135.2	65.7	-0.1	35.0	74.6	35.0	0	48.9	107.4	49.0	+0.1
44.1	95.0	44.1	0	29.6	63.1	29.6	0	39.2	86.0	39.0	-0.2
29.3	62.9	29.3	0	19.8	42.0	20.0	+0.2	27.7	61.2	27.6	-0.1
20.7	44.3	20.9	+0.2	14.0	28.9	14.1	+0.1	19.5	43.8	19.6	+0.1
14.6	30.2	14.5	-0.1	INVERT SUGAR +0.1 g OF SUCROSE				INVERT SUGAR +3 g OF SUCROSE			
134.1	290.2	133.5	-0.6	148.4	306.6	149.0	+0.6	148.4	308.4	147.5	-0.9
120.2	264.9	120.3	+0.1	140.8	290.1	140.4	-0.4	140.8	296.8	141.5	+0.7
106.4	238.3	106.8	+0.4	133.0	275.7	133.0	0	133.0	281.4	133.4	+0.4
88.7	202.5	89.0	+0.3	117.7	245.3	117.5	-0.2	117.7	250.6	117.4	-0.3
71.3	165.4	71.0	-0.3	98.3	207.4	98.4	+0.1	98.3	213.6	98.6	+0.3
53.3	127.1	53.1	-0.2	78.8	167.6	78.8	0	78.8	173.6	78.8	0
44.3	107.7	44.2	-0.1	59.0	126.5	59.0	0	59.0	132.0	58.8	-0.2
35.5	80.3	35.9	+0.4	49.1	105.4	49.0	-0.1	49.1	110.7	48.8	-0.3
25.1	64.3	24.9	-0.2	39.3	84.6	39.3	0	39.3	89.6	39.0	-0.3
17.7	47.9	17.7	0	27.7	59.6	27.7	0	27.7	64.7	27.6	-0.1
				11.8	24.6	11.8	0	19.6	47.3	19.8	+0.2
								11.8	29.5	11.9	+0.1

¹ Dextrose=0.786+0.4476 Cu+0.000135 Cu².
 Levulose=1.009+0.4432 Cu+0.000110 Cu².
 Invert sugar=0.936+0.4435 Cu+0.000131 Cu².
 When 0.1 g of sucrose is present, invert sugar=0.792+0.4442 Cu+0.000128 Cu².
 When 0.3 g of sucrose is present, invert sugar=0.498+0.4457 Cu+0.000118 Cu².
 When 1 g of sucrose is present, invert sugar=-0.023+0.4438 Cu+0.000119 Cu².
 When 3 g of sucrose is present, invert sugar=-0.922+0.4308 Cu+0.000165 Cu².
 When 10 g of sucrose is present, invert sugar=-2.429+0.4117 Cu+0.000195 Cu².

TABLE 3.—Invert-sugar analyses

Invert sugar taken	Invert sugar found in series—						Maximum error		Average error	
	1	2	3	4	5	6	mg	%	mg	%
mg 140	mg	mg	mg	mg	mg	mg	mg	0.29	mg	0.23
-----	-----	-----	140.4	139.7	-----	139.6	0.4	0.29	0.32	0.23
120	119.8	120.1	140.2	120.4	120.0	119.9	.4	.33	.15	.12
100	99.9	100.1	100.5	100.0	100.4	99.8	.5	.50	.20	.20
80	79.9	80.0	80.3	80.2	80.2	99.9				
60	59.7	60.0	60.2	59.9	-----	80.0				
							.3	.38	.12	.15
							.3	.50	.20	.33

The pure sugars, dextrose, levulose, and invert sugar, yield roughly parallel curves. As the concentration of sugar is diminished from 150 mg, the reducing powers pass through maxima in the vicinity of 40 mg of sugar or approximately 90 mg of copper, and then abruptly and very rapidly diminish, as shown in figure 4. The form of these curves is similar to that produced by levulose⁸ in its reaction with Ost's solution at 55° C, the maximum reducing power in the latter case being at about 60 mg of levulose. The very rapid change of the ratios with concentration of sugar at the lower concentrations makes it questionable whether a satisfactory precision of analysis can be attained with these dilute solutions. For this reason, and because the number of experiments is insufficient to define these rapidly varying curves accurately, there is recorded in table 4 the milligrams of sugar corresponding to a minimum value of 100 mg of copper.

The precision of this method can be estimated by considering the deviation of analytical results from the calculated values. Of the 76 analyses falling within the range included in table 4, the number of analyses in which results deviate from the calculated values by specific amounts are as follows:

Number of analyses	Deviation
	<i>Percent</i>
3	0.6 to 0.5
7	0.5 to 0.4
15	0.3 to 0.2
22	0.2 to 0.1
22	0.1 to 0

Ninety-six percent vary less than 0.5 percent, with 77 percent less than 0.3 percent.

The analyses of invert-sugar solutions given in table 3 are in agreement with these findings. When working with pure sugar solutions under carefully controlled conditions, the accuracy attained within the range given in table 4 agrees well with the precision of the method. If one is working with very dilute solutions or those in which more than about 300 mg of copper is reduced (the total amount of copper present is 318.2 mg), slight variations in the conditions may shift the curves given in figure 4 so that the results become less accurate, although duplicate analyses may agree well within the limits of the precision of the method. The precision of a reducing-sugar method in the analysis of products other than pure sugar solutions is so dependent upon the composition of the product that a general statement cannot be made. However, it can be said that in the analysis of five molasses samples investigated by this method, the end point could readily be determined and duplicate determinations agreed within 0.5 percent.

⁸ R. F. Jackson and J. A. Mathews, *J. Assn. Official Agr. Chem.* **15**, 204 (1932).

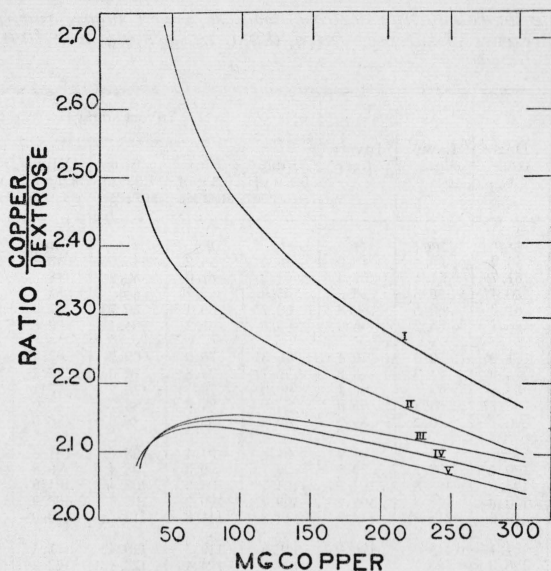


FIGURE 4.—Change of the copper-sugar ratio with variation in the amounts of reduced copper.

Curve I, Invert sugar+10 g of sucrose; II, invert sugar+3 g of sucrose; III, levulose; IV, invert sugar; V, dextrose.

It is interesting to note that quite in contrast to their relative reducing powers in the caustic alkali-copper reagents, levulose has in the copper carbonate solutions a greater reducing power than dextrose.

Sucrose alone has a reducing action, but the effect is much smaller in these copper carbonate solutions than in the caustic alkali-copper solutions. When analyzed alone pure sucrose solutions showed the following reducing powers, no corrections for blanks being applied:

Sucrose	Copper	Copper per gram of sucrose
<i>g</i>	<i>mg</i>	<i>mg</i>
10	10.9	1.09
8	10.1	1.26
6	8.3	1.38
4	6.0	1.5
2	4.2	2.1

When mixtures of sucrose and invert sugar are analyzed the reducing power of sucrose is apparently greater than when analyzed alone. At lower concentrations of invert sugar the reducing power of sucrose becomes an increasingly larger proportion of the total reducing power as the concentration of invert sugar is diminished.

TABLE 4.—Table for calculating dextrose, levulose, invert sugar, and invert sugar in the presence of sucrose, (0.1 g, 0.3 g, 1.0 g, 3.0 g, and 10.0 g)

Copper	Dex- trose	Levu- lose	Invert sugar	Invert sugar				
				Plus 0.1 g of sucrose	Plus 0.3 g of sucrose	Plus 1.0 g of sucrose	Plus 3.0 g of sucrose	Plus 10.0 g of sucrose
<i>mg</i>	<i>mg</i>	<i>mg</i>	<i>mg</i>	<i>mg</i>	<i>mg</i>	<i>mg</i>	<i>mg</i>	<i>mg</i>
100	46.9	46.4	46.6	46.5	46.2	45.5	43.8	40.7
110	51.7	51.1	51.3	51.2	51.0	50.2	48.5	45.2
120	56.4	55.8	56.0	55.9	55.7	54.9	53.2	49.8
130	61.3	60.5	60.8	60.7	60.4	59.7	57.9	54.4
140	66.1	65.2	65.6	65.5	65.2	64.4	62.6	59.0
150	71.0	70.0	70.4	70.3	70.0	69.2	67.4	63.7
160	75.9	74.7	75.2	75.1	74.8	74.0	72.2	68.4
170	80.8	79.5	80.1	80.0	79.7	78.9	77.1	73.2
180	85.7	84.4	85.0	84.9	84.6	83.7	82.0	78.0
190	90.7	89.2	89.9	89.8	89.4	88.6	86.9	82.8
200	95.7	94.1	94.9	94.8	94.4	93.5	91.8	87.7
210	100.4	98.9	99.8	99.7	99.3	98.4	96.8	92.6
220	105.8	103.8	104.8	104.7	104.3	103.4	101.8	97.6
230	110.9	108.8	109.9	109.7	109.2	108.4	106.9	102.6
240	116.0	113.7	114.9	114.8	114.3	113.4	112.0	107.6
250	121.1	118.7	120.0	119.8	119.3	118.4	117.1	112.7
260	126.3	123.7	125.1	124.9	124.4	123.4	122.2	117.8
270	131.5	128.7	130.2	130.1	129.4	128.5	127.4	123.0
280	136.7	133.7	135.4	135.2	134.5	133.6	132.6	128.2
290	141.9	138.8	140.6	140.4	139.7	138.7	137.9	133.4
300	147.2	143.9	145.8	145.6	144.8	143.9	143.2	138.7

WASHINGTON, December 19, 1944.