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ERRORS OF MUNSON AND WALKER'S REDUCING-SUGAR TABLES AND THE PRECISION OF THEIR METHOD

By Richard F. Jackson and Emma J. McDonald

ABSTRACT

In a recent investigation (RP1301) Hammond has undertaken a comprehensive revision of the Munson and Walker reducing-sugar tables and has disclosed discrepancies much greater than the experimental errors of analysis. It is now shown that the main cause of these discrepancies was the contamination of the cuprous oxide, which Munson and Walker weighed directly for the estimation of copper. An independent series of analyses made by the authors, who determined copper iodometrically, was found to be in good agreement with the analyses of Hammond, who determined the copper electrolytically. The recommendation is made that Hammond's copper equivalents be adopted in substitution of those of Munson and Walker.

A series of determinations by Erb and Zerban of invert sugar in the presence of sucrose in aliquots containing 0.4 g of total sugars in 50 ml of solution are in agreement with those of Hammond at high concentrations of invert sugar, but deviate at lower concentrations. The present series of analyses are in agreement with those of Hammond at these discrepant points.

Detailed methods of copper analyses are described. A new modification of the dichromate method is given in which the end point is determined both colorimetrically and electrometrically.

Methods for the preparation of standard invert sugar by hydrolysis of sucrose are discussed.

CONTENTS

I. INTRODUCTION

In recent years, advances in reducing-sugar analysis have consisted mainly in improvements in volumetric processes, especially those which permit the entire analysis to be completed in a single reaction vessel. While these methods are of most general service, the older methods which require filtration of cuprous oxide still serve a useful purpose, particularly when but an occasional analysis is required or the sample is seriously contaminated or discolored. The gravimetric methods have the disadvantage of being more time-consuming than the volumetric processes, but when modified by the introduction of volumetric methods for the determination of reduced copper, they approach the volumetric methods with respect to convenience and rapidity.

Practically the only one of the older gravimetric methods which has survived in this country and which indeed is still extensively employed at the present time is the method of Munson and Walker [1].[1] This method has the advantages of extreme simplicity and, as will be shown, high precision. Munson and Walker, in establishing their fundamental tables in 1906, analyzed the respective pure sugars, determining the reduced copper by weighing as cuprous oxide, which they then converted to copper by the stoichiometrical factor. Erb and Zerban [2] redetermined the copper values for sucrose-invert sugar mixtures containing 0.4 g of total sugar. Their results were in agreement with those of Munson and Walker for the middle range of concentrations, but in disagreement at the higher concentrations.

Recently Hammond [3] has made a comprehensive revision of the tables for dextrose, levulose, invert sugar, and three series of mixtures of sucrose and invert sugar containing, respectively, 0.3, 0.4, and 2.0 g of total sugar. Many of the copper values found by Hammond differ from those of Munson and Walker by amounts far greater than any probable experimental error. In view of the importance of these tables, it seemed advisable that a third series of analyses be made in order that one or the other set of values might be corroborated. It was the purpose of the present investigation to contribute a third series and to determine, if possible, the source of the discrepancies between Hammond's and Munson and Walker's copper equivalents.

II. METHODS OF ANALYSIS

1. MATERIALS AND REAGENTS

(a) SOXHLET REAGENT

The Soxhlet reagent was prepared in the usual manner by mixing 25 ml of each of the two constituent solutions immediately before the analysis. The copper solution contained 34.639 g of copper sulfate crystals in 500 ml of solution. The solution was allowed to stand for several days and was then filtered through fritted glass. The crystals were usually deficient in copper by 1 to 2 percent, and thus the copper content of the filtrate required adjustment to 440.9 mg in 25 ml. For the median range of sugar concentrations it is apparently unimportant that the copper be adjusted accurately to the specified value; but at the higher concentrations when the copper

[1] Figures in brackets indicate the literature references at the end of this paper.

approaches exhaustion, it is important that the copper content conform to the specification.

The alkaline tartrate solution contained 173 g of Rochelle salt and 50.0 g of sodium hydroxide in 500 ml of solution. The sodium hydroxide was freed from carbonate by allowing a 50-percent solution to stand overnight and filtering through asbestos or fritted glass. The alkali content of the filtrate was determined by titration of weighed samples.

(b) SUGARS

Dextrose and levulose.—The dextrose (Standard Sample No. 41 of this Bureau), which had been prepared by crystallization from aqueous solution in the anhydrous crystalline form, showed no loss in weight upon heating at 110° C for 2 hours. The levulose, which had been prepared by crystallization from aqueous solution, was further purified by twice recrystallizing from aqueous alcohol [4]. During the first recrystallization a volume of nitric acid stoichiometrically equivalent to the inorganic impurities was added. The final crystals, dried at 70° C, showed an ash content of less than 0.002 percent and an absence of moisture when dried in a thin layer for 2 hours at 70° C.

Invert sugar.—For most of the analyses invert sugar was prepared by taking equal weights of pure dextrose and levulose. Hammond prepared his invert sugar in the same way; but his data, in spite of the high purity of the substance, showed considerably lower yields of copper than those of Munson and Walker, who prepared invert sugar by hydrolysis of sucrose with 0.02 *N* hydrochloric acid for "one-half hour on the water bath." It was thought at first that the differences in the observed reducing power might be due to differences in the invert sugar. An effort was accordingly made to reproduce Munson and Walker's measurements.

The velocity of inversion of cane sugar is in the highest degree a function of the temperature. Hence merely placing a solution on the water bath without regarding the temperature actually attained leads to uncertain results. In one instance such an experiment produced a preparation which was but 83 percent inverted. Jackson and Gillis [5] derived formulas which enabled us to calculate the velocity of inversion as a function of temperature and concentration of hydrochloric acid. It was calculated that at 93° C in the presence of 0.02 *N* acid, inversion was 99.99 percent complete in 16 minutes.

A solution was prepared in an Erlenmeyer flask containing 1.9665 g of sucrose, 78.2 ml of water, and 20 ml of 0.1 *N* hydrochloric acid. The position of the flask was so adjusted in the steam bath that the solution attained a temperature of 92° C in about 7 minutes. It was allowed to remain in the bath for an additional 23 minutes, during which time the temperature remained between 92° and 94° C. The solution was cooled, neutralized carefully with sodium hydroxide (brom-cresol green), and made up to a volume of 500 ml. The average of five analyses showed that 50 ml of this solution containing 207 mg of invert sugar precipitated 375.0 mg of copper. On the other hand, 207 mg of pure synthetic invert sugar precipitated 375.6, as shown by the mean of four determinations. Evidently Munson and Walker's high values for invert sugar are not due to the greater reducing power of hydrolyzed sucrose. The explanation of their excessive precipitations will be discussed in a later paragraph.

The deficiency in reducing power of sucrose hydrolyzed at a high temperature is in harmony with our measurements of the rotatory powers of invert sugar prepared by hydrolysis of sucrose [6]. We endeavored to show that the rotation of invert sugar is a function of the temperature at which the inversion is carried out, and the higher the temperature of inversion the greater the decomposition of invert sugar, even when the time of inversion is carefully chosen to avoid decomposition of the reaction products. Hence, to prepare a standard invert sugar solution from sucrose, elevated temperatures of inversion must be avoided.

Table 1 shows the reducing power of invert sugar prepared under various conditions of inversion. Each solution was prepared by inverting 1.9665 g of sucrose and making up to a final volume of 500 ml. A 50-ml aliquot contained 207 mg of invert sugar. The acid in each preparation was neutralized with sodium hydroxide, yielding small amounts of sodium chloride. Parallel experiments with mixtures of invert sugar and as much as 500 mg of sodium chloride showed that the salt had a negligible influence on the weight of copper reduced. As appears from the tabulated values, standard invert sugar solutions having the same reducing power as pure synthetic invert sugar can be prepared by room-temperature inversion. If the inversion is carried out at higher temperatures, a slightly diminished reducing power results.

TABLE 1.—*Reducing power of 207 mg of invert sugar prepared from sucrose*

Temperature of inversion	Acidity	Time of inversion	Volume of solution containing 1.9665 g of sucrose	Copper reduced
°C		hr	ml	mg
93	0.02 *N* HCl	0.5	100	375.0
76	.01 *N* HCl	3.2	11	374.8
55	.1 *N* HCl	4	11	374.9
24	.65 *N* HCl	70	100	375.5
23	1.0 *N* H_2SO_4	50	50	375.7
Synthetic invert sugar				375.6

For the inversion at laboratory temperature the procedure of Lane and Eynon [7] is suitable. "A solution of 9.5 g of pure sucrose is treated with 5 ml of hydrochloric acid (sp. gr. 1.19) made up to about 100 ml, left at room temperature for about a week at 12° to 15° or 3 days at 20° to 25° C and then made up to 1 liter. A known volume of the standard solution is neutralized with sodium hydroxide and suitably diluted immediately prior to use."

In the present series 3.933 g of sucrose was dissolved in 87.5 ml of water and acidified with 10 ml of 6.34 *N* hydrochloric acid (Clerget acid). It was allowed to stand at 23.5° to 24.5° C for 71 hours and was then neutralized with sodium hydroxide and made to a volume of 1 liter. Fifty milliliters of this solution contained 207 mg of invert sugar and 185 mg of sodium chloride.

2. DETERMINATION OF REDUCED COPPER

(a) CUPROUS OXIDE METHOD

Munson and Walker determined the precipitated copper by directly weighing the cuprous oxide and converting to metallic copper by the stoichiometrical factor. They stated that the method had been checked against the electrolytic and the thiosulfate methods and the accuracy of the cuprous oxide procedure demonstrated. The applicability of this stoichiometrical factor has been extensively debated in subsequent literature and apparently agreement has been reached that, while cuprous oxide is a true measure of copper in the analysis of pure sugars, it is unreliable for the analysis of crude substances because of the likelihood of contamination by impurities derived from the substance undergoing analysis. This was demonstrated by Sherwood and Wiley [8] in an extended series of analyses.

In an effort to explain the source of the considerable discrepancies between Hammond's and Munson and Walker's copper values, we duplicated Munson and Walker's cuprous oxide procedure in detail, and in addition determined the true reduced copper by thiosulfate titration or by electrolysis.

A series of platinum Gooch crucibles was available which had very fine perforations and held the asbestos with but negligible loss during analysis. The asbestos was prepared by the method of Brewster and Phelps [9]. The analytical results are shown in table 2, each figure in the table representing the mean of at least four analyses. In every instance but one the copper calculated from the cuprous oxide is higher than the true copper as shown by thiosulfate titration or electrolysis. Thus even with pure sugars the cuprous oxide is contaminated with organic decomposition products.

TABLE 2—*Contamination of cuprous oxide*

Weight of sugar	Cuprous oxide	Copper		
		by factor	by electrolysis	by thiosulfate
mg	*mg*	*mg*	*mg*	*mg*
207 (Invert, synthetic)	423.8	376.4	376.0	375.7
207 (Invert, sucrose)	424.7	377.2	----	375.5
117 (Dextrose)	258.5	229.6	----	229.7
207 (Dextrose)	438.1	389.1	388.1	388.0
207 (Levulose)	412.3	366.3	364.5	----
207 (Invert, plus 1.793 g of sucrose)	439.3	390.2	385.7	385.7

The cuprous oxide from 207 mg of invert sugar prepared from sucrose shows from table 2 a contamination of 1.7 mg. The difference between Hammond's and Munson and Walker's copper is 1.8 mg. The cuprous oxide from 2.0 g of total sugar containing 207 mg of invert sugar has a contamination of 4.5 mg, while Hammond differs from Munson and Walker by 4.7 mg. A similar comparison of the dextrose values fails because Hammond obtained more copper than Munson and Walker. The average contamination of all the cuprous oxide precipitates in table 2 is 1.6 mg, while the average difference between Hammond and Munson and Walker in 46 analyses is 1.8 mg. In other words, it appears highly probable that the entire difference between the two series of analyses is due to the fact that Munson

and Walker's cuprous oxide was contaminated by appreciable quantities of organic decomposition products.

It is of interest to inquire why a so easily demonstrable contamination has not been revealed before. It is doubtless due to the fact that in most instances the comparison has been made with dextrose, which alone among the sugars causes a negligible contamination, at least at median concentrations of sugar. Thus up to a concentration of 180 mg of sugar Hammond differs from Munson and Walker by an average of only 0.2 mg of copper. In table 2, 117 mg of dextrose produced an uncontaminated precipitate, but the contamination became appreciable at 207 mg of sugar.

The conclusion seems justified that Munson and Walker's tables, so far as they relate sugar to metallic copper, are in error by the amount of the contamination. The apparent cuprous oxide-sugar equivalents are probably substantially correct if the amount of contamination is a constant quantity. This constant relation will hold in general for relatively pure sugars, but will fail for crude substances. On the other hand, Hammond's electrolytic copper is uncontaminated, but he has introduced into his tables the calculated weights of cuprous oxide. These latter values lead to erroneous results when cuprous oxide is weighed for the very reason that they are not contaminated.

It seems highly advisable that all reducing-sugar analyses be referred to metallic copper. We have therefore in the following paragraphs given attention to the analytical methods for the estimation of copper.

(b) THIOSULFATE METHOD

The iodometric determination of copper, when conducted under conditions insuring complete reduction of copper, is capable of an accuracy comparable to that of the best volumetric methods. The reaction on which the method depends, namely $2\,CuI_2 \rightarrow 2\,CuI + I_2$, is reversible; but if precautions are taken to remove cuprous ions as completely as possible and to maintain a sufficient concentration of iodide ions, the reaction runs quantitatively from left to right within the errors of measurement. Under suitable conditions the reaction can be made to run quantitatively from right to left. Shaffer and Hartmann [10] have shown that this, the cuprous titration, can be applied directly to the Munson and Walker method, but such a procedure causes a divergence from the specified routine of the method and will not be considered here.

Shaffer and Hartmann [10] studied the equilibria involved and showed that for amounts of copper up to 318 mg per 100 ml the final concentration of potassium iodide must be 4.2 g (or more) in 100 ml at the end of the titration. For greater amounts of copper the concentration of potassium iodide must be greater in direct proportion.

In the analyses reported here the volume of the copper solution was so adjusted by previous calculation and marking the Erlenmeyer flask that the volumes of the potassium iodide solution, thiosulfate, starch, and washings made the proper final concentration of potassium iodide. For the lowest concentration of copper the final concentration of potassium iodide was 2.1 g in 50 ml; for the highest (425 mg), 6.7 g in 115 ml.

Foote and Vance [11] have shown that the sharpness of the end point and the precision of analysis are enhanced by the addition of

ammonium thiocyanate at the approximate end point of the thiosulfate titration. Since cuprous thiocyanate is more insoluble than cuprous iodide, the thiocyanate has the effect of more completely removing cuprous ions from the solution and thus furthering the reaction from left to right. Another important result of the addition of thiocyanate is that apparently the surface portions of the precipitated cuprous iodide are converted to cuprous thiocyanate and the small quantity of adsorbed iodine is released to react with the thiosulfate. At the end point the precipitate is white, whereas without the thiocyanate it is slightly purple.

Although, as shown by Whitehead and Miller [12], the effect of small concentrations of strong acids is not considerable, that of acetic acid is even smaller. In our experience the reliability of the analysis is increased when the titration is carried out in the presence of acetic acid.

In reducing-sugar analysis the cuprous oxide is dissolved in 5 ml of 1:1 nitric acid. It has been the practice to neutralize the excess of nitric acid with sodium hydroxide and then add a few drops of acetic acid. This procedure is tedious and, as will be shown, unnecessary. The same object could be accomplished by adding a sodium acetate solution, if the acetic acid which is released has no appreciable effect on the titer. A 1:1 solution of nitric acid is about 8.3 *N*. Five milliliters of this solution would be completely buffered by 10 ml of a 4.22 *N* solution of sodium acetate. A maximum of 2.4 ml of acetic acid is released by the prescribed volumes of nitric acid and sodium acetate. The data showing that the effect of this procedure is inappreciable are given in table 3. The results must be considered merely comparative because the same figures are used to standardize the thiosulfate.

Of a pure copper sulfate solution 50-ml portions were titrated with 0.1573 *N* thiosulfate (1 ml=10 mg of Cu) to a final volume of about 100 ml, 2 g of thiocyanate being added near the end of the titration.

TABLE 3.—*Effect of acetic acid on the thiosulfate titer*

Reagent	Number of analyses	Copper
		mg
0.1 ml of acetic	7	318.8
2 ml of acetic	5	318.8
5 ml of acetic	1	318.7
5 ml of 1:1 nitric + 10 ml of sodium acetate[a]	3	318.7

[a] This mixture releases 2.37 ml of glacial acetic acid.

In order to assure ourselves that the ratio of thiosulfate to copper was constant with both large and small amounts of copper, the series of analyses of a copper sulfate solution was made with the results shown in table 4. To each solution were added 5 ml of 1:1 nitric acid and 6 ml of 8 *N* ammonium acetate. The analyses showing the greatest departure from the mean, namely the second, third, and fifth, were in error by 0.01, 0.01, and 0.02 ml, respectively.

TABLE 4.—*Constancy of the ratio of thiosulfate to copper covering the range of volumes used in the determinations*

Volume of copper sulfate	Volume of final solution	Weight of potassium iodide	Titer	Ratio: thiosulfate to copper sulfate
ml	*ml*	*g*	*ml*	
15.026	54	2.2	12.634	0.8408
20.094	64	2.6	16.884	.8403
25.042	74	3.0	21.070	.8414
33.895	93	3.4	28.502	.8409
45.455	116	4.8	38.244	.8414
50.004	127	5.7	42.039	.8407
58.937	144	6.3	49.562	.8409
Average				0.8409

Procedure.—Collect the reduced copper on a Gooch crucible and wash the beaker and precipitate free from unreduced copper. From a 5-ml pipette add a few drops of 1:1 nitric acid to the reaction beaker and drop the remainder carefully on the precipitate, covering the crucible closely with a watchglass. Rinse the pipette and watchglass, catching the rinsings in the beaker. Allow the cupric nitrate and washings from the beaker to drain into an Erlenmeyer flask. Remove the nitrogen oxides by prolonged digestion on the steam bath or follow the usual procedure, using bromine water [13].

Cool and add 10 ml of sodium acetate solution (574 g of trihydrate per liter). Add a volume of potassium iodide solution (40 g per 100 ml) such that at the end of the titration the concentration of potassium iodide shall be 4.0 to 4.5 g per 100 ml. The potassium iodide should be added slowly and with continuous agitation.

Titrate with 0.1573 *N* thiosulfate, preferably adding the solution at the rate of about 13 ml per minute. Add a starch solution when the iodine color approaches disappearance and continue the titration until the blue starch iodide is just decolorized. Add about 2 g of ammonium thiocyanate and agitate until the salt is completely dissolved. Continue the titration to the disappearance of the blue color.

Standardize the thiosulfate (39 g of crystals per liter=0.1573 *N*) by titration against 0.2 to 0.4 g of pure copper dissolved in 5 ml of 1:1 nitric acid and treated as described for the cuprous oxide or against a measured volume of a copper sulfate solution which has been analyzed by electrolysis. In the latter case add 2 ml of acetic acid before titration. One milliliter of thiosulfate should be equivalent to approximately 10 mg of copper.

(c) PERMANGANATE METHOD

The original method of determining cuprous oxide by means of permanganate was devised by Mohr [14], who dissolved the precipitate in acidified ferric sulfate and titrated the resulting ferrous iron with permanganate which had been standardized against sodium oxalate. It was eventually discovered that the results were from 1 to 2 percent too low. Schoorl and Regenbogen [15] modified the method by dissolving the cuprous oxide in neutral ferric sulfate, subsequently acidifying and titrating. The modification has apparently eliminated the errors of the original method. The analytical results here reported substantiate this conclusion.

In this investigation the method of Schoorl and Regenbogen was used with but one essential modification. For the purpose of oxidizing reducing impurities in the stock ferric sulfate solution, the procedure has been to treat the neutral solution with an amount of permanganate determined by titration of an acidified sample. This seems a doubtful expedient because, as is well known, permanganate behaves differently in acid and alkaline (or neutral) solution. The procedure now recommended is to titrate 50 ml of the ferric sulfate solution, acidified exactly as in the analytical process, and to use the determined titer as a zero-point correction. This has the added advantage that the small excess of permanganate which is required to produce the color change and which in general will be approximately the same in the analysis is included in the correction.

Standardization.—[16] Prepare a solution, approximately 0.1573 *N*, containing 4.98 g of potassium permanganate per liter. After several days' aging, filter through asbestos or fritted glass.

Transfer 0.35 g of pure sodium oxalate (dried at 103°C) to a 600-ml beaker. Add 250 ml of sulfuric acid (1 volume of acid diluted with 19 volumes of water) previously boiled for 10 minutes and cooled to 27° C±3°. Stir until the oxalate is dissolved. Add 29 to 30 ml of permanganate at a rate of 25 to 35 ml per minute while stirring slowly. Allow the mixture to stand until the pink color disappears (about 45 seconds). Heat to 55° or 60° C and complete the titration by adding permanganate dropwise until a faint pink color persists for 30 seconds. Allow each drop to become decolorized before adding the next.

Determine the excess of solution (usually 0.03 to 0.05 ml)required to impart the same pink color to the same volume of acid boiled and cooled to 55° to 60° C.

Weight, in grams, of oxalate×948.7÷titer=mg of copper per ml.

An equally satisfactory standard is arsenious oxide [17].

Ferric sulfate.—Dissolve 135 g of ferric ammonium alum or 55 g of ferric sulfate (anhydrous) and dilute to 1 liter. The ferric sulfate dissolves very slowly. Determine $Fe_2(SO_4)_3$ in the stock supply by strong ignition to Fe_2O_3. Acidify 50 ml with 20 ml of 4 *N* sulfuric acid and titrate with permanganate to the slightest perceptible color change. Apply the titer (usually 0.03 to 0.08 ml) as a zero-point correction in analytical titrations.

Determination.—[13] Filter the cuprous oxide on a Gooch crucible and wash the beaker and precipitate thoroughly. Transfer the asbestos film to the beaker with the aid of a glass rod. Add 50 ml of the ferric sulfate solution and stir vigorously until the cuprous oxide is completely dissolved. Examine for complete solution, holding the beaker above the level of the eye. Add 20 ml of 4 *N* sulfuric acid and titrate with standard permanganate to the same color change as in the titration for zero-point correction.

The end point is rendered sharper by the addition of one drop of ferrous phenanthroline indicator (0.7425 g of orthophenanthroline monohydrate in 25 ml of 0.025 *M* ferrous sulfate).

Discussion.—The permanganate method as modified by Schoorl and Regenbogen yields highly accurate results. It is eminently suitable for small amounts of cuprous oxide and has indeed found its greatest serviceability for such small amounts that the titration can be carried out with 0.033 *N* permanganate. When larger amounts of cuprous oxide (from 200 to 400 mg) are precipitated, great difficulty

is encountered in dissolving the precipitate. In many of the analyses reported here, a period of 45 minutes was required before the cuprous oxide was dissolved. Table 5 shows some of the analytical results obtained.

TABLE 5.—*Comparative analyses by permanganate and thiosulfate*

Substance	Copper by permanganate	Number of analyses	Difference between extremes	Copper by thiosulfate	Number of analyses	Difference between extremes	Difference permanganate minus thiosulfate
	mg		*mg*	*mg*		*mg*	*mg*
69 Invert	133.12	4	0.89	133.29	5	0.67	−0.17
220 Invert	396.82	3	.39	397.08	3	.51	−.28
184 Dextrose	349.62	4	.70	349.35	4	.69	+.27

(d) DICHROMATE METHOD

In a previous article Jackson and Mathews [4] described a method by which cuprous oxide was dissolved in an excess of acidified potassium dichromate and titrated back to an electrometric end point with ferrous sulfate. Since the advent of the indicator, orthophenanthroline [18], the same titration can be made colorimetrically. Some modification of the method is necessary because, whereas the electrometric titration can be made in 1.2 *N* acid, the colorimetric procedure requires that the final solution be about twice normal in hydrochloric acid. Below this acid concentration the end point is uncertain and slow of attainment.

The colorimetric and electrometric end points do not occur at exactly the same titer of ferrous sulfate. On back titration the color change occurs first and the large potential change when an additional 0.02 ml (in average) of 0.1573 *N* ferrous sulfate has been added.

All of the earlier electrometric measurements were made with an instrument constructed in the laboratory, similar to the one described by Forbes and Bartlett [19]. The later measurements were made with a Serfass Electron-Ray Titrimeter [20]. Comparative analyses showed that the earlier instrument had served satisfactorily.

The dichromate method is the most expeditious for determining copper. The cuprous oxide dissolves readily in the hydrochloric acid–dichromate solution, in contrast to the difficulty of dissolving in ferric sulfate. In precision it approaches the thiosulfate method very closely at the median and lower concentrations of sugar. At the high concentrations of sugar the results are from 0.1 to 0.2 percent lower. The method would appear particularly serviceable when a considerable number of analyses are required quickly.

Reagents.—Standard dichromate solution, 0.1573 *N* (containing 7.7135 g of crystals of pure potassium dichromate dried at 150° C in 1 liter). One milliliter of this solution is equivalent to 10 mg of copper.

Approximately 6 *N* hydrochloric acid.

Ferrous ammonium sulfate solution, containing 61.9 g of the hexahydrate and 5 ml of concentrated sulfuric acid in 1 liter.

Phenanthroline-ferrous complex. Dissolve 0.7425 g of orthophenanthroline monohydrate in 25 ml of 0.025 *M* ferrous sulfate solution (6.95 g of $FeSO_4 \cdot 7H_20$ in 1 liter).

Procedure. –Estimate the volumes of dichromate, ferrous sulfate, hydrochloric acid, and water which will give an assured excess of dichromate and yield a concentration of about 2 *N* acid in about 200 ml of final volume. An error of 5 percent, or in most cases even 10 percent, in acid concentration, can be tolerated. Fill graduated cylinders with the required volumes of water and acid.

Collect the precipitated cuprous oxide on a Gooch crucible and wash thoroughly. Detach the mat with a glass rod and transfer to the reaction beaker. Add a small volume of water and disintegrate the mat. Pipette accurately a volume of standard dichromate in excess of the quantity required to oxidize the cuprous oxide. In general the approximate weight of copper will be known or can be roughly estimated, but in any case a sufficient volume must be added to supply an assured excess. Add rapidly the whole required volume of hydrochloric acid with continuous stirring and continue to stir until all the cuprous oxide is dissolved. Immerse the crucible in the solution and be assured that the adhering cuprous oxide is dissolved. Remove the crucible with the glass rod and wash it with the water from the graduate. Add one drop of phenanthroline solution and titrate with ferrous sulfate to the permanent appearance of the brown ferrous-phenanthroline complex. As the end point is approached, the brown color appears and fades as each of the last few drops is added; and the ferrous sulfate must be added until the color is permanent, the additions finally being in fractions of drops.

Determine the ratio of concentrations of ferrous sulfate and dichromate and from this ratio compute the volume of dichromate required for the oxidation of cuprous oxide. This volume multiplied by 10 gives directly the number of milligrams of copper reduced.

The titration can be conducted electrometrically, in which case the addition of the indicator is unnecessary. Both colorimetric and electrometric end points can be determined in the same solution.

3. THE REDUCTION REACTION

The reduction reaction was conducted in rigorous accordance with Munson and Walker's specifications. The analyses were made in 400-ml beakers covered with watchglasses. The solutions were brought to the boiling point in about 4 minutes by a previous adjustment of conditions. When a solution approached the boiling point, there usually occurred a few sporadic periods of apparent boiling which were followed by a very definite moment when the whole solution suddenly started to boil vigorously. This latter appearance was taken as the starting time of the 2-minute period. It was found important that the beakers after use be allowed to dry on a rack for at least 1 day and preferably several days. If they are thoroughly aerated in this way, the time of boiling can be definitely recognized and no superheating or bumping occurs.

Since at the expiration of the 2-minute period of boiling the solutions remain approximately at the boiling temperature, it is important that the time required for filtration be uniform. By adjusting the vacuum and the thickness of the asbestos mat, the rate of filtration was roughly controlled so that 30 to 50 seconds elapsed between the end of the boiling period and the completion of the filtration. Naturally in

many analyses the time varied from this standard and such variations probably contributed to the error of analysis, but in most instances imperceptibly.

Blank determinations were made of the amount of copper precipitated by boiling the mixed reagents without addition of sugar and collecting the precipitate on a Gooch crucible. This was moistened with 1:1 nitric acid, washed into a flask, and evaporated to dryness. The residue was dissolved in 0.25 ml of acetic acid, transferred to a test tube, and made up to 5 ml with water. A drop of potassium ferrocyanide was added, and the sample was compared colorimetrically with standards containing known weights of copper. Quite uniformly throughout the investigation this blank test showed 0.3 mg of copper, with maximum deviations of less than 0.05 mg. Munson and Walker [1] determined the copper reduced in blank experiments by weighing cuprous oxide. The average value from 67 determinations was 0.24 mg of copper. They obtained many blanks of negative value. If these are rejected, together with the equally improbable ones of 1 mg or more, their average is 0.33 mg. Both of these averages are in essential agreement with the values found in our analyses.

The copper reduced by the reagents is caused by the oxidizing effect of cupric copper on the alkaline tartrate. The amount of reduction probably varies with the concentration of copper and would not be the same if much of the copper is rapidly reduced by sugar. It therefore seems a questionable procedure to apply a uniform blank correction to all analyses regardless of the concentration of sugar. Moreover, the analyst who uses the empirical tables seldom determines or applies a blank correction, and for his purpose the uncorrected tables are more directly applicable. For these reasons we shall in the following pages report the weights of copper precipitated by sugar exactly as determined without application of a blank correction. This procedure is in harmony with that of Hammond, who published the copper values obtained without correction for blanks.

The foregoing statements apply to Soxhlet reagents that are not more than a few months old. After long standing, the blank becomes increasingly greater. If the analyst determines a blank correction and finds a value different from 0.3 mg, he has merely to deduct all but 0.3 mg from his copper precipitate in order to be in correspondence with Hammond's or our data.

III. EXPERIMENTAL RESULTS

By the methods which have been described, analyses were made of the three sugars, dextrose, levulose, and invert sugar at 10 concentrations ranging from 23 to 230 mg in 50 ml of solution. At each concentration usually eight determinations were made, in four of which the reduced copper was determined by thiosulfate titration and in the remaining four by colorimetric dichromate titration.

The results of the analyses of dextrose solutions are shown in table 6. The experimental results for the thiosulfate analysis of copper (column 2) were correlated by the method of least squares, the computation yielding the formula

$$\text{Cu (by thiosulfate)} = 2.0820\, d - 0.001005\, d^2, \qquad (1)$$

in which copper and dextrose (d) are expressed in milligrams. The residuals (column 4) are satisfactorily small.

It is of interest to compare this series of analyses with a series completed and published [21] previously in which the copper was related to the dextrose by the formula

$$\text{Cu (by thiosulfate)} = 2.0800\ d - 0.000989\ d^2. \quad (2)$$

The two independent series show no deviation (column 6) as great as 1 part in a thousand. For tabulation, the mean of these two formulas will be taken as the best result of this investigation:

$$\text{Cu (by thiosulfate)} = 2.0810\ d - 0.000997\ d^2. \quad (3)$$

TABLE 6.—*Milligrams of copper reduced by dextrose*

Weight of dextrose	Copper by thiosulfate					Copper from Hammond's table	Difference of mean [c] from Hammond's table		Copper by dichromate (colorimetric)			
	Series II			Calculated [b] from Series I	Series II minus series I (calculated)							
	Found	Calculated [a]	Found minus calculated						Found	Calculated [d]	Found minus calculated	Dichromate minus thiosulfate (calculated values)
mg							*mg*	*percent*				
23	46.3	47.3	−1.0	47.3	0	47.8	−0.5	1.0	46.8	47.3	−0.5	−0.0
46	94.0	93.7	+0.3	93.6	+0.1	94.0	−.4	0.4	94.3	93.5	+.8	−.1
69	138.6	138.9	−.3	138.8	+.0	139.0	−.2	.1	138.3	138.7	−.4	−.1
92	183.2	183.0	+.2	183.0	0	183.0	0	0	182.7	182.8	−.1	−.2
115	225.9	226.1	−.2	226.1	0	225.8	+.3	.1	225.6	225.8	−.2	−.3
138	268.0	268.2	−.2	268.2	0	267.6	+.6	.2	268.0	267.8	+.2	−.4
161	309.3	309.2	+.1	309.2	0	308.5	+.7	.2	308.5	308.7	−.2	−.5
184	349.1	349.1	0	349.2	−.1	348.3	+.8	.2	348.9	348.5	+.4	−.7
207	388.3	387.9	+.4	388.2	−.3	387.2	+.8	.2	387.2	387.3	−.1	−.9
230	425.4	425.7	−.3	426.1	−.4	424.8	+1.1	.3	425.0	425.0	0	−1.1

[a] Formula 1, Cu (by thiosulfate) = $2.0820\ d - 0.001005\ d^2$.
[b] Formula 2, Cu (by thiosulfate) = $2.0800\ d - 0.000989\ d^2$.
[c] Formula 3, Cu (by thiosulfate) = $2.0810\ d - 0.000997\ d^2$ (mean of 1 and 2).
[d] Formula 4, Cu (by dichromate) = $2.0792\ d - 0.001005\ d^2$.

In the last four columns of table 6 are shown the results of the analysis by colorimetric dichromate titration. A least-square computation yielded the formula

$$\text{Cu (by dichromate)} = 2.0792\ d - 0.001005\ d^2. \quad (4)$$

In the low and medium ranges of concentration the dichromate titrations agree essentially with the thiosulfate values for total copper, but in the higher concentrations the dichromate values fall slightly below those by thiosulfate, the discrepancy rising to a maximum of 0.26 percent. This difference occurs not only for dextrose but also, as will appear below, for levulose and invert sugar. It cannot therefore be ascribed to analytical error, but must be explained by some more fundamental property of the reaction which was not further investigated.

In the article previously cited [21] a series of dichromate-dextrose equivalents was published. These are not directly comparable with those given in table 6 because they were determined by electrometric titration at a hydrochloric-acid acidity of about 1.2 to 1.4 *N*. Systematic experimentation showed a consistent difference of 0.06 ml of dichromate between the electrometric end point in the presence of 1.2

N hydrochloric acid, and the colorimetric end point in 2 N hydrochloric acid, whereas the mean experimental difference between the two respective series of sugar analyses proved to be 0.054, the electrometric titration giving in both cases the lower volume of dichromate. The two series of analyses thus yielded consistent results.

In table 7 are assembled the data on the reducing action of levulose. Least-square computations yielded the respective formulas

$$\text{Cu (by thiosulfate)} = 1.8818\ l - 0.000596\ l^2 \quad (5)$$

and

$$\text{Cu (by dichromate)} = 1.8840\ l - 0.000614\ l^2. \quad (6)$$

TABLE 7.—*Milligrams of copper reduced by levulose*

Weight of levulose	Copper by thiosulfate			Copper from Hammond's table	Difference from Hammond's table (calculated values)		Copper by dichromate (colorimetric)			
	Found	Calculated [a]	Found minus calculated				Found	Calculated [b]	Found minus calculated	Dichromate minus thiosulfate (calculated values)
mg					*mg*	*percent*				
23	43.3	43.0	+0.3	43.4	−0.4	1.0	43.7	43.0	+0.7	0
46	85.5	85.3	+.2	85.8	−.5	0.6	85.2	85.4	−.2	+0.1
69	126.9	127.0	−.1	127.4	−.4	.3	127.4	127.1	+.3	+.1
92	168.1	168.1	0	168.4	−.3	.2	168.2	168.1	+.1	0
115	208.5	208.5	0	208.7	−.2	.1	208.2	208.5	−.3	0
138	248.2	248.3	−.1	248.5	−.2	.1	248.3	248.3	0	0
161	287.6	287.5	+.1	287.5	0	0	287.1	287.4	−.3	−.1
184	326.2	326.1	+.1	326.0	+.1	0	325.9	325.9	0	−.2
207	363.9	364.0	−.1	363.8	+.2	.1	363.6	363.7	−.1	−.3
230	401.5	401.3	+.2	400.9	+.4	.1	401.3	400.9	+.4	−.4
243	421.9	422.1	−.2	421.1	+1.0	.2	421.4	421.6	−.2	−.5

[a] Formula 5, Cu (by thiosulfate) = $1.8818l - 0.000596l^2$.
[b] Formula 6, Cu (by dichromate) = $1.8840l - 0.000614l^2$.

The small values of the residuals show that the formulas represent the data satisfactorily.

The reduction data on invert sugar are given in table 8. Least-square adjustment yielded the formulas

$$\text{Cu (by thiosulfate)} = 1.9834\ i - 0.000818\ i^2 \quad (7)$$

and

$$\text{Cu (by dichromate)} = 1.9828\ i - 0.000827\ i^2. \quad (8)$$

While the measurements were in progress, it was expected that the rule of mixtures would apply to the problem and that the copper reduced by invert sugar would be the mean of the amounts precipitated by dextrose and by levulose. Such proved not to be the case. The copper reduced by invert sugar is less than the mean by the two constituents (with the exception of the lower concentrations), the deficiency rising to somewhat more than 0.1 percent at the higher concentrations in the analysis by thiosulfate and to more than 0.2 percent in those by dichromate.

TABLE 8.—*Milligrams of copper reduced by invert sugar*

Weight of invert sugar	Copper by thiosulfate			Copper from Hammond's table	Difference from Hammond's table (calculated values)		Copper by dichromate			
	Found	Calculated [a]	Found minus calculated				Found	Calculated [b]	Found minus calculated	Dichromate minus thiosulfate (calculated values)
mg					*mg*	*percent*				
23	45.0	45.2	−0.2	45.2	0	0	45.2	45.1	+0.1	−0.1
46	89.3	89.5	−.2	89.6	−0.1	0.1	89.1	89.4	−.3	−.1
69	132.5	133.0	−.5	133.0	0	0	132.3	132.7	−.4	−.3
92	175.1	175.5	−.4	175.6	−.1	.1	175.5	175.3	+.2	−.2
115	217.3	217.3	0	217.2	+.1	0	217.0	216.9	+.1	−.4
138	258.1	258.1	0	258.0	+.1	0	257.3	257.7	−.4	−.4
161	298.4	298.1	+.3	298.0	+.1	0	298.1	297.5	−.4	−.6
184	337.2	337.2	0	337.0	+.2	.1	336.4	336.5	−.1	−.7
207	375.9	375.5	+.4	375.2	+.3	.1	374.9	374.7	+.2	−.8
230	412.7	412.9	−.2	412.4	+.5	.1	411.6	411.9	−.3	−1.0

[a] Formula 7, Cu (by thiosulfate) = $1.9834\,i - 0.000818\,i^2$.
[b] Formula 8, Cu (by dichromate) = $1.9828\,i - 0.000827\,i^2$.

The amount of copper reduced is a function not only of the concentration of sugar but also of the concentration of copper. If one constituent of a sugar mixture reacts upon the copper more rapidly than the other, it will diminish the copper concentration before the second constituent has reacted. The latter then will undergo reaction in a lower concentration of copper when in a mixture than when alone, and theoretically should reduce less copper [22]. This theory is completely in accord with the very exact results of Quisumbing and Thomas [23], whose deviations from the rule of mixtures are in the same direction and of about the same magnitude as those in this article.

The weight of copper precipitated in all methods in which the reduction is carried out at the boiling point is influenced by the barometric pressure. During the experiments described here a complete record of the barometric readings was kept. When a considerable change in pressure occurred, its effect was definitely noticeable in the amount of copper precipitated. In extreme cases the determination was repeated under more favorable atmospheric pressure conditions. The mean barometric reading for dextrose was 757 mm; for levulose, 756 mm; and for invert sugar, 752 mm (corrected to 0°C and for latitude).

The data in tables 6 to 8 permit an appraisal of the dichromate method. Compared with the thiosulfate method the results are slightly low. In the dextrose table there is an average deficiency of 0.14 percent; in the invert sugar table, of 0.19 percent; and in the levulose table, of 0.05 percent. The mean deficiency in all 31 analyses is 0.13 percent. If this difference should prove to be constant, it could readily be corrected empirically, most simply by diminishing the concentration of dichromate in the standard solution by 0.13 percent. The residuals would then have both positive and negative signs, and the errors would be of the order of 0.1 percent.

IV. COMPARISON OF DATA WITH THOSE OF HAMMOND

In tables 6, 7, and 8 are shown the deviations of the results from those of Hammond, the difference being given both in milligrams and in percentage of the copper reduced. A definite trend is noticeable, the results of the present measurements being in general slightly lower than Hammond's at the low concentrations of sugar and higher at the high concentrations. This suggests some small systematic difference in procedure. In conference it was revealed that Hammond's crucibles were probably more closely packed than the authors', thus causing a slight increase in the time required for filtration. Hammond required 90 to 100 seconds to filter the reaction mixture, while the authors required 30 to 50 seconds.

At the end of the 2-minute period of boiling there are two competing reactions which occur during the filtration. One is the back oxidation of cuprous oxide by air, resulting in re-solution of precipitated copper; the other is the continued reduction of copper. The additional amount of copper reduced by an extension of time of the reaction is dependent on the amount of sugar taken for analysis. Thus, as shown in table 9, the weight of reduced copper increases considerably with extension of time for low concentrations of dextrose, less so for intermediate concentrations, and not at all for high concentrations.

The slower filtration in Hammond's analyses is equivalent to a slight extension of reaction time. Hence at low concentrations of sugar he obtained, as expected, slightly greater reduction of copper. Throughout the wide range of intermediate concentrations these two competing effects counterbalance each other and the agreement between the two sets of analyses is highly satisfactory. In the range of the greater weights of sugar the only remaining effect of delayed filtration is the back oxidation by air, and thus Hammond obtained slightly less copper than the authors.

TABLE 9.—*Effect of duration of boiling on the amount of copper precipitated at varying concentrations of sugar*

Dextrose (mg)------	46	138	230
Duration of boiling	Copper		
Minutes	*mg*	*mg*	*mg*
1	91.4	265.3	424.3
2	93.5	268.1	423.9
3	94.8	269.0	424.6
4.5	96.9	271.6	423.9
6	98.5	273.7	424.6

It would perhaps be possible to add more closely defined specifications for the Munson and Walker method. This appears inadvisable since it would detract from the simplicity of the method.

A comparison of these two investigations serves fairly to evaluate the precision obtainable by Munson and Walker's method, since they represent two quite independent series of analyses. The percentage deviations of the authors' values from those of Hammond are given in the respective columns of tables 6 to 8. Of the 31 values given, 19, or 61 percent, are in agreement within 0.1 percent, and 25, or 81 percent,

within 0.2 percent. The remaining six values are all at the extreme high or low concentrations of sugar. All the values for invert sugar and 6 of the 11 values for levulose agree within 0.1 percent. Curiously enough the deviations are greater in the dextrose analyses than in those of the other sugars. The mean difference from Hammond throughout the whole series of three sugars is, regardless of sign, 0.19 percent. The greater part of this difference is contributed by the extreme high and low concentrations of sugar.

Munson and Walker's procedure is essentially a macromethod. If, therefore, we confine our attention to those determinations where there is sufficient precipitation to insure accuracy, and not so great a precipitation that the copper approaches exhaustion, say between 69 and 207 mg of sugar, we find an average deviation from Hammond of 0.5 mg, or 0.17 percent, for dextrose; 0.2 mg, or 0.11 percent, for levulose; and 0.1 mg, or 0.05 percent, for invert sugar. The mean deviation for all three sugars within this range of concentration is 0.26 mg, or 0.11 percent.

It is probable that a close collaboration would result in a reconciliation of the small differences observed, but the value of such an attempt would be problematical. The conclusion seems justified that the copper equivalents found in the present investigation corroborate those of Hammond well within the limits of experimental error. We therefore recommend that Hammond's tables be substituted for those of Munson and Walker in all analyses where the reduced copper is determined analytically.

Of the sucrose-invert sugar mixtures, we have undertaken only a small number of analyses for verifying Hammond's data and assisting in making a decision between his data and those of Erb and Zerban [2]. These latter authors have established formulas relating copper to 0.4 g of mixtures of sucrose and invert sugar. At the higher concentrations of invert sugar they are in almost perfect agreement with Hammond, but at certain lower concentrations their values diverge by a maximum of 1.7 mg of copper. Since their discrepant values are in agreement with Munson and Walker's copper precipitates, which, as has been shown above, are contaminated, they are probably too high.

In table 10 are given the weights of copper obtained from various sucrose-invert sugar mixtures. Our values follow the general tendency found in the previous analyses, an agreement with Hammond at the middle range of concentrations and a slightly greater recovery of copper at the high concentrations. In the analysis of 0.4 g of total sugar our results are in practically perfect agreement with those of Hammond.

TABLE 10.—*Copper equivalents of some sucrose-invert sugar mixtures*

Total sugar	Sucrose	Invert sugar	By thiosulfate	By electrolysis	From Hammond's table	From Erb and Zerban's formula	By dichromate	
							Colorimetric	Electrometric
g	*g*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*
2.0	1.880	120	----------	238.9	238.7	----------	238.8	238.6
2.0	1.793	207	385.6	384.7	384.2	----------	----------	----------
2.0	1.770	230	421.6	421.2	420.3	----------	----------	----------
0.4	0.340	60	156.4	----------	156.5	158.2	156.9	156.8
.4	.320	80	119.6	----------	119.7	121.3	----------	----------

V. SUMMARY AND CONCLUSIONS

In a recent revision of the Munson and Walker reducing-sugar tables [1] by Hammond [3], important deviations in many of the copper values were disclosed. The present investigation, constituting a third series of analyses, corroborates Hammond's measurements within the limits of experimental error. The differences between Hammond's and Munson and Walker's values for copper are due almost entirely to the respective methods of estimating copper. Hammond determined the reduced copper by electrolysis, whereas Munson and Walker weighed the precipitated cuprous oxide. It is now shown that the cuprous oxide is contaminated with organic decomposition products even when pure sugars are analyzed. The amount of contamination is almost exactly equal to the difference between Hammond's and Munson and Walker's copper values.

The authors have shown that reduced copper should be determined by analysis and not by direct weighing of cuprous oxide and that Hammond's tables should be substituted for those of Munson and Walker.

Erb and Zerban's analyses of 0.4 g of sucrose-invert sugar mixtures are in agreement with those of Hammond except within a short range of lower concentrations. The analyses here presented are in agreement with Hammond's within this range.

The various methods for the determination of copper are discussed. The main reliance during the investigation was the iodometric method in acetic acid solution, with the concentration (4.2 g per 100 ml) of potassium iodide specified by Shaffer and Hartmann [10], and with the addition of thiocyanate at the end of the titration as specified by Foote and Vance [11].

A method of determining cuprous oxide by oxidation with an excess of dichromate and back titration with ferrous sulfate to a colorimetric or electrometric end point is described.

The permanganate method as modified by Schoorl and Regenbogen [15] is shown to give accurate results.

The precision of Munson and Walker's method, as indicated by Hammond's and the authors' independent analyses, is shown to be about 0.2 percent. If the concentrations of sugar are restricted to the range between 69 and 207 mg of reducing sugar, the average precision appears to be about 0.1 percent.

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