

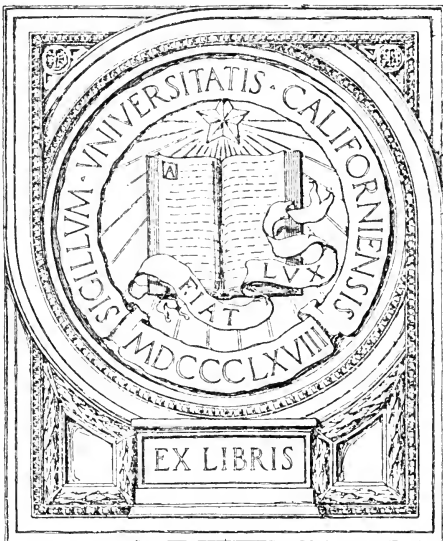
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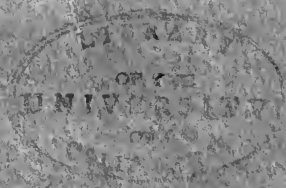
Comparative Study of Conditions
Affecting the Determination of Reducing
Sugars by Fehling Solution

DISSERTATION

Submitted in Partial Fulfillment of the Requirements for the
Degree of Doctor of Philosophy in the Faculty of
Pure Science of Columbia University

By

FRANCISCO A. QUISUMBING, M.S.



NEW YORK CITY

1921



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The author wishes also to extend his appreciation to Professor J. M. Nelson for helpful suggestions.

TO THE
ANNUAL

EXCHANGE

CONTENTS

- I. Introductory
 1. General Statement
 2. Historical
 3. Object and Plan of the Research
- II. Preliminary Experiments
 1. Atmospheric Pressure and Temperature of Boiling
 2. Apparatus
 3. Preparation and Analysis of the Sugars used
 4. Influence of Temperature and Time of Heating
- III. Surface Oxidation and Reduction
- IV. Fehling's Reagent
 1. Influence of the Nature of the Alkali
 2. Influence of the Concentration of Alkali
 3. Influence of Varying the Amount of Copper Sulphate in Fehling Solution
 4. The Effect of Varying the Amounts of Sodium Potassium Tartrate in Fehling Solution with constant Amounts of Alkali and Copper Sulphate
 5. Photosensitiveness and Keeping Quality of Fehling Solution
 6. Colloidal Cuprous Oxide
 7. Preparation of Proposed Modification of Fehling Solution Based on Results of Experiments Cited Above
- V. Technique of Proposed Method
- VI. Comparison of Methods for the Determination of Copper
- VII. Accuracy of Proposed New Method for Determination of Reducing Sugars
- VIII. Reducing Action of Sucrose on Fehling Solution and Reducing Sugars
- IX. Sugar Table
- X. Reducing Ratios
- XI. Summary

ABSTRACT

Object of the Research

To establish conditions for the quantitative analysis of common sugars such that the errors due to atmospheric pressure and temperature of boiling, difficulty in observation of exact time of boiling, surface oxidation, auto-reduction of Fehling solution, and reducing action of sucrose might be eliminated.

Results Accomplished

The errors inherent to current methods due to changes in atmospheric pressure and consequently temperature, difficulty in observation of exact time of treatment have been eliminated by carrying out the digestion in a vessel submerged in a water bath kept at a definite temperature.

From detailed investigation of the effect of variation of temperature and time of reaction as well as variation in content of copper sulphate, alkali and Rochelle salt, those conditions have been selected which lead to optimum precipitation of cuprous oxide in kind and quantity under which there is no auto-reduction of the reagent and the reduction by sucrose is eliminated. The conditions are reduction at 80° C., for 30 minutes using a mixed modified Fehling solution of composition as outlined on page 26.

Careful study of the relation of surface exposed to air and glass of container has been made showing a definite positive catalytic effect of the lateral area of glass exposed to the reagent. The error due to surface oxidation has been reduced to a minimum.

What Results Are New to the Science of Chemistry?

1. The modified Fehling solution gives no "blank" reduction.
2. The reducing action of sucrose is eliminated.
3. Highly accurate and reproducible results are possible.

I. INTRODUCTORY

I. GENERAL STATEMENT

Upon review of the different modifications of methods for the determination of reducing sugars it appears that, if greater accuracy is desired a re-standardization of conditions for reduction must be made.

A more accurate method for the determination of sugars in which copper reducing methods are involved depends, primarily, upon a well standardized set of conditions; upon a rigorous specification of procedure to be followed in operations where these conditions are involved; and upon a more rapid and accurate method for the determination of copper. Next in importance, is the study of the composition of Fehling's reagent, method of preparation and keeping quality. A study of the reducing action of sucrose on Fehling solution and effect of presence of sucrose upon reducing sugars with a view to eliminate the use of empirical formulae as a means of correcting for the reducing action of sucrose, and factors for calculating copper to invert sugar for the different ratios of sucrose and invert sugar is of timely interest.

2. HISTORICAL

The oxidation of sugars by alkaline copper salts has been known since 1841, when Trommer¹ first introduced alkaline copper sulphate as a reagent to distinguish dextrose from sucrose.

In 1844, Barreswil² improved Trommer's reagent by the addition of potassium tartrate. The latter prevents the subsequent precipitation of cupric hydroxide, thus increasing the stability of the reagent.

In 1848, Fehling³ worked out the details of the method, giving some of the stoichiometrical equivalents between copper and dextrose. The ratio of one molecule of dextrose to five of copper was regarded as constant by Fehling, and was so employed by all chemists, until Soxhlet⁴ in 1878, showed that the ratio was not

¹ Ann., 39, 360 (1841).

² Journal de Pharmacie (3) 6, 301.

³ Ann., 72, 106 (1849); 106, 75 (1858).

⁴ J. prakt. Chem., (2) 21; 227.

constant but varied according to the amount of copper in solution.

The literature relative to modifications of methods of sugar analysis by Fehling solution is so voluminous that a detailed discussion cannot be given here. The many modifications are described by Browne.¹ Of these, possibly the most important and widely used is the Unified Method of Munson and Walker.²

3. OBJECT AND PLAN OF THE RESEARCH

The purpose of this investigation was to establish conditions for the quantitative analysis of common sugars such that the errors due to atmospheric pressure and temperature of boiling, difficulty in observation of exact time of boiling, surface oxidation, auto-reduction of Fehling solution, and reducing action of sucrose might be eliminated.

1. In the preliminary experiments, a short discussion of the inadequacy of the current methods to adopt a well established set of conditions for temperature control will be considered from the standpoint of the effect of atmospheric pressure and temperature of boiling in determinations of reducing sugars.

2. Description of the apparatus for temperature control is given.

3. A study of the interrelationship of temperature and time of heating to determine the best time and optimum temperature for reduction. The effect of range of temperature, from 60° to 110° and time of heating, from 10 minutes to 2 hours on reduction has been investigated. Moreover, auto-reduction of Fehling solution at these different temperatures has been measured.

4. A study of surface oxidation and reduction of Fehling solution. Different methods of heating have been tried to determine the amount of copper lost due to surface oxidation. Beakers of different capacities have been used to determine the most convenient size of receptacle for carrying on reduction.

5. Fehling's reagent has been studied from the standpoint of nature and concentration of alkali, concentration of copper and tartrate. The main point in view was to find out the maximum and minimum concentrations of Cu^{++} and OH^- ions necessary to give greatest reduction of Fehling solution, and to ensure the formation of the complex cupric tartrate ion. Furthermore, it has been

¹ Browne, Handbook of Sugar Analysis (1912).

² J. Am. Chem. Soc., 28, 663 (1906); 29, 541 (1907).

endeavored to study some of the physical properties of colloidal cuprous oxide, photosensitiveness and keeping quality of Fehling solution, and preparation of Fehling's reagent.

6. A procedure for the proposed method of reduction and comparison of results for the determination of copper is described.

7. Discussion of the degree of accuracy of the method.

8. A study of the reducing action of sucrose on Fehling solution and effect on reducing sugars has been made with the view to eliminate the use of empirical formulae and factors for calculations of errors involved:

II. PRELIMINARY EXPERIMENTS

I. ATMOSPHERIC PRESSURE AND TEMPERATURE OF BOILING

It is the experience in this laboratory as well as in others that entirely satisfactory determinations of reducing sugars are not realized by the current methods. Part of the irregularity of results is due to the effect of atmospheric pressure on temperature of boiling coupled with ill-defined set of conditions for reduction.

Traphagen and Cobleigh¹ have pointed out that variable temperatures of boiling at different altitudes above sea level is a cause of difference in determination of reducing sugars. Rosenkranz², who studied the influence of atmospheric pressure upon reducing power of invert sugar, showed that the sugar had the tendency to increase in reducing power with increase of pressure. The difference is due to change in temperature of boiling water with change of pressure. At high temperatures, a change of a few degrees has a marked effect on the oxidation of sugars, especially when sucrose is present.

Maquenne³ working on the comparative action of sucrose and invert sugar on "cupro-potassic" reagent found a marked change of reduction with change of temperature showing how barometric pressure influences the results of determinations made in a boiling water bath.

Tables 1 and 2 show how different atmospheric pressures influence the variability of results due to change of temperature of boiling.

¹ J. Am. Chem. Soc., 21, 369 (1899).

² Z. Ver. Deut. Zuckerind., 61, 426 (1911).

³ Compt. rend., 162, 145-9 (1916); 161, 617-23 (1915)

TABLE I

Influence of Atmospheric Pressure on the Oxidation of Invert Sugar by Fehling solution (from Rosenkranz ¹)

Pressure	Temp. of Boiling	25 c.c. of Invert Sugar Plus	
		25 c.c. Water 50 c.c. Fehling Solution	25 c.c. of 1% Sucrose Solution 50 c.c. Fehling Solution
600 mm	90-95°C	232.5	244.9
775 "	103-105°C	236.5	260.4
760 "	103-104 "	235.6	277.7
925 "	109-110 "	236.1	296.3

TABLE II

Comparison of Reducing Values for Dextrose Obtained by Current Methods of Heating

	Munson & Walker's 2 min. Boiling over a Flame		12 min. Heating in Boiling Water	
			Observed Barometric Pressure	Observed Temp. of boiling water
100 mg. of dextrose	189.7 mg. Cu	188.9 mg. Cu	756.0 mm	98.0°C
	194.0 " "	192.1 " "	758.0 "	98.5 "
	190.2 " "	192.7 " "	759.0 "	99.0 "
	191.3 " "	192.4 " "	759.5 "	99.0 "
	192.8 " "	192.7 " "	759.0 "	99.0 "
	203.6 " "	209.9 " "	753.0 "	97.0 "
150 mg. of dextrose	202.2 " "	205.9 " "	752.5 "	96.0 "
	203.0 " "	211.0 " "	759.0 "	98.0 "
	206.2 " "	211.8 " "	758.8 "	97.5 "

*92.12
16 mg. Cu
Per 1 mm
Dextrose*

The experiments tabulated above were performed at different times of the year.

The great difference of results obtained by the Munson and Walker's method is only partly due to change of boiling temperature of the solution. A greater error inherent to the method is the difficulty for the observer to decide when to begin to reckon the time of boiling. Furthermore, the temperature of boiling for reduction under these conditions is not constant. It ranges from 101 to 105°C. depending how one controls the heating power. While these last mentioned faults do not apply to the method of heating in boiling water, even in this method concordant results cannot be obtained on account of changes in atmospheric pressure and consequently temperature of boiling.

¹ Z. Ver. Deut. Zuckerind, 61, 426 (1911).

It is apparent that variation in boiling point due to locations of laboratories at different altitudes might seriously affect the direct use of Allihn's, Munson and Walker's or Defren's Methods.

To overcome the uncertainties of temperature, in all experiments cited herein, a water thermostat as described below served as method of heating, in which water was used as the liquid medium for temperatures below 90°C., and rapeseed oil for temperatures above 90°C.

2. APPARATUS

A 50 liter constant temperature bath similar to the one described by H. C. Sherman and A. W. Thomas¹, and J. M. Nelson and F. M. Beegle² was constructed. The water in the bath was heated to 80°C. by a 15 ampere Simplex heater and kept at this temperature by two 250 watt radiator lamps which were in series with a relay and a mercury constant temperature regulator containing a nickle wire in the neck which served as the contact. The temperature was maintained at 80°C. \pm 0.1°. Excessive evaporation of the water was prevented by the addition of 2 to 3 drops of colorless paraffin oil. The water in the bath was stirred constantly and vigorously by means of a stirrer driven by a 1/10 HP motor.

When the bath was set at a definite temperature, a 350 c.c. beaker containing the solution was placed in it, supported on an iron ring, so that the levels of the liquid in the container and the water in the bath were practically identical. Occasional shaking with rotatory motion of the beaker was made to ensure thorough mixing of the solution. At the end of the desired time of heating, the beaker was removed, and the solution containing the precipitated cuprous oxide filtered through a mat of asbestos in a Gooch crucible. The precipitate was washed with warm water, then dissolved with 10 to 20 c.c. of 16 N HNO₃, and copper determined either by Beans and Stillman's³ Electrolytic Method or E. C. Kendall's⁴ Modified Iodide Method.

3. PREPARATION AND ANALYSIS OF THE SUGARS USED

Sugar solutions for measurement of optical rotation were made by dissolving 5 grams of the sugar with distilled water and made up

¹ J. Am. Chem. Soc. 37, 623-648 (1915).

² *Ibid.*, 41, 559 (1919).

³ J. W. Stillman: New Direct Method for the Electrolytic Determination of Copper. Dissertation, Columbia Univ., 1920.

⁴ J. Am. Chem. Soc., 33, 1947 (1911).

to 100 c.c. at 25°C. in a 100 c.c. calibrated flask. The solutions were allowed to stand until the mutarotation had come to equilibrium.

For measurement of specific rotatory power a Schmidt and Haensch polarimeter sensitive to 0.01° was used. The solutions in 2 dcm. polariscope tubes were maintained during reading at 25°C. ± 0.01°, by use of the thermostat described by Nelson and Beegle.¹

Mercury vapor lamp and sodium flame were used as sources of light. The light of the sodium flame was not purified. The light of the mercury lamp was passed through a Wratten No. 74 filter giving a wave length, $\lambda = 546 \mu \mu$.²

Bureau of Standards, Sucrose Sample No. 17: This sample was of highest degree of purity obtainable. Analysis showed it to contain,—

Ash	0.003%
Moisture	0.003%

400 milligrams of the sugar sample did not show any reducing action on Fehling solution at 80°C. for 30 minutes.³

$$[\alpha]_{\lambda=546 \mu \mu}^{25} = 78.^\circ 200 \quad \frac{[\alpha]_D^{25}}{[\alpha]_{\lambda=546 \mu \mu}^{25}} = 0.84916 \quad \frac{[\alpha]_{\lambda=546 \mu \mu}^{25}}{[\alpha]_D^{25}} = 1.1776$$

$$[\alpha]_D^{25} = 66.^\circ 405$$

Dextrose: C.P. Dextrose obtained from Corn Products Refining Company, was purified according to the Bureau of Standards method⁴ of preparing pure dextrose. Analysis of the sample showed it to contain,—

Ash	0.003%
Moisture	0.150%

The sample exhibited slight hygroscopicity.

100 milligrams of this dextrose reduced 201.0 milligrams of copper (30 minutes, 80°C. Method).³

$$[\alpha]_{\lambda=546 \mu \mu}^{25} = 62.^\circ 021 \quad \frac{[\alpha]_D^{25}}{[\alpha]_{\lambda=546 \mu \mu}^{25}} = 0.84610 \quad \frac{[\alpha]_{\lambda=546 \mu \mu}^{25}}{[\alpha]_D^{25}} = 1.1818$$

$$[\alpha]_D^{25} = 52.^\circ 480$$

Levulose: The original sample of levulose was kindly furnished by Dr. J. M. Nelson. The sample was crystalline and white in color.

¹ J. Am. Chem. Soc., 41, 559 (1919).

² W. C. Vosburgh, J. Am. Chem. Soc., 42, 1698 (1920).

³ See page 47 of this paper.

⁴ U. S. Bur. of Stand., Cir. No. 44, 92 (1919).

It was further purified by recrystallization from concentrated acetic acid solution as recommended by Hudson and Dale¹ for glucose. Slight odor of acetic acid was removed by drying the sample in vacuo over concentrated solution of sodium hydroxide. Analysis of the sample showed it to contain,—

Ash 0.060%

Moisture 0.002%

100 milligrams of this levulose reduced 185.0 milligrams of copper (30 minutes, 80°C. Method).²

$$[\alpha]_{\lambda=546\mu\mu}^{25} = -105.30 \quad \frac{[\alpha]_D^{25}}{[\alpha]_{\lambda=546\mu\mu}^{25}} = 0.8490 \quad \frac{[\alpha]_{\lambda=546\mu\mu}^{25}}{[\alpha]_D^{25}} = 1.1778$$

$$[\alpha]_D^{25} = -89.40$$

Invert Sugar: Invert sugar prepared by the invertase method is not recommended for reduction by Fehling solution. Invertase and Fehling solution when heated form a complex copper invertase compound, the so called "copper yeast gum," (Salkowski).³ This compound was also obtained by Nelson and Born.⁴ On hydrolysis, the polysaccharide invertase yields mannose and dextrose (Hesseland).⁵ The gelatinous copper compound was very hygroscopic. Analysis showed that the copper compound increases in weight with increasing concentration of invertase. It is interesting to note that 1, 2, 4 c.c. of invertase plus 50 c.c. of Fehling solution when heated for 30 minutes at 80°C. gave a "copper gum," the copper contents of which were as follows:—1.7, 2.9, and 5.5 milligrams. For these reasons the invertase method was abandoned and the invert sugar solution was prepared by mixing equal numbers of molecules of dextrose and levulose.

100 milligrams of this invert sugar reduced 191.5 milligrams of copper. (30 minutes, 80°C. Method).²

$$[\alpha]_{\lambda=546\mu\mu}^{25} = -21.50 \quad \frac{[\alpha]_D^{25}}{[\alpha]_{\lambda=546\mu\mu}^{25}} = 0.8577 \quad \frac{[\alpha]_{\lambda=546\mu\mu}^{25}}{[\alpha]_D^{25}} = 1.1691$$

$$[\alpha]_D^{25} = -18.39$$

Lactose: Lactose was prepared from Squibb's crystallized lactose. It was purified by dissolving in a small volume of boiling water,

¹ J. Am. Chem. Soc., 39, 320 (1917).

² See page 47 of this paper.

³ Z. physiol. Chem., 31, 305 (1900).

⁴ J. Am. Chem. Soc., 36, 393-403 (1914).

⁵ Z. Ver. Rübenzuckerind., 42, 671 (1892).

filtering, and when cold, a mixture of equal volumes of ether and alcohol was added with constant stirring, and the lactose was allowed to crystallize in the cold for two days. The crystals were filtered, washed with alcohol and ether and dried in a vacuum oven at 70°C. for several hours. The dried material was ground to powder and dried 100°C. for 4 to 5 hours. Analysis showed it to contain,—

Ash	0.03%
Moisture	5.22% (loss at 125° — 130°C)

One molecule of water, corresponding to the formula $C_{12}H_{22}O_{11}$. H_2O requires 5.0% of water, therefore the sample thus prepared contained 0.22% excess water calculated as lactose hydrate.

100 milligrams of this lactose reduced 123.0 milligrams of copper (30 minutes, 80°C. Method).¹

$$\begin{array}{l} [\alpha]_{\lambda=546 \mu\mu}^{25} = 61.^\circ 36 \\ [\alpha]_D^{25} = 52.^\circ 90 \end{array} \quad \frac{[\alpha]_D^{25}}{[\alpha]_{\lambda=546 \mu\mu}^{25}} = 0.8621 \quad \frac{[\alpha]_{\lambda=546 \mu\mu}^{25}}{[\alpha]_D^{25}} = 1.1599$$

Maltose: Maltose was prepared from Lintner² soluble starch as follows: A 10% solution of starch was prepared and autoclaved for one-half hour. To two liters of starch solution, 25 c.c. of soluble active enzyme of malt amylase prepared according to Sherman and Schlesinger³ were added. The saccharogenic power of the enzyme was activated by adjusting the hydrogen ion concentration of the solution approximately to $10^{-4.2}$ moles per liter by means of hydrochloric acid.⁴ Saccharification took place at 50°C. for 5 hours, and then at room temperature for several hours. The final product of conversion was fermented with 5 c.c. of pure culture of *Saccharomyces Ludwiggii* to remove glucose and fermentable sugars other than maltose. When fermentation was completed, the sugar solution was autoclaved, cooled, and 95% alcohol added until the alcoholic strength of the solution was about 80%. The latter was allowed to stand for 2 days then decanted from the precipitated dextrans; alcohol distilled and solution evaporated under vacuum to a thin syrup. The syrup was inoculated with pure maltose, and the sugar allowed to crystallize. The crystals were filtered

¹ See page 47 of this paper.

² J. prakt. Chem. (2) 34, 378 (1886).

³ J. Am. Chem. Soc., 35, 1617-23 (1913).

⁴ H. C. Sherman & A. W. Thomas, J. Am. Chem. Soc., 37, 623 (1915).

and recrystallized from hot 95% alcohol, using acid-purified cocoa-nut charcoal as decolorizing agent.

Maltose thus prepared was dried at 60°C. in a vacuum oven for 4 hours, then ground to powder and dried at 90°C. for 10 hours. Analysis showed it to contain,—

Ash	0.02%
Moisture	5.05%

As maltose hydrate, $C_{12}H_{22}O_{11} \cdot H_2O$ requires 5% water there was present 0.05% water in excess.

100 milligrams of this maltose reduced 100.9 milligrams of copper (30 minutes, 80°C. Method).¹

$$[\alpha]_{\lambda=546 \mu}^{25} = 153.75$$

$$[\alpha]_D^{25} = 131.25 \text{ (hydrated)} = 137.81 \text{ for anhydrous maltose}$$

4. INFLUENCE OF TEMPERATURE AND TIME OF HEATING

Temperature and time of heating prove to be the most important factors in the oxidation of sugars by Fehling solution.

A series of experiments to determine the most suitable temperature and length of time of heating for the oxidation of sugars by Soxhlet-Fehling solution are shown in Table III (See Curves in Figs. 1 and 2). The solution consisted of 50 c.c. of mixed Soxhlet-Fehling solution,² 50 milligrams of glucose. The total volume was made up to 100 c.c. with distilled water.

TABLE III

Reduction of Fehling Solution by Dextrose

Time of Heating in min.	60°C	70°C	80°C	90°C	100°C	110°C
10	5.2	14.1	26.3	61.2	71.2	81.6
20	27.5	51.7	75.1	81.3	86.8	91.1
30	50.3	76.6	81.3	84.1	88.9	97.0
60	77.1	79.9	82.3	86.3	93.0	103.7
120	75.1	79.0	82.0	86.3	99.7	105.7

(The above figures represent averages of duplicate determinations which varied not more than 0.6 mg. of Cu.)

¹ See page 47 of this paper.

² Soxhlet's formula: A. 34.639 g. $CuSO_4 \cdot 5H_2O$ in 500 c.c. solution

B. 173 g. of Rochelle salts, 51.6 g. NaOH in 500 c.c. of solution.

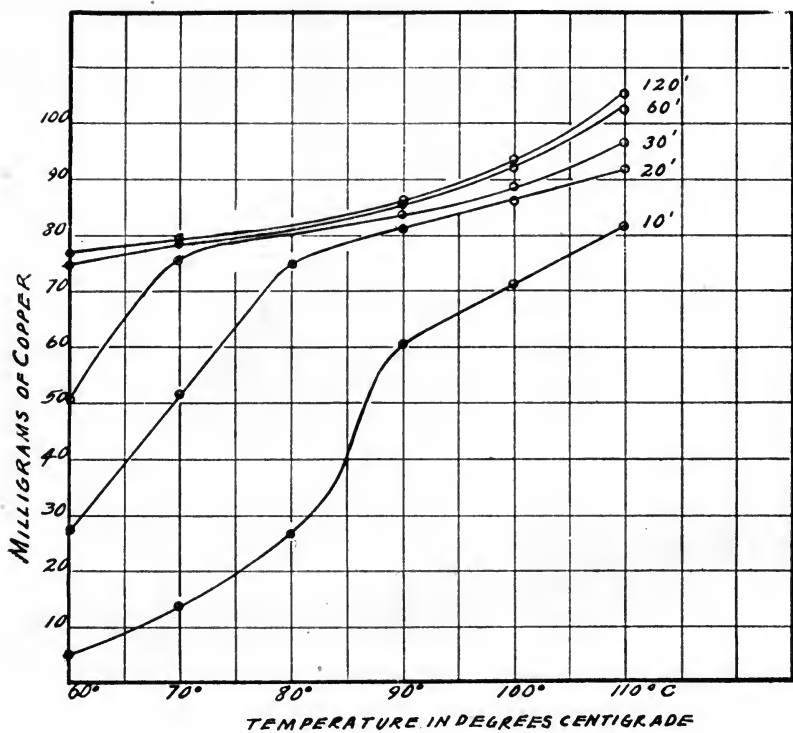
REDUCTION BY GLUCOSE AT DIFFERENT
TEMPERATURES

Fig. 1

CURVES SHOWING THE INFLUENCE OF TIME ON
REDUCTION BY GLUCOSE

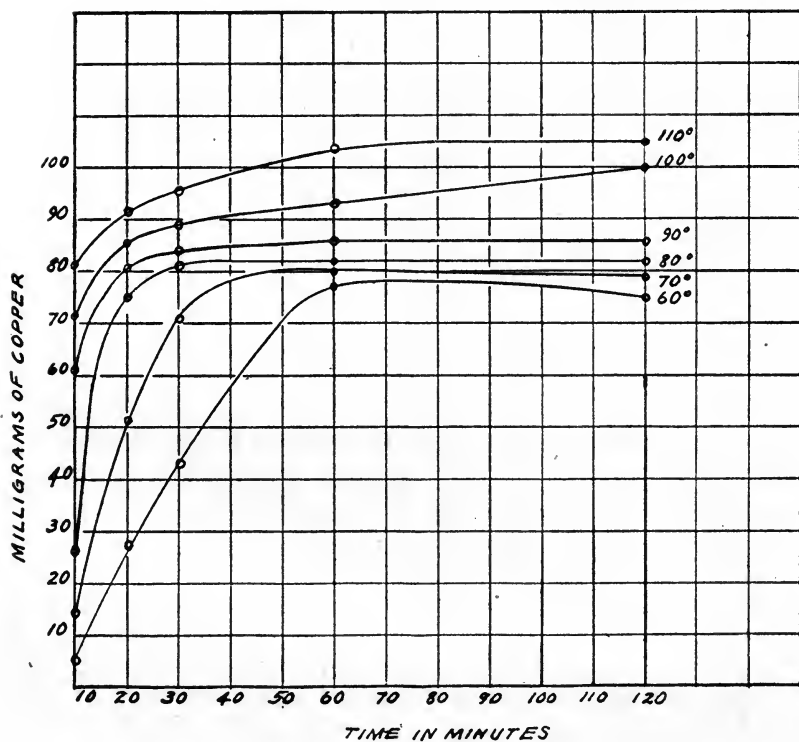


Fig. 2

Upon critical examination of figures in Table III and curves in Figures 1 and 2, it is seen that temperature and time of heating exert a great influence on the oxidation of dextrose. Reduction increases with rise of temperature and it appears complete at temperatures below 90°C. after an hour's heating. At temperatures above 100°C. there is an appreciable increase in reduction which may be due to auto-reduction of Fehling solution.

TABLE IV

Auto-Reduction of Fehling Solution at Different Temperatures and Times of Heating

Time of Heating	MILLIGRAMS OF COPPER					
	60°C	70°C	80°C	90°C	100°C	110°C
10 min.	0	0	0	0	0.2	0.4
20 "	0	0	0	0	0.4	0.9
30 "	0	0	0	0.6	2.8	3.1
60 "	0	0	0.3	1.2	3.2	3.5
120 "	0	0.3	0.6	1.8	3.9	5.2

AUTO-REDUCTION OF FEHLING SOLUTION

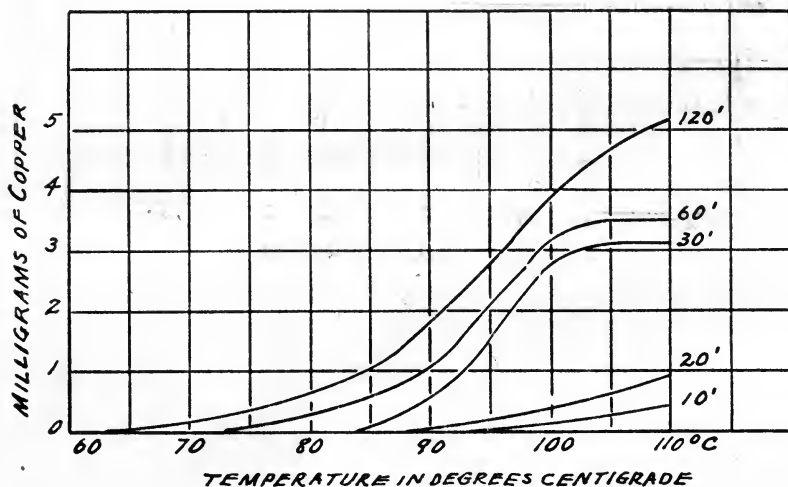


Fig 3

Table IV and curves in Figure 3 show that Soxhlet's Fehling solution is reduced at high temperatures when no sugar is present. The detection of cuprous oxide in so-called traces in the "blanks" by methods of 2 minutes boiling over a flame or heating in boiling water is due to this auto-reduction of Fehling solution. Attention is called to the absence of such auto-reduction at 80°C. up to 30 minutes time of heating, and furthermore at this temperature there is but slight formation of cuprous oxide even after heating for 1 hour.

Selection of Conditions for Reduction. In Figure 2, the ordinates represent the total amount of copper reduced, the abscissae, the time of heating in minutes. The asymptotic nature of the curves is at once apparent. If it is feasible to determine when the process of reduction is complete, that point would be the most appropriate one at which to terminate the treatment, but if the time element is considered, it matters not whether the terminus point is reached for the purpose of an analytical method. The most desirable point may be gotten by selecting a set of standardized conditions so that the amount of reduction obtained is sharply defined at a selected point by great constancy. Upon critical study of the curves in Figures 2 and 3, the conditions of 80° and 30 minutes appear to be most ideal, because (1) Fehling solution at this temperature and time of heating does not give a "blank" reduction and (2) beyond 30 minutes heating at 80° the curve rises very slightly. This point can therefore be safely called the "terminus point." Temperatures other than 80°C. might have been selected, but the reason for not selecting lower temperatures is that it would require a longer time of heating to give a reasonable amount of copper for quantitative determination, and at higher temperatures, the difficulty to regulate the bath to within $\pm 0.1^\circ$ would make it impracticable, and the error due to sucrose would appear.

Objection may be raised that 30 minutes reduction requires too much time for the analysis, but experience shows that if sets of seven samples are run under these conditions, a sample's reducing power can be determined, counting from the time it is placed in the bath to washing of the precipitate, within eight to ten minutes.

A great advantage claimed for this procedure over the flame heating method is that it does not require continuous observation to note the time of boiling. Furthermore, this method offers an

important improvement over the current methods because there is no auto-reduction of Fehling solution nor interference by sucrose. Moreover, longer time of heating has the advantage of producing a coarser and more granular cuprous oxide precipitate, which is easily washed and removed from the beaker without the aid of a rubber "police-man."

III. SURFACE OXIDATION AND REDUCTION

The shape and size of receptacles in which Fehling solution and reducing sugars are heated has some influence on the amount of copper reduced. The nature of the material of which the receptacle is made has also some accelerating effect on the velocity of deposition of cuprous oxide.

Urecht's¹ measurements on the rate of precipitation of cuprous oxide showed that the rate of reduction of Fehling solution by invert sugar was augmented by the use of vessels of large internal capacity.

Kjeldahl² made direct measurements of surface of liquid exposed to the air of receptacles of different shapes and capacities, and found that the greater the surface of liquid exposed to the air during reduction, the less the amount of copper reduced.

Kendall³ performed a series of experiments on the amount of cuprous oxide lost due to surface oxidation by weighing definite weights of cuprous oxide and adding alkaline salicylate solution. The flasks containing the cuprous oxide and Kendall-Fehling solution were subjected to different methods of heating and after a time, the cuprous oxide was filtered and the amount of copper recovered, determined. His results showed no appreciable loss due to surface oxidation when the solution was heated in boiling water for 20 minutes. There was, however, some loss when the solution was boiled for 2 minutes over a flame.

While an appreciable loss of cuprous oxide is known to result from surface oxidation, no attempt has been made to correlate surface area of the reagent exposed to the walls of the glass container and reduction. To get definite figures for such relationship, the following experiments were made. They were performed in

¹ Ber., 15, 2687 (1882).

² Comptes Rendus des Travaux du Laboratoire de Carlsberg (1895).

³ J. Am. Chem. Soc., 34, 317-341 (1912).

beakers where the surface of the reagent was exposed to direct action of the air. The solution consisted of 200 milligrams of the sugar and 50 c.c. of Fehling solution, made up to 100 c.c. with distilled water.

Table V shows that reduction is directly proportional to lateral area of liquid exposed to the glass and inversely proportional to surface area of liquid exposed to the air. That is, the more surface area of the liquid exposed to the air, the less the amount of copper reduced.

Boiling for 2 minutes over a flame gives reduction which varies to the extent of 5.8 milligrams of copper with beakers in which the surface area of the liquid exposed to the air ranged from 43.7 to 85.6 sq. cm. Heating for 20 minutes in boiling water gives a difference of 3.2 milligrams, and for 30 minutes in water at 80°C., 2.9 milligrams.

It should be observed that in all these three methods of heating 350 and 550 c.c. beakers gave practically the same reduction, although the surface areas of the liquid exposed to the air differed by 13.3 sq. cm. This is due to the fact that their *total surface areas* ($2\pi rh + \pi r^2$) are equal.

A second series of experiments was planned to study this relationship further. The solutions consisted of 100 milligrams of dextrose and 50 c.c. of Fehling solution, made up to 100 c.c. with distilled water. In (1) the liquid was covered with a layer of toluene, (2) the beakers were covered with watch glass, and in (3) the liquid was left exposed to the air. Copper was determined electrolytically.

Table VI shows that if the action of air is excluded, reduction is proportional not to *total* but to *lateral* area of liquid exposed to the glass, that is, the higher the column of liquid exposed to the glass surface, the more cuprous oxide is precipitated. It appears that the most plausible explanation for this phenomenon is that reduction is accelerated more along the sides than at the bottom of the container because as the precipitate drops down from the sides to the bottom, the layer of cuprous oxide thus formed tends to cut down the accelerating effect of the glass surface at the bottom, and leaves a free surface along the sides for catalytic action. The table also shows that the amount of cuprous oxide lost due to surface oxidation is proportional to the area of liquid exposed to the air.

TABLE V
Effect of Surface Area of Liquid Exposed to the Air and Lateral Area of Liquid Exposed to the Glass
on the Amount of Copper Reduced

Receptacle	Diameter	Height of column of liquid	Lateral Area of liquid exposed to glass	Surface Area of liquid exposed to air	(200 MILLIGRAMS OF DEXTROSE USED IN EACH CASE)		
					2 min. boiling over a flame	Methods of Heating 20 min. heating in boiling water	30 min. heating in water at 80° C.
Beaker	750 c.c.	2.0 cm.	65.2 sq. cm.	85.6 sq. cm.	305.0	304.7	302.9
"	550 "	2.7 "	79.5 "	70.1 "	305.8	306.4	304.6
"	350 "	3.5 "	92.2 "	56.8 "	305.7	306.7	304.7
"	250 "	4.0 "	93.0 "	43.7 "	310.8	307.9	305.8
Flask	250 "	3.0 "	89.4 "	70.4 "	306.7	310.2	304.5

(Figures represent averages of duplicate determinations which varied not more than 0.7 mg. of Cu.)

TABLE VI

Beaker	Diameter	Height of column of liquid	Lateral Area of liquid exposed to glass	Surface Area of liquid exposed to air	(100 MILLIGRAMS OF DEXTROSE USED IN EACH CASE)			Loss (1)-(3)
					(1) Covered with toluene	(2) Covered with watch glass	(3) Uncovered	
Beaker	1000 c.c.	1.7 cm.	63.2 sq. cm.	111.6 sq. cm.	196.7	196.3	191.6	5.1
350 "	8.5 "	3.5 "	92.2 "	56.8 "	198.7	198.2	196.2	2.5
140 "	6.25 "	5.5 "	110.8 "	36.0 "	199.4	200.9	198.8	0.6

(Figures represent averages of duplicate determinations which varied not more than 0.4 mg. of Cu.)

The effect of surface oxidation was further investigated by adding to definite weights of chemically pure cuprous oxide 50 c.c. of Fehling solution, the total volume of the solution being adjusted to 100 c.c. with distilled water, and placed in 350 c.c. beakers of same diameters. One c.c. of toluene was added to each. After the mixtures had been heated for 30 minutes in water at 80°C., the cuprous oxide was filtered off, washed and copper determined electrolytically.

	Covered with toluene	Covered with watch glass	Uncovered	
Weight of Cu_2O used	147.9 mg	234.2 mg	230.6 mg	239.6 mg
" " " recovered	147.8 "	234.0 "	227.2 "	237.0 "

Cuprous oxide used and recovered was calculated from the weight of copper determined electrolytically.

These experiments show that the loss of cuprous oxide due to surface oxidation can be obviated by either covering the liquid with some inert liquid such as toluene, or by covering the beaker with a watch glass. The latter serves to prevent evaporation of the liquid as well as surface oxidation. Kjeldahl¹ eliminated this error by use of an atmosphere of hydrogen or of oxygen-free illuminating gas. Although Kjeldahl's method undoubtedly gives more nearly the true reduction, on the other hand, the inconveniences attending such manipulation would preclude its general use. Covering the beaker with watch glass approximates quite as well the true reduction as the other expedients suggested and, of course, is preferable due to its simplicity.

IV. FEHLING'S REAGENT

In consequence of Nef's² elaborate study of the action of alkalis on sugars extending over a period of ten years, a more complete knowledge is available concerning the fate of sugars when treated with Fehling solution and other alkaline oxidizing agents. According to Nef any sugar in alkaline solution undergoes such a complicated change, that it is eventually transformed into a mixture in which not less than 116 different substances are present. The mechanism of the reaction of sugars with Fehling solution is not

¹ Comptes Rendus des Travaux du Laboratoire de Carlsberg (1895).

² Ann. 357, 214-312 (1907); 376, 1-119 (1910); 403, 204-383 (1914).

well understood. As a result of Nef's work we know that the sugar molecule in the presence of Fehling solution undergoes oxidation, reduction, condensation or further decomposition giving rise to a multiplicity of aldehydes and acids as end products, the kind, as well as amount of which, varies with concentration of sugar used.

I. INFLUENCE OF THE NATURE OF THE ALKALI

The nature and amount of alkali in Fehling solution influence reduction and physical character of the copper oxide formed. Sodium hydroxide and potassium hydroxide of equivalent concentrations give practically the same reduction with dextrose and invert sugar. Glendining¹ finds that maltose shows a higher reducing power when potassium hydroxide is substituted for sodium hydroxide in Fehling solution. Kjeldahl,² studying the effect of different strengths of sodium hydroxide upon reduction, found that between limits of 0.5 to 2 normal sodium hydroxide solution, the variation was not more than 1% with glucose, while with maltose and lactose there was a wide variation.

It is the author's experience that Fehling solution containing sodium hydroxide gives a more satisfactory precipitation than when potassium hydroxide or the carbonates are used.

TABLE VII

Showing the Effect of the Nature of Alkali in Fehling Solution upon Reduction (Fig. 4)

Normality of Fehling solution	0.6N	0.8N	1.2N	1.6N	2.0N	3.0N	4.0N	6.0N	8.0N
No. grams of alk. in 50 c.c. Fehling sol.	1.2	1.6	2.4	3.2	4.0	6.0	8.0	12.0	16.0
NaOH	173.0	177.1	—	179.5	—	178.6	176.7	174.2	172.2
KOH	103.3	173.7	—	177.8	—	—	175.6	148.2	99.5
Na ₂ CO ₃	—	52.9	59.7	—	108.6	—	167.4	217.3	140.7
K ₂ CO ₃	—	10.9	61.5	—	122.5	—	187.7	201.0	174.7

Table VII and curves in Fig. 4 show that alkali hydroxides exert great differences in the oxidation of sugars by Fehling solution of different alkalinity. Sodium hydroxide appears to show the greatest influence on reduction. By "1.6 normal Fehling solution" is

¹ Jour. Chem. Soc. Trans., 67, 999-1002 (1895).

² Comptes Rendus des Travaux du Laboratoire de Carlsberg (1) 4, (1895).

meant that 50 c.c. of mixed Fehling solution contain 3.2 grams of sodium hydroxide.

Figures show that there exists a certain minimum concentration of OH^- ions necessary to ensure the formation of the complex cupric tartrate ion and a maximum to give the greatest reduction of Fehling solution.

2. INFLUENCE OF THE CONCENTRATION OF ALKALI

TABLE VIII

Reduction of Fehling Solution of Different Alkalinity by Dextrose, Levulose, Invert Sugar, Lactose, and Maltose (Fig. 5)

Normality of Fehling sol.	0.4N	0.6N	0.8N	1.6N	3.0N	4.0N	6.0N	8.0N
No. grams NaOH* in 50 c.c. Fehling solution	0.8	1.2	1.6	3.2	6.0	8.0	12.0	16.0
Dextrose	—	173.1	177.1	179.5	178.6	176.7	174.2	172.2
Levulose	—	171.6	176.7	178.6	178.8	178.9	175.6	174.0
Invert Sugar	20.8	—	144.8	144.5	144.3	143.8	143.6	141.5
Lactose	11.4	—	129.8	129.6	127.5	119.4	115.5	114.0
Maltose	—	86.8	106.7	106.4	99.9	97.4	95.5	92.5

*Alkali prepared as described on page 47.

According to Table VIII and curves in Fig. 5, the most effective alkalinity of Fehling solution is 1.6 normal in terms of sodium hydroxide. This alkalinity has also been found best by Kjeldahl¹ and Brown, Morris and Millar,² when reduction was carried on in a boiling water bath. Fehling solution of this alkalinity corresponds to 65 grams of sodium hydroxide per 500 c.c. of the alkaline tartrate solution.

The curves show that the concentration of OH^- ions influences the rate and amount of oxidation of the sugars. The amount of copper reduced increases with increasing concentration of OH^- ions up to a certain point, where oxidation appears to be at its maximum, and from that point further increase in OH^- ions tends to decrease reduction. (Curves in Fig. 5). The most plausible explanation for the decrease in reduction is due to the formation in large quantities of compounds of lower reducing power. The same phenomenon is exhibited regardless of the nature of alkali. Greater decomposition of the sugar is more apparent with lactose and maltose.

¹ Comptes Rendus des Travaux du Laboratoire de Carlsberg (1895).

² Jour. Chem. Soc., 999-1002 (1895).

CURVES SHOWING THE INFLUENCE OF DIFFERENT ALKALIES
IN FENLING SOLUTION ON THE OXIDATION
OF GLUCOSE

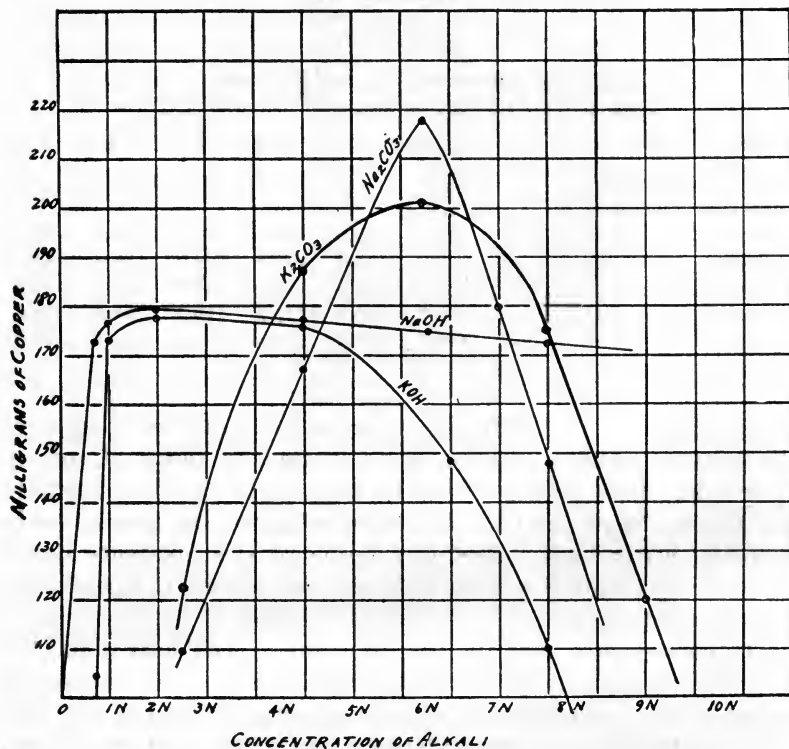
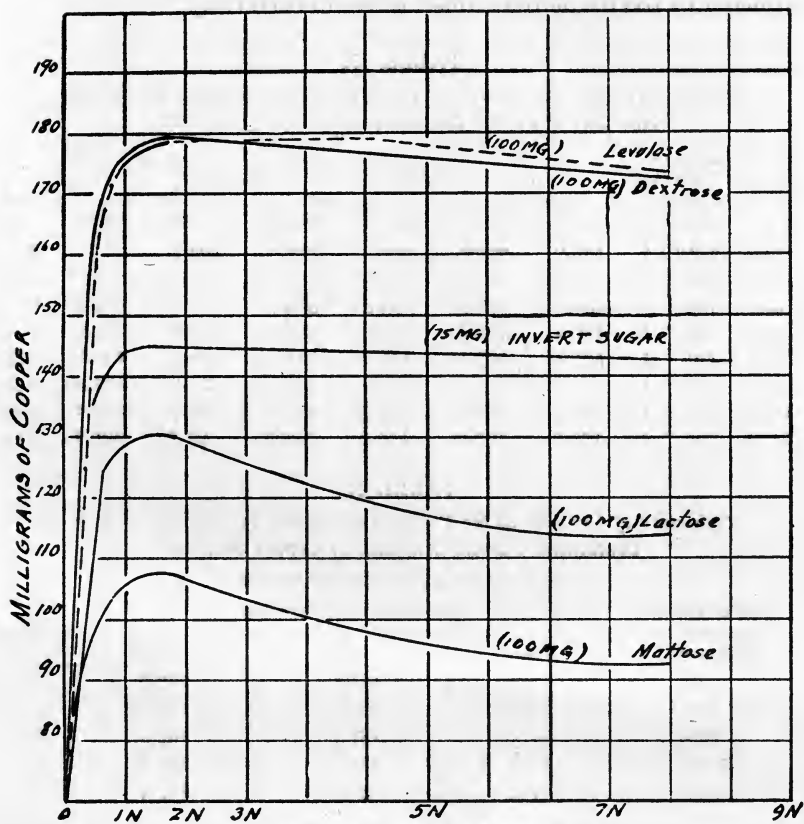


Fig. 4

CURVES SHOWING THE INFLUENCE OF DIFFERENT CONCENTRATIONS OF ALKALI IN FEHLING SOLUTION ON THE OXIDATION OF SUGARS



CONCENTRATION OF ALKALI
Fig. 5

3. INFLUENCE OF VARYING THE AMOUNT OF COPPER SULPHATE IN FEHLING SOLUTION

The solution consisted of 50 c.c. of Fehling solution containing varying amounts of copper sulphate, 3.2 grams of sodium hydroxide, 8.65 grams of the sodium potassium tartrate and 100 or 200 milligrams of the sugar. The total volume of the solution was adjusted to 100 c.c. with distilled water in each case.

TABLE IX

Oxidation of Reducing Sugars by Fehling Solution Containing Varying Amounts of Copper Sulphate, at 80°C for 30 minutes

Wt. of Cu in 50 c.c. mg.	Ratio of NaOH to Cu by weight	Reduction in terms of mgs. of Cu						Blank
		Dextrose		Levulose		Lactose		
		100 mg.	200 mg.	100 mg.	200 mg.	100 mg.	200 mg.	
210	15.2 to 1	142.6	—	—	—	108.7	—	0
315	10.0 " 1	148.3	—	152.6	—	114.4	—	0
420	7.6 " 1	152.9	225.9	154.5	304.6	118.0	224.7	0
525	6.2 " 1	156.1	303.4	157.0	308.8	119.6	227.9	0
630	5.0 " 1	158.8	307.9	158.9	312.3	120.5	232.6	0.3
840	4.0 " 1	157.6	311.8	158.6	314.3	121.7	233.4	0.9
1050	3.0 " 1	155.8	311.1	157.7	311.4	120.7	232.1	3.0
1260	2.4 " 1	150.2	307.0	154.6	309.6	97.7	226.1	3.9

TABLE X

Oxidation of Dextrose in the Presence of Sucrose by Fehling Solution Containing Varying Amounts of Copper Sulphate

REDUCTION BY DEXTROSE ALONE

Wt. of Copper in Fehling sol. Milligrams	Reduction in terms of mgs. of Cu			Blank
	Milligrams of Dextrose in 10 c.c. of solution			
	5 mg.	50 mg.	95 mg.	
525	17.8	161.0	297.8	0
630	17.6	160.8	299.2	0.3
840	19.2	157.2	297.2	0.9
1050	18.5	155.8	291.3	3.0
1260	17.5	151.8	287.4	3.5

TABLE X (Continued)

REDUCTION OF DEXTROSE IN THE PRESENCE OF SUCROSE

Weight of Copper in Fehling sol. Milligrams	Reduction in terms of mgs. of Cu		
	5 mgs. Dextrose	50 mgs. Dextrose	95 mgs. Dextrose
	95 " Sucrose	50 " Sucrose	5 " Sucrose
525	17.8	161.0	297.6
630	20.2	161.0	299.4
840	21.1	159.0	296.8

Upon examination of Tables IX and X, the most suitable ratio is from 5 to 6 of sodium hydroxide to 1 of copper by weight. In other words, with the alkalinity of 1.6 (3.2 grams of NaOH per 50 c.c. of mixed reagent), 0.525 to 0.630 gram of copper gives the most satisfactory results for reduction. It is also interesting to note that with increasing concentration of copper sulphate, total reduction is decreased, while auto-reduction of the solution is increased. Furthermore, Table X shows that in the presence of sucrose, increasing the concentration of copper sulphate tends to increase reduction. It seems therefore that concentration of copper has a direct bearing on the decomposition of sucrose. If enough reducing sugar is present to precipitate nearly all of the copper in Fehling solution, the presence of sucrose plays no part in the reduction.

4. THE EFFECT OF VARYING THE AMOUNTS OF SODIUM POTASSIUM TARTRATE IN FEHLING SOLUTION WITH CONSTANT AMOUNTS OF ALKALI AND COPPER SULPHATE

The solution consisted of 25 c.c. of copper sulphate solution containing 525 milligrams of copper (as cupric sulphate), 3.2 grams of sodium hydroxide, 100 milligrams of the sugar and varying amounts of the tartrate. The total volume of each solution was made up to 100 c.c. with distilled water.

A saturated solution of sodium potassium tartrate was prepared from Merck's Rochelle Salt (Highest Purity), and filtered. To the clear solution, 95% alcohol was added with constant stirring, and the salt was allowed to crystallize. The crystals were filtered, washed with alcohol, and dried in an oven at 50°C. for 15 hours, and then at 60°-65°C. for 10 hours. The salt as analyzed contained 4 molecules of water of crystallization ($\text{NaKC}_4\text{H}_4\text{O}_6 \cdot 4 \text{H}_2\text{O}$).

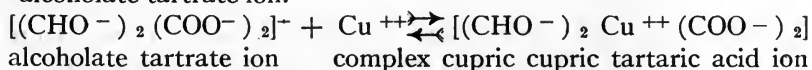
TABLE XI

Influence of Concentration of Rochelle Salt

Weight of Rochelle Salt in grams ($\text{NaKC}_4\text{H}_4\text{O}_6 \cdot 4 \text{H}_2\text{O}$)	Dextrose	Milligrams of Copper Reduced			Maltose
		Levulose	Lactose		
7.96	159.0	155.4	112.3	90.3	
8.65	159.5	154.2	112.9	92.5	
11.38	158.8	155.3	113.1	93.0	
15.84	157.7	154.0	112.5	92.6	
17.30	155.3	155.0	112.6	92.8	

Table XI shows that there is no material effect in increasing the concentration of the tartrate beyond that called for by Soxhlet's Fehling solution formula. The preventive action of the tartrate upon the precipitation of the cupric hydroxide can be safely guarded by the presence of 8.65 grams of the tartrate in 50 c.c. of mixed Fehling solution. Less than this amount appears to give less reduction for lactose and maltose. With levulose, there appears to be no effect, but the reduction by dextrose decreases with increasing concentration of the tartrate.

A theory of the rôle of Rochelle salt in Fehling solution has been suggested by Küster¹ who found that when cupric sulphate is added to the alkaline tartrate solution, a slightly *ionizable complex cupric tartrate ion* is formed by the union of the cupric ion with the "alcoholate tartrate ion."



The greater portion of the copper is present as part of the *complex negative ion of cupric tartaric acid and its salt*.

5. PHOTOSENSITIVENESS AND KEEPING QUALITY OF FEHLING SOLUTION

Byk² showed that Fehling solution is reduced by light of the wave length, of approximately 400 $\mu\mu$ and is not sensitive to light corresponding to absorption band in the red and yellow.

The photosensitiveness of Fehling solution to light and the consequent precipitation of cuprous oxide was noticed by Fehling³ himself. Eder⁴ made the first careful experiments of the sensitiveness of Fehling's reagent by preparing a Fehling solution which was easily decomposed at ordinary conditions. To prevent decomposition of his solution, he used different ray filter solutions in order to cut off certain portions of the solar spectrum.

Leighton⁵ found that Fehling solution absorbed the red and yellow ends of the spectrum and let the violet and blue through. His experiments showed that the photochemical change was due to ultra violet rays, and the formation of cuprous oxide must be pre-

¹ Z. Elektrochem., 4, 117 (1897).

² Z. physik. Chem., 40, 659, 679 (1904).

³ Ann., 72, 108 (1849).

⁴ Sitzungsber. Akad. Wiss. Wien, 92, 344 (1885), through J. Phys. Chem. 17, 204 (1913).

⁵ J. Phys. Chem., 17, 204 (1913).

vented by using ray filter solutions that will cut off the ultra violet rays.

The author's experiments show that 50 c.c. of mixed Fehling solution exposed to direct sunlight will give traces of cuprous oxide precipitate within ten to twenty hours, while if kept in the dark, or in opaque bottles, or in ray filter solutions such as potassium dichromate, or acidified quinine sulphate, decomposition does not take place until a month or longer. 50 c.c. of mixed Fehling solution exposed to direct sunlight will be completely decolorised after two months, showing only a slight amount of copper present.

Fehling solution as ordinarily prepared shows a slight tendency to auto-reduction.

Munson and Walker contended that unless the spontaneous reducing power of the alkaline tartrate solution is determined and allowed for, the results obtained will be considerably too high. In their original article¹ Munson and Walker gave a series of figures showing the reducing power of their alkaline tartrate solution from day to day during the course of their investigation. Their results varied from - 0.8 to + 1.6 milligrams of cuprous oxide. Apparently the reducing power of the alkaline tartrate solution varies with different grades of Rochelle salt, and it is necessary to make some corrections for all samples of Rochelle salt used, unless they are purified as described on page 23. A commercial "Highest Purity" grade of Rochelle salt proved entirely satisfactory.

Our experiments with Fehling solutions of varying copper, tartrate and alkali content show conclusively that the reducing power of the solutions is not entirely due to impurities of the alkaline tartrate solution as generally claimed, but to a great extent to the method of heating. Samples of Fehling solution prepared according to the formula given in page 47, showed no reducing power, *i.e.*, no "blank" after they have been kept for five months. But, if reduction was carried out according to Munson and Walker's method, the solutions gave considerable precipitate, which varied from 1.5 to 4.0 milligrams of copper.

6. COLLOIDAL CUPROUS OXIDE

When a reducing sugar is treated with an alkali, a series of degradation products is formed, and these are responsible for the

¹ J. Am. Chem. Soc. 28, 663 (1906); 29, 541 (1907).

reduction of the cupric salt. The cuprous oxide precipitate that comes down first in the hydrate form has different degrees of dispersion. The different colors of the same chemical compound, Cu_2O when in the colloidal state, are claimed by Svedberg¹ and Wo. Ostwald² to be nothing more than the color changes coincident with the gradual increase of the size of the particles, the red being the largest.

Zsigmondy³ prepared colloidal cuprous oxide from glycerin, copper sulphate and sugar which showed the Tyndall phenomenon.

Experiments performed in this laboratory show that the concentration of the reducing substance, nature of the alkali, and the degree of alkalinity of the latter, temperature and time of heating influence the production and physical character of cuprous oxide. The colloidal particles are produced even at six normal alkalinity with the carbonates, while with the alkali hydroxides, the same dispersions are noticeable only at concentrations below 0.5 normal. Reduction at high temperatures augments the production of the colloidal copper oxide. Low temperature and a fairly high concentration of alkali in Fehling solution increases the size characteristic to the red cuprous oxide precipitate.

7. PREPARATION OF PROPOSED MODIFICATION OF FEHLING'S SOLUTION BASED ON RESULTS OF EXPERIMENTS CITED ABOVE

1. *Copper Sulphate Solution.* The crystals of C. P. copper sulphate were washed free from dust, etc., with distilled water and dissolved in hot water to make a saturated solution and filtered. The copper was determined electrolytically and the solution diluted so that 25 c.c. of it contained 525 milligrams of copper or 41.2 grams of $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ in 500 c.c. of the solution.

2. *Alkaline Tartrate solution.*

Sodium hydroxide. A saturated solution was prepared from sodium hydroxide (purified by alcohol), and let stand for several days until the carbonates and other impurities had settled out. The clear solution was siphoned off and its alkalinity established by titration with standard acid.

¹ The Svedberg: Herstellung Kolloider Losungen.

² Kolloid—Chem. Beihefte 2, 409 (1911).

³ Zeit. physiol. Chem., 101, 133, (1918).

Zeit. anal. Chem., 5, 193 (1919).

173 grams of crystallized Rochelle salt ($\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$) (Highest Purity) were dissolved in water in a 500 c.c. graduated flask and the calculated amount of sodium hydroxide solution added so that 500 c.c. of this alkaline tartrate solution contained exactly 65 grams of *NaOH*.

It is absolutely necessary that sodium hydroxide for the alkaline tartrate solution should be prepared as described above because purity of the reagent and exact concentration of the alkali in Fehling solution has such a decided influence on the oxidizing power of the reagent. Weighing of sodium hydroxide sticks is not recommended. Advantages claimed for this formula and method of preparation of Fehling solution:

1. It gives no "blank" reduction.
2. Sucrose shows no reducing action up to 425 mg. of sucrose present.
3. Has greater range of reducible copper.
4. Colloidal cuprous oxide is not formed.
5. Gives a bright granular red precipitate of cuprous oxide easily removed from beaker with water alone.

V. TECHNIQUE OF PROPOSED METHOD

25 c.c. each of the cupric sulphate and alkaline tartrate solution are placed in a 400 c.c. Bohemian or Pyrex beaker. The solution containing the reducing sugar is then added plus sufficient distilled water to make a total volume of 100 c.c. The beaker, covered with a watch glass, is submerged in the water bath regulated at 80°C. At the expiration of 30 minutes the cuprous oxide is immediately filtered through a mat of asbestos in a Gooch crucible, using suction. The precipitate is washed with hot water, then with small amounts of alcohol and ether, and dried in an oven at 100°C. for 30 minutes.

The copper may be determined by weighing the cuprous oxide, or more accurately by Beans and Stillman's Electrolytic Method. E. C. Kendall's Modified Iodide Method also has certain advantages. Full discussion and comparison of these methods follows in Section VI.

The amounts of Dextrose, Levulose, Invert Sugar, Lactose and Maltose corresponding to different weights of copper and cuprous oxide are given in Table XXVII.

VI. COMPARISON OF METHODS FOR THE DETERMINATION OF COPPER

During the progress of this investigation, three different methods for the determination of copper have been used: Direct Weighing of Cuprous Oxide, E. C. Kendall's Modified Iodide Method,¹ and H. L. Beans and J. W. Stillman's Electrolytic Method.²

The copper in Tables III, IV, VII, VIII, and IX was determined by E. C. Kendall's Iodide Method, and in the other Tables by Beans and Stillman's Electrolytic Method.

Direct weighing of cuprous oxide method is the simplest of the gravimetric methods for the determination of copper but it is liable to be less accurate when solutions other than of pure sugars are used due to contamination of the precipitate with mineral and organic impurities.

The extent of error in estimating copper from the weight of cuprous oxide is shown by the comparative analyses made by Sherwood and Wiley³ upon a variety of sugar-containing products.

In consideration of the fact that Stillman has demonstrated his electrolytic method to be of a very high degree of accuracy, it will be seen (Table XII) that the method of direct weighing of cuprous oxide from analyses of even highly purified sugar solutions is subject to slight error. In the nine comparative tests cited which are typical of 50 tests made, differences ranged from -0.8 to $+1.1$ mg. of Cu. High results may be ascribed to oxidation while low results are traceable to the loss of asbestos which is mechanically carried through the Gooch or dissolved by the hot Fehling solution. When using the direct weighing procedure, the Gooch crucible should be treated with hot Fehling solution in order to determine the correction to be applied to subsequent tests.

Kendall's Modified Iodide Method is convenient and rapid, but has the disadvantage of poor end-point and it will be seen (Table XIII) that in the nine cases given which are typical of 43 tests made, it gave consistently low results in comparison with the direct weighing method.

When determining the reducing power of pure sugars dissolved in distilled water, the methods for determination of copper reduced

¹ J. Am. Chem. Soc., 33, 1947 (1911).

² Stillman: New Direct Method for the Electrolytic Determination of Copper, Dissertation, Columbia University (1920).

³ U. S. Bur. of Chem. Bull. 105, 120 (1907).

are in order of their degree of accuracy, (1) Electrolytic, (2) Direct weighing of cuprous oxide and (3) E. C. Kendall's Iodide Method. Where the solutions contain substances other than pure sugar, the methods are in order of preference for accuracy, (1) Electrolytic, (2) E. C. Kendall's Iodide, and (3) Direct weighing.

The E. C. Kendall Iodide Method is sufficiently accurate for commercial analyses.

An abstract of Beans and Stillman's Electrolytic Method is appended below. The original dissertation should be consulted for full description of the apparatus and discussion of the method.

Beans and Stillman's Technique for the Electrolytic Determination of Copper.

The cuprous oxide is dissolved with 10 to 20 c.c. of 16 M. nitric acid, and the solution diluted to about 200 c.c. with distilled water. Ten c.c. of a 3% hydrogen peroxide are added. The solution is then electrolyzed at room temperature (18–25°C.) with a current of 1 ampere. The E.M.F. will usually be from 2.5 to 3.5 volts. A 50 mesh platinum gauze cathode and a platinum wire spiral anode serve as electrodes. A mechanical stirrer is used so that there will be rapid agitation of the electrolyte. During the electrolysis a 3% solution of hydrogen peroxide is constantly dropped into the electrolyte. When the solution has become colorless, the sides of the beaker and the cover glasses are washed with distilled water, thus raising the level of the electrolyte and exposing a new surface of the platinum cathode. The process is continued until this new surface shows no deposit of copper.

When the electrolysis is complete, the cathode is quickly lifted from the solution without interrupting the current and placed in a beaker containing distilled water.

The electrode with its deposit of copper is washed with 95% alcohol, dried in an oven at 100°C. for a minute or two, then cooled in a desiccator, and weighed.

TABLE XII

Comparison Between Beans and Stillman's Electrolytic Method and Direct Weighing of Cuprous Oxide

Sample No.	Direct Weighing of Cu_2O		Electrolytic Wt. of Cu mg	Abs. Diff. in weight mg
	Weighed Cu_2O	Cu calc. from Cu_2O		
	mg	mg		
15	457.2	406.1	405.2	+0.9
18	418.3	371.5	371.7	-0.2
24	300.6	267.0	267.8	-0.8
25	258.2	229.3	229.5	-0.2
26	218.6	194.2	194.2	0.0
27	173.8	154.4	154.8	-0.4
30	89.6	79.6	79.8	-0.2
31	44.7	39.7	38.6	+1.1
32	22.6	20.1	19.7	+0.4

TABLE XIII

Comparison Between Kendall's Iodide Method and Direct Weighing of Cuprous Oxide

Sample No.	Direct Weighing of Cu_2O		Kendall's Wt. of Cu mg	Abs. Diff. in weight mg
	Weighed Cu_2O	Cu calc. from Cu_2O		
	mg	mg		
1	92.6	82.3	81.3	+1.0
2	93.2	82.8	82.2	+0.6
3	95.3	84.7	84.1	+0.6
4	98.3	87.3	86.3	+1.0
5	94.2	83.7	82.3	+1.4
6	94.4	83.9	82.6	+1.3
7	85.9	76.3	75.2	+1.1
8	98.3	87.3	86.3	+1.0
9	95.8	85.1	84.1	+1.0

VII. ACCURACY OF PROPOSED NEW METHOD FOR DETERMINATION OF REDUCING SUGARS

Under the conditions defined in V. and VI. for reduction, a series of experiments with dextrose and lactose were performed to study the degree of accuracy of the method.

TABLE XIV

Reduction of Fehling solution by Reducing Sugars

Wt. of Sugar in Milligrams	Reduction in mgs. of copper				
	(1)	(2)	(3)	(4)	(5)
Dextrose					
20	42.2	42.0	42.6	42.3	42.3
50	102.3	102.4	102.4	102.9	102.6
100	201.2	201.2	201.0	201.2	201.1
150	295.2	...	295.1	295.4	...
200	385.1	385.6	385.0	385.4	384.8
250	472.7	...	473.2	472.6	472.7
Lactose	(1)	(2)	(3)		
10	10.7	11.3	10.6		
50	61.2	61.3	61.6		
100	122.5	122.1	123.0		
150	184.0	184.2	184.4		
200	245.7	246.6	245.9		

From the above Table, the mean error of the mean computed from the least square equation, $m = \pm \sqrt{\frac{\sum d^2}{n(n-1)}}$ was calculated, where,

- m = mean error of the mean
- d = deviation from the mean
- n = number of determinations

The mean error of the mean for dextrose and lactose under these conditions appears to be ± 0.08 and ± 0.17 milligram of copper respectively. The percentage error is approximately $\pm 0.12\%$. Besides the "mean error," of the mean there may be constant errors which arise either during the preparation of the solution and weighing of samples, etc. These results show that the method is highly accurate compared with the current methods of sugar analysis, and the slight differences may be called negligible.

TABLE XV
The Effect of Temperature Deviations

Wt. of Sugar In Milligrams	Temperature				
	78°C	79°C	80°C	81°C	82°C
Dextrose					
50	102.1	102.0	102.5	102.3	103.7
100	201.5	201.9	202.2	202.7	203.4
200	385.7	385.9	386.2	386.7	387.2
Lactose					
100	120.7	121.6	122.2	123.5	124.5
200	244.3	245.0	245.6	246.6	247.2
300	366.8	367.3	368.2	368.7	370.2

The mean error of the mean as calculated from these figures appears to be ± 0.23 and ± 0.44 mg. of Cu for dextrose and lactose respectively for every degree of temperature change around 80°C., and ± 0.50 and ± 0.94 milligram of copper for dextrose and lactose for every two degrees. This error is approximately equal to $\pm 0.3\%$ for $\pm 1^\circ$ and $\pm 0.65\%$ for $\pm 2^\circ$ around 80°C. The greater difference in reducing value for lactose may be ascribed to its greater susceptibility to decomposition by Fehling solution. Experiments show that the temperature can be regulated with a set of burners, and results indicate that the error introduced by fluctuations in temperature of $\pm 1^\circ\text{C}$. does not materially affect the degree of accuracy required for most purposes. For high accuracy an automatically temperature controlled bath should be used.

VIII. REDUCING ACTION OF SUCROSE ON FEHLING SOLUTION AND REDUCING SUGARS

Preliminary study of the effect of the presence of sucrose has been made by Maquenne,¹ Pellet,² Saillard,³ Browne⁴ and others, but no definite efforts have been made to eliminate its reducing action on Fehling solution.

Experiments show that when the current methods of heating are used, the error in the glucose and invert sugar determinations,

¹ Comptes rendus, 162, 207-13, 145-9 (1916); 161, 617-23 (1915).

² Ann. Chem. Anal., 20, 169-75 (1915).

Bull. Assoc. Chem. sucr. dist., 31, 183-205.

³ Comptes rendus, 161, 591 (1915).

J. Fabr. sucre, 56, 1 (1915).

⁴ J. Am. Chem. Soc., 28, 439-453 (1906).

when sucrose is present, is considerable. Browne¹ gives an empirical formula for the correction of errors due to reducing action of sucrose on glucose. Meissl and Hiller² give tables of factors for calculating copper to invert sugar for different ratios of sucrose to invert sugar. Munson and Walker³ give tables for the determination of invert sugar and lactose in the presence of sucrose.

The inadequacy of the use of these tables, factors for correction, empirical formula and other unnecessary calculations involved, led to the following study of the comparative action of sucrose on Fehling solution and reducing sugars with the view to establishing the best conditions by which the reducing action of sucrose could be entirely eliminated or reduced to a minimum.

TABLE XVI

*Reducing Action of Sucrose on Fehling
Solution*

Wt. of Sucrose in Milligrams	Methods of heating		
	2 min. boiling over a flame mg. Cu	12 min. boiling in boiling water mg. Cu	30 min. heating in water at 80°C. mg. Cu
0	1.2	0.9	0
50	0.9	0.6	0
100	1.5	1.0	0
200	2.2	2.5	0
250	3.6	7.5	0
400	4.2	8.2	0
425	5.0	9.4	0.5
450	6.7	10.2	0.7
475	7.5	11.5	1.7
500	8.6	12.3	2.1
600	12.1	17.5	3.0
800	15.0	22.4	3.4
1000	19.5	27.5	6.2
1500	25.9	35.2	8.6
2000	37.0	39.2	9.3

¹ Browne, Handbook of Sugar Anal., p. 428 (1912).

² Z. Ver. Deut. Zuckerind., 735 (1889).

³ J. Am. Chem. Soc., 28, 663 (1906).

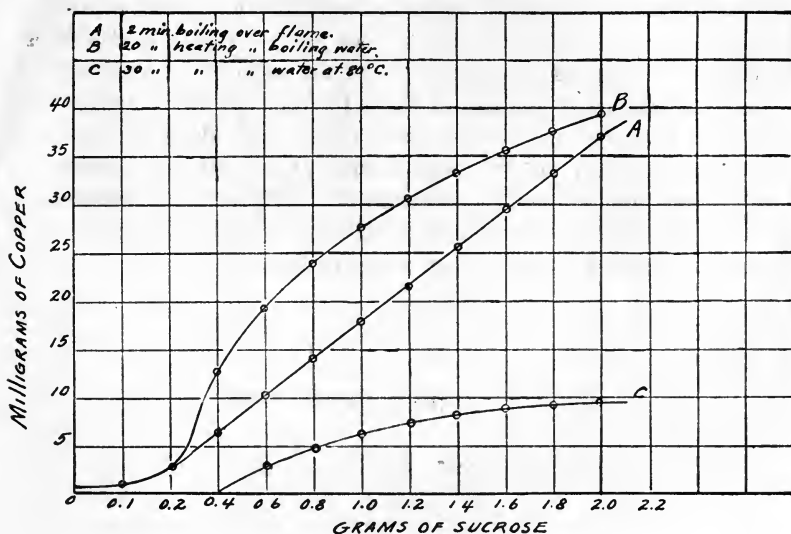
REDUCTION BY SUCROSE UNDER DIFFERENT
METHODS OF HEATING

Fig. 6

The reducing values above were corrected for "blank" reductions.

Table XVI shows that sucrose reduces Fehling solution, reduction increasing with greater concentration of the sugar. It is evident that the reducing action of sucrose is considerable if the current methods are used. Reduction of Fehling solution is shown even at low concentrations of sucrose. Under the newly standardized method of heating, the reducing action of sucrose is eliminated if less than 425 milligrams are present. It begins to show slight reduction at 425 milligrams, and increases gradually with increasing concentration.

The reducing action of sucrose was further investigated by running reductions with different mixtures of sucrose and reducing sugars. The following tables will show the effect of sucrose.

TABLE XVII
Reduction by Sucrose in the Presence of Dextrose

Dextrose in Milli- grams	Sucrose in Milligrams								
	100	200	400	600	800	1000	1500	2000	
0	0	0	0	3.0	3.4	6.2	8.7	9.3mg. Cu	
20	42.2	42.6	42.3	42.4	45.5	45.8	48.1	53.2	56.3
50	102.3	103.2	102.8	102.5	106.0	107.3	107.4	113.1	114.3
100	201.2	201.1	201.2	201.1	204.9	205.5	206.5	211.7	212.2
150	295.2	294.4	295.0	295.4	298.5	299.6	299.5	304.4	306.3
200	385.1	384.9	385.2	385.7	388.2	389.6	389.0	393.5	395.7
250	472.7	473.3	472.5	472.7	475.7	476.4	476.3	477.0	482.9

TABLE XVIII
Influence of Sucrose Upon Reduction by Dextrose

Sucrose Taken mg.	Dextrose Taken mg.	Dextrose Found (Total reduction calc. as dextrose)	Error mg. Cu
		mg. Cu	
100	20	20.6	+0.6
100	50	50.2	+0.2
100	100	100.1	+0.1
100	150	149.5	-0.5
100	200	199.2	-0.8
100	250	250.0	0.0
200	20	20.2	+0.2
200	50	50.0	0.0
200	100	100.0	0.0
200	150	149.9	-0.1
200	200	195.5	-0.5
200	250	249.5	-0.5
400	20	20.4	+0.4
400	50	49.9	-0.1
400	100	100.1	+0.1
400	150	150.0	0.0
400	200	199.8	-0.2
400	250	249.7	-0.3
600	20	21.9	+1.9
600	50	51.5	+1.5
600	100	101.8	+1.8
600	150	151.5	+1.5
600	200	201.2	+1.2
600	250	251.2	+1.2

Sucrose Taken mg.	Lactose Taken mg.	Dextrose Found	Error mg. Cu
		(Total reduction calc. as dextrose) mg. Cu	
800	20	22.0	+2.0
800	50	52.2	+2.2
800	100	102.2	+2.2
800	150	152.3	+2.3
800	200	202.0	+2.0
800	250	251.6	+1.6
1000	20	23.2	+3.2
1000	50	52.2	+2.2
1000	100	102.2	+2.2
1000	150	152.3	+2.3
1000	200	201.7	+1.7
1000	250	251.4	+1.4
1500	20	25.6	+5.6
1500	50	55.0	+5.0
1500	100	105.0	+5.0
1500	150	154.8	+4.8
1500	200	204.2	+4.2
1500	250	252.5	+2.5
2000	20	27.2	+7.2
2000	50	55.7	+5.7
2000	100	105.6	+5.6
2000	150	155.8	+5.8
2000	200	205.4	+5.4
2000	250	255.5	+5.5

TABLE XIX

Reduction by Sucrose in the Presence of Levulose

Levulose in Milligrams	Sucrose in Milligrams						
	0	200	300	400	500	1000	2000
0	0	0	0	0	2.1	6.2	9.3 mg. Cu
10	19.0	19.5	20.2	21.3	22.3	28.3	32.8
40	75.5	76.2	78.1	79.2	80.1	85.0	88.0
80	150.5	150.5	151.7	153.0	154.7	157.1	161.7
120	220.2	220.5	221.8	223.5	225.3	228.1	232.5
160	291.8	291.8	292.0	293.3	295.6	298.6	303.3
200	360.6	359.6	360.8	361.1	363.4	365.0	371.5
240	428.1	427.1	428.2	428.9	430.1	432.4	439.1

TABLE XX

Influence of Sucrose upon Reduction by Levulose

Sucrose Taken mg.	Levulose Taken mg.	Levulose Found (Total reduction calc. as levulose) mg. Cu	Error mg. Cu
200	10	10.5	+0.5
200	40	40.5	+0.5
200	80	80.7	+0.7
200	120	119.8	-0.2
200	160	160.2	+0.2
200	200	199.5	-0.5
200	240	239.1	-0.9
300	10	10.7	+0.7
300	40	41.4	+1.4
300	80	81.2	+1.2
300	120	120.5	+0.5
300	160	160.4	+0.4
300	200	200.2	+0.2
300	240	239.8	-0.2
400	10	11.2	+1.2
400	40	42.0	+2.0
400	80	82.2	+2.2
400	120	121.5	+1.5
400	160	161.1	+1.1
400	200	200.2	+0.2
400	240	240.0	0.0
500	10	11.8	+1.8
500	40	42.5	+2.5
500	80	83.2	+3.2
500	120	122.5	+2.5
500	160	162.5	+2.5
500	200	201.7	+1.7
500	240	241.0	+1.0
1000	10	15.0	+5.0
1000	40	45.1	+5.1
1000	80	84.4	+4.4
1000	120	124.1	+4.1
1000	160	164.0	+4.0
1000	200	202.7	+2.7
1000	240	242.3	+2.3

Sucrose Taken mg.	Levulose Taken mg.	Levulose Found	Error mg.
		(Total reduction calc. as levulose) mg.	
2000	10	16.6	+6.6
2000	40	46.8	+6.8
2000	80	87.0	+7.0
2000	120	126.5	+6.5
2000	160	166.7	+6.7
2000	200	206.5	+6.5
2000	240	246.1	+6.1

TABLE XXI

Reduction by Sucrose in the Presence of Invert Sugar

Invert Sugar in Milligrams	Sucrose in Milligrams						
	0	200	300	400	500	1000	2000
0	0	0	0	0	2.1	6.2	9.3
10	20.0	21.0	21.7	22.5	23.1	31.4	39.2
20	39.1	40.5	41.0	41.4	42.3	50.2	58.2
40	79.5	79.1	79.2	78.8	80.5	87.8	95.9
80	154.6	155.0	155.1	155.2	156.6	162.1	170.9
120	229.3	230.0	230.4	229.8	231.0	235.9	245.4
160	301.5	301.4	301.9	302.6	303.6	307.1	318.1
200	373.0	373.2	373.0	373.0	373.1	376.9	386.0
240	441.5	441.5	441.7	441.5	441.2	445.5	452.7

TABLE XXII

Influence of Sucrose upon Reduction by Invert Sugar

Sucrose Taken mg.	Invert Sugar Taken mg.	Invert Sugar Found	Error mg.
		(Total reduction calc. as invert sugar) mg.	
200	10	10.6	+0.6
200	20	20.5	+0.5
200	40	40.4	+0.4
200	80	80.0	0.0
200	120	120.3	+0.3
200	160	160.2	+0.2
200	200	200.4	+0.4
200	240	240.0	0.0
300	10	11.0	+1.0
300	20	20.8	+0.8
300	40	40.5	+0.5
300	80	80.2	+0.2
300	120	120.5	+0.5
300	160	160.4	+0.4
300	200	200.4	+0.4
300	240	240.1	+0.1

Sucrose Taken mg.	Invert Sugar Taken mg.	Invert Sugar Found	Error mg.
		(Total reduction calc. as invert sugar) mg.	
400	10	11.4	+1.4
400	20	21.1	+1.1
400	40	40.2	+0.2
400	80	80.1	+0.1
400	120	120.4	+0.4
400	160	160.2	+0.2
400	200	200.4	+0.4
400	240	240.0	0.0
500	10	11.6	+1.6
500	20	21.5	+1.5
500	40	41.2	+1.2
500	80	81.0	+1.0
500	120	121.1	+1.1
500	160	161.2	+1.2
500	200	200.5	+0.5
500	240	239.9	-0.1
1000	10	16.0	+6.0
1000	20	25.4	+5.4
1000	40	45.0	+5.0
1000	80	84.0	+4.0
1000	120	123.9	+3.9
1000	160	163.2	+3.2
1000	200	202.9	+2.9
1000	240	202.2	+2.2
2000	10	19.2	+9.2
2000	20	29.4	+9.4
2000	40	49.2	+9.2
2000	80	88.9	+8.9
2000	120	128.9	+8.9
2000	160	169.3	+9.3
2000	200	208.0	+8.0
2000	240	246.4	+6.4

TABLE XXIII

Reduction by Sucrose in the Presence of Lactose

Lactose in milligrams	Sucrose in Milligrams				
	0	200	500	1000	2000
0	0	0	2.1	6.2	9.3 mg. Cu
10	12.2	13.0	13.7	19.4	28.5
50	61.5	62.9	64.9	70.5	80.1

Lactose in milligrams	Sucrose in Milligrams				
	0	200	500	1000	2000
100	123.0	124.5	126.4	132.3	141.6 mg. Cu
150	184.5	186.0	188.0	194.2	203.4
200	246.0	247.6	249.2	255.9	264.4
250	307.1	307.7	310.5	315.4	324.8
300	368.0	368.3	371.2	376.3	384.5
350	429.5	429.8	432.7	436.6	445.7

TABLE XXIV
Influence of Sucrose upon Reduction by Lactose

Sucrose Taken mg.	Lactose Taken mg.	Lactose Found (Total reduction calc. as lactose)	Error mg.
		mg.	
200	10	10.4	+0.4
200	50	51.0	+1.0
200	100	101.2	+1.2
200	150	151.2	+1.2
200	200	201.1	+1.1
200	250	250.3	+0.3
200	300	300.0	0.0
200	350	349.8	-0.2
500	10	11.0	+1.0
500	50	52.8	+2.8
500	100	102.8	+2.8
500	150	152.9	+2.9
500	200	202.3	+2.3
500	250	252.6	+2.6
500	300	302.1	+2.1
500	350	352.1	+2.1
1000	10	15.6	+5.6
1000	50	57.2	+7.2
1000	100	108.0	+8.0
1000	150	158.1	+8.1
1000	200	208.1	+8.1
1000	250	256.7	+6.7
1000	300	306.2	+6.2
1000	350	355.2	+5.2
2000	10	23.0	+13.0
2000	50	65.0	+15.0
2000	100	115.0	+15.0
2000	150	165.4	+15.4
2000	200	215.4	+15.4
2000	250	264.6	+14.6
2000	300	314.0	+14.0
2000	350	363.8	+13.8

TABLE XXV

Reduction by Sucrose in the Presence of Maltose

Maltose in Milligrams	Sucrose in Milligrams					
	0	50	100	200	500	1000
0		0	0	0	2.1	6.2 mg. Cu
10	10.0	10.2	10.0	10.2	11.3	17.2
50	50.5	50.5	50.8	51.1	53.5	58.4
100	100.8	100.7	101.4	102.0	104.3	110.7
150	151.5	151.8	152.0	152.3	155.0	162.1
200	202.0	202.2	202.6	203.2	206.0	213.1
250	252.5	252.5	253.0	255.1	357.4	263.5
300	302.6	303.1	303.8	306.2	308.6	313.9
350	353.4	354.2	354.9	358.7	360.5	365.1

TABLE XXVI

Influence of Sucrose upon Reduction by Maltose

Sucrose Taken mg.	Maltose Taken mg.	Maltose Found (Total reduction calc. as Maltose)	Error mg. Cu
		mg. Cu.	
50	10	10.0	0.0
50	50	50.0	0.0
50	100	99.8	-0.2
50	150	150.2	+0.2
50	200	200.2	+0.2
50	250	250.1	+0.1
50	300	300.0	0.0
50	350	350.5	+0.5
100	10	9.8	-0.2
100	50	50.2	+0.2
100	100	100.4	+0.4
100	150	150.5	+0.5
100	200	200.6	+0.6
100	250	250.7	+0.7
100	300	300.8	+0.8
100	350	351.1	+1.1
200	10	10.0	+0.0
200	50	50.5	+0.5
200	100	101.0	+1.0
200	150	151.0	+1.0
200	200	201.2	+1.2
200	250	252.8	+2.8
200	300	303.0	+3.0
200	350	355.1	+5.1

Sucrose Taken mg.	Maltose Taken mg.	Maltose Found (Total reduction calc. as Maltose) mg. Cu	Error mg. Cu
500	10	10.8	+0.8
500	50	52.8	+2.8
500	100	103.2	+3.2
500	150	153.5	+3.5
500	200	204.0	+4.0
500	250	255.0	+5.0
500	300	305.5	+5.5
500	350	356.3	+6.3
1000	10	16.8	+6.8
1000	50	57.8	+7.8
1000	100	109.3	+9.3
1000	150	160.3	+10.3
1000	200	211.0	+11.0
1000	250	261.0	+11.0
1000	300	310.8	+10.8
1000	350	361.3	+11.3

Discussion of Results.

Tables XVII and XVIII show that the reducing action of sucrose in the presence of dextrose is negligible at concentrations below 400 milligrams. The greater the proportion of dextrose, the less the action of sucrose, that is, the reducing action of sucrose upon Fehling solution is proportional to concentration of sucrose and amount of copper left unreduced. If enough dextrose is present to precipitate nearly all of the copper in Fehling solution, reduction due to sucrose is slight.

Upon examination of the reducing values of sucrose and dextrose, it appears that for certain concentrations of the two sugars, total reduction is the sum of the two; while at certain concentrations, it is less. Past a certain point, the phenomenon reverses and instead of acting as a protector, dextrose accelerates the decomposition of sucrose. This increase in reduction of sucrose decreases however with increasing amounts of dextrose. The decrease in reduction at certain points indicates probably the formation of complex copper sodium sucates, the dissociation constants of which decrease in the presence of increasing concentrations of the sugar, and this diminishes the alkalinity and concentration of

ionizable copper of the reagent. It is obvious therefore, that reduction by sucrose is not entirely a matter of hydrolysis of its molecule, but is a direct function of the molecule itself.

Tables XIX and XX show that the action of sucrose on levulose is negligible at concentrations below 300 milligrams. Greater reducing power of sucrose appears to be inhibited by levulose at concentrations between 40 and 160 milligrams of levulose. Furthermore, they show that levulose has an accelerating action on the decomposition of sucrose, its action being greatest at concentrations between 40 and 160 milligrams. The error in the levulose determination is directly proportional to the amount of sucrose, and inversely proportional to the amount of levulose.

Tables XXI and XXII show that the error due to the reducing action of sucrose is negligible at concentrations below 400 milligrams. They also show that the protective action of invert sugar on sucrose is more apparent at 500 milligrams of sucrose. The accelerating action of invert sugar on sucrose is indicated at 1.0 and 2.0 grams of sucrose, showing decomposition of the latter. Total reduction is seen to be less with increasing amounts of reducing sugar.

Tables XXIII and XXIV show greater reducing power of sucrose in the presence of lactose. The partial decomposition of sucrose is due to some catalytic effect of lactose, or probably reduction may be due to the combined effect of the two disaccharides. Reduction attributed to sucrose decreases progressively with increasing amounts of lactose.

Tables XXV and XXVI show that maltose greatly influences the decomposition of sucrose. Reduction of sucrose increases considerably with increasing amounts of maltose. It is most probable that maltose acts catalytically on sucrose. This appears to be a clear case of hydrolysis of sucrose. The other sugars do not behave the same in the presence of sucrose as maltose does.

Summary. The presence of as much as 400 milligrams of sucrose does not affect the accuracy of determinations of dextrose and invert sugar by this new method. In case of mixtures of sucrose and levulose, 250 milligrams of sucrose show no reducing effect, while of sucrose and lactose, 200 milligrams show slight reduction, and of sucrose and maltose, 100 milligrams.

IX. SUGAR TABLE

Figures in sugar table were calculated from the equation

$$y = a + bx + cx^2, \text{ where } y = \text{mg. of sugar}$$

$$x = \text{ " " copper}$$

By method of least square (Mellor: Higher Math. p. 329)

$$b = \frac{\Sigma (x^4) \Sigma (xy) - \Sigma (x^3) \Sigma (x^2y)}{\Sigma (x^2) \Sigma (x^4) - [\Sigma (x^3)]^2}$$

$$c = \frac{\Sigma (x^2) \Sigma (x^2y) - \Sigma (x^3) \Sigma (xy)}{\Sigma (x^2) \Sigma (x^4) - [\Sigma (x^3)]^2}$$

$$\text{Dextrose. } y = 0.474x + 0.000115x^2$$

$$\text{Levulose. } y = 0.526x + 0.000078x^2$$

$$\text{Lactose. } y = 0.813x + 0.000003x^2$$

$$\text{Maltose. } y = 0.990x + 0.0000005x^2$$

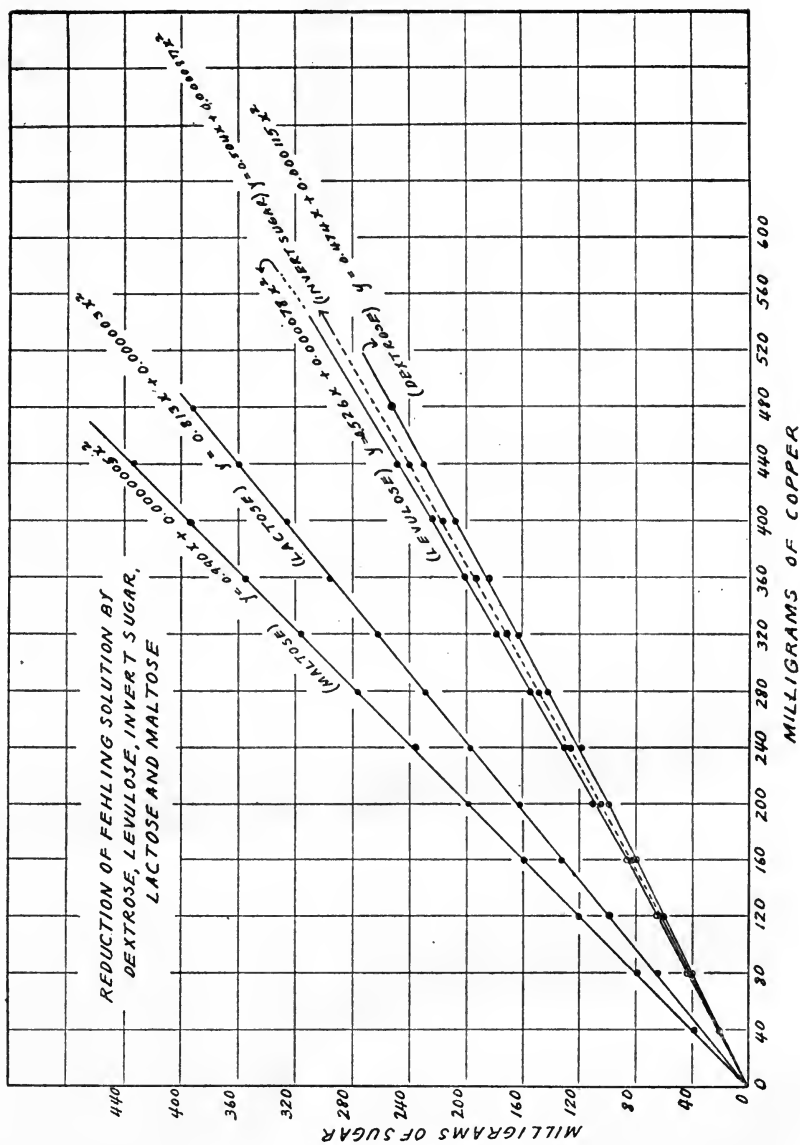
$$\text{Invert Sugar. } y = 0.504x + 0.0000870x^2$$

TABLE XXVII

Table for Calculating Dextrose, Levulose, Invert Sugar, Lactose, and Maltose

Copper (Cu)	Cuprous oxide (Cu ₂ O)	Dextrose (d-glu- cose)	Levulose (d-fruc- tose)	Invert Sugar	Lactose		Maltose	
					C ₁₂ H ₂₂ O ₁₁	C ₁₂ H ₂₂ O ₁₁ .H ₂ O	C ₁₂ H ₂₂ O ₁₁	C ₁₂ H ₂₂ O ₁₁ .H ₂ O
10	11.1	4.8	5.3	5.0	7.7	8.1	9.4	9.9
20	22.5	9.5	10.5	10.1	15.5	16.3	18.8	19.8
30	33.8	14.3	15.8	15.2	23.2	24.4	28.2	29.7
40	45.0	19.1	21.2	20.3	30.9	32.5	37.6	39.6
50	56.3	24.0	26.5	25.4	38.7	40.7	47.0	49.5
60	67.6	28.9	31.9	30.6	46.4	48.8	56.4	59.4
70	78.8	33.7	37.2	35.7	54.0	56.9	65.8	69.3
80	90.1	38.7	42.6	40.9	61.7	65.0	75.2	79.2
90	101.3	43.6	48.0	46.1	69.5	73.2	84.6	89.1
100	112.6	48.6	53.4	51.3	77.2	81.3	94.0	99.0
110	123.8	53.5	58.8	56.5	85.0	89.5	103.4	108.9
120	135.1	58.5	64.3	61.8	92.7	97.6	112.8	118.8
130	146.4	63.6	70.7	67.0	100.4	105.7	122.2	128.7
140	157.6	68.6	75.2	72.3	108.2	113.9	131.6	138.6
150	168.9	73.7	80.7	77.6	116.0	122.0	141.0	148.5
160	180.1	78.8	86.2	82.9	123.7	130.1	150.4	158.4
170	191.4	83.9	91.7	88.3	131.4	138.3	159.8	168.3
180	202.6	89.1	97.2	93.7	139.1	146.4	169.2	178.2
190	213.9	94.2	102.8	99.1	146.9	154.6	178.8	188.1
200	225.2	99.4	108.4	104.4	154.6	162.7	188.2	198.0
210	236.4	104.6	114.0	109.8	162.3	170.9	197.6	207.9
220	247.7	109.9	119.6	115.2	170.0	179.0	207.0	217.8
230	258.9	115.1	125.2	120.6	177.8	187.2	216.4	227.7
240	270.2	120.4	130.8	126.1	185.5	195.3	225.8	237.6
250	281.5	125.7	136.4	131.6	193.2	203.4	235.2	247.5
260	292.7	131.0	142.1	137.1	201.0	211.6	244.6	257.4
270	304.0	136.4	147.8	142.6	208.8	219.8	254.0	267.3
280	315.2	141.7	153.5	148.2	216.5	227.9	263.4	277.2
290	326.5	147.1	159.2	153.7	224.2	236.0	272.8	287.1
300	337.8	152.6	165.0	159.3	232.0	244.2	282.2	297.0
310	349.0	158.0	170.7	164.9	239.7	252.3	291.6	306.9
320	360.3	163.5	176.5	170.5	247.5	260.5	301.0	316.8
330	371.5	168.9	182.3	176.1	255.3	268.7	310.4	326.7
340	382.8	174.5	188.1	181.8	263.0	276.8	319.8	336.6
350	394.0	180.0	193.9	187.4	270.7	285.0	329.2	346.5
360	405.3	185.5	199.7	193.1	278.4	293.1	338.6	356.4
370	416.6	191.1	205.5	198.8	286.2	301.3	348.0	366.3
380	427.8	196.7	211.4	204.5	293.9	309.4	357.4	376.2
390	439.1	202.3	217.3	210.2	301.6	317.5	366.8	386.1
400	450.3	208.0	223.2	216.0	309.4	325.7	376.2	396.0
410	461.6	213.7	229.1	221.8	317.1	333.8	385.6	405.9
420	472.9	219.4	235.0	227.6	324.9	342.0	395.0	415.8
430	484.1	225.1	240.9	233.4	332.6	350.1	404.4	425.7
440	495.4	230.8	246.9	239.2	340.4	358.3	413.8	435.6
450	506.6	236.6	252.9	245.0	348.1	366.4	423.2	445.5
460	517.9	242.4	258.9	250.9	355.9	374.6	432.6	455.4
470	529.1	248.1	264.9	256.8	363.6	382.7	442.0	465.3
480	540.4	250.8	270.9	262.7	371.3	390.9	451.4	475.2

Figure 7



X. REDUCING RATIOS

TABLE XXVIII

Showing the Reducing Ratios of Levulose, Invert Sugar, Lactose
and Maltose under the new Method

Copper mg	Dextrose Levulose	Dextrose Invert Sugar	Dextrose Lactose	Dextrose Lactose hydrate	Dextrose Maltose	Dextrose Maltose hydrate
50	0.905	0.945	0.620	0.589	0.510	0.489
100	0.906	0.947	0.629	0.598	0.517	0.491
150	0.913	0.950	0.635	0.604	0.522	0.496
200	0.917	0.952	0.642	0.611	0.528	0.502
250	0.921	0.955	0.650	0.618	0.534	0.508
300	0.924	0.957	0.657	0.624	0.540	0.513
350	0.928	0.960	0.665	0.631	0.546	0.519
400	0.932	0.963	0.672	0.638	0.552	0.525
450	0.935	0.965	0.679	0.645	0.559	0.531
	0.920	0.955	0.651	0.618	0.534	0.508

In Table XXVIII, the ratios were calculated from sugar values corresponding to several weights of copper. (See Table XXVII).

The ratios for levulose and invert sugar are approximately equal to those obtained by Browne¹ using Allihn's method. Ratios for lactose and maltose are lower than those obtained by others.²

METHOD

Measure accurately 25 c.c. each of copper sulfate and alkaline tartrate solutions (prepared as described on page 26) into a 400 c.c. Pyrex or Bohemian glass beaker, the diameter of which is approximately 9 cm. Add 50 c.c. of sugar* solution making a total volume of 100 c.c. Cover the beaker containing the mixed solutions, with a watch glass and submerge it in a water bath which is maintained at 80°C. After exactly 30 minutes digestion filter the cuprous oxide precipitate by suction through a mat of asbestos in a Gooch crucible. The precipitate is washed in the usual manner with warm water and the copper determined by one of the methods

¹ Browne: Handbook of Sugar Analysis, p. 421 (1912).
J. Am. Chem. Soc., 28, 436 (1906).

² Browne: Handbook of Sugar Analysis, p. 421 (1912).
Sherman: Organic Analysis, p. 73 (1918).

discussed on page 28. From the weight of copper or cuprous oxide obtained, find the corresponding weight of reducing sugar from Table XXVII on page 45 or calculate it by means of the equations on page 44.

**Weights of the sugars to be taken for analysis:*

For dextrose, levulose and invert sugar, take 50 to 150 milligrams; for lactose and maltose, take 100 to 300 milligrams. These weights of the sugars will reduce about 100 to 350 milligrams of copper.

In the presence of sucrose, the weight of sugar for analysis can be judged by a preliminary test of the sample, but in any case if not more than 100 milligrams of sample be used, there will be no error due to sucrose.

XI. SUMMARY

1. From this investigation, it is concluded that the current methods for the determination of reducing sugars are not satisfactory, because conditions for temperature control have not been well established.

2. The amount of reduction of Fehling solution by dextrose has been measured at temperatures from 60° to 110°C., and time of heating from 10 minutes to 2 hours and conditions at 80°C. for 30 minutes have been found best for reduction. The degree of accuracy in determination by this method is approximately $\pm 0.12\%$ when the temperature is maintained at 80°C. $\pm 0.1^\circ$. If the heating power is regulated by a set of burners, the error introduced by fluctuation in temperature is approximately $\pm 0.3\%$ for $\pm 1^\circ$, and $\pm 0.65\%$ for $\pm 2^\circ$ around 80°C.

3. Auto-reduction of Fehling solution at different temperatures and lengths of time of heating has been measured and found that the error inherent to these factors can be eliminated.

4. Surface oxidation and reduction have been studied and found that the amount of cuprous oxide lost due to surface oxidation is proportional to the area of the liquid exposed to the air, and the error inherent thereto is obviated by the use of watch glass. Reduction is proportional to the lateral area of the glass; that is, the higher the column of liquid exposed to the glass surface, the more cuprous oxide is precipitated.

5. A thorough study of Fehling's reagent has been made and shown how the nature and amount of alkali in Fehling solution influence reduction and physical character of cuprous oxide formed. Sodium hydroxide in Fehling solution gives a more satisfactory precipitation than when potassium hydroxide or the carbonates are used. "1.6 normal Fehling solution" has been found best for the oxidation of the sugars. The concentrations of Cu^+ and OH^- ions influence the rate amount of reduction of sugars. The amount of copper reduced increases with increasing concentrations of Cu^+ and OH^- ions up to a certain point, where oxidation is at its maximum, and from that point, further increase in Cu^+ and OH^- ions tends to decrease reduction. Increasing the concentration of Rochelle salt beyond that called for by Soxhlet-Fehling solution formula shows no material effect. A modified Fehling solution has been prepared that gives no "blank" reduction.

6. A comparative study of the reducing action of sucrose on Fehling solution and reducing sugars has been made and found that the reducing action of sucrose can be eliminated. The presence of as much as 400 milligrams of sucrose does not affect the accuracy of determinations of dextrose and invert sugar. In case of mixtures of sucrose and levulose, slight reduction due to sucrose is shown by 300 milligrams; sucrose and lactose, 200 milligrams; and sucrose and maltose, 100 milligrams. The greater proportion of these sugars with the exception of maltose in Fehling solution, the less the reducing action of sucrose. Maltose has a marked catalytic effect on the reducing action of sucrose. It is concluded therefore, that reduction by sucrose is not entirely a matter of hydrolysis of its molecule, but is a direct function of the molecule itself.

7. Reduction by dextrose, levulose, invert sugar, lactose and maltose of different concentrations has been made and a sugar table constructed from the calculated equations,—

$$\text{(Dextrose)} y = 0.474 x + 0.000115 x^2$$

$$\text{(Levulose)} y = 0.526 x + 0.000078 x^2$$

$$\text{(Invert Sugar)} y = 0.504 x + 0.000087 x^2 \text{ where } y = \text{mg. of sugar}$$

$$\text{(Lactose)} y = 0.813 x + 0.000003 x^2 \quad x = \text{ " " copper}$$

$$\text{(Maltose)} y = 0.990 x + 0.0000005 x^2$$

VITA

Francisco A. Quisumbing was born in Sta. Cruz, Laguna, P. I., December 3, 1893. He prepared for college entrance in St. Juan de Letran College, Manila, and entered the College of Agriculture, University of the Philippines in June, 1909. He received the degree of B. Agr. in 1914. He was Assistant in Chemistry at the University of the Philippines from June, 1914 to July, 1918. He received the degree of M.S. in April, 1918, and was awarded a travelling fellowship by the University of the Philippines in May, 1918. He began his graduate study of Chemistry under the Faculty of Pure Science of Columbia University in September, 1918.







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