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CONDITIONS AFFECTING THE QUANTITATIVE DETERMINATION OF REDUCING SUGARS BY FEHLING SOLUTION.  
ELIMINATION OF CERTAIN ERRORS INVOLVED  
CURRENT IN METHODS.<sup>1</sup>

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The reduction of alkaline cupric solutions by certain sugars has been extensively utilized in qualitative and quantitative analyses of saccharine mixtures since 1841, when Trommer<sup>2</sup> first introduced alkaline copper sulfate as a reagent to distinguish dextrose from sucrose.

Barreswil<sup>3</sup> improved Trommer's reagent by the addition of potassium tartrate which prevented the precipitation of cupric hydroxide, thus increasing the stability of the reagent. Fehling<sup>4</sup> worked out the details of the quantitative method, giving some stoichiometrical equivalents between copper and dextrose. The ratio of 1 molecule of dextrose to 5 of copper was regarded constant by Fehling and was so employed until Soxhlet<sup>5</sup> showed that the ratio was not constant, but varied according to the amount of copper in solution.

The literature relative to modifications of methods of sugar analysis is so voluminous that a detailed discussion cannot be given here. Many of the modifications are described by Browne.<sup>6</sup>

Despite numerous attempts to improve upon the original methods, there is none which will give perfect quantitative results in the analysis of mixtures of common sugars. The errors involved are (1) reducing action of sucrose, (2) "blank" or auto-reduction of Fehling solution, including all modifications of same, and (3) lack of exact control of temperature.

A study of the reducing action of sucrose on Fehling's solution and the effect of the presence of sucrose upon reducing sugars with a view of eliminating the use of empirical formulas and correction tables as a means of correcting for the effect of sucrose is of timely interest.

If the necessity of running "blank" tests to determine the amount of auto-reduction of the Fehling solution (which may be as much as 5 mg.

<sup>1</sup> Part of the thesis submitted by F. A. Quisumbing in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science of Columbia University, 1921.

<sup>2</sup> Trommer, *Ann.*, 39, 360 (1841).

<sup>3</sup> Barreswil, *J. Pharm.*, [3] 6, 301 (1844).

<sup>4</sup> Fehling, *Ann.*, 72, 106 (1849); 106, 75 (1858).

<sup>5</sup> Soxhlet, *J. prakt. Chem.*, [2] 21, 227 (1878).

<sup>6</sup> Browne, "Handbook of Sugar Analysis," John Wiley & Sons, 1912.

of cuprous oxide and is not constant) could be dispensed with, it is admitted that greater accuracy and speed would be realized.

In all methods the reaction between reducing sugars and the complex cupric compound is carried out at the indefinite temperature obtained by heating in a bath of boiling water, or boiling the mixture directly over a flame. It is evident that since the amount of reduction varies with the temperature and time of digestion, errors are unavoidable due to changes in barometric pressure. Further, the methods involving direct boiling of the mixture are subject to an additional lack of accuracy due to the difficulty in determining exactly when the solution begins to boil. Apart from the inaccuracy of this sort of procedure, the manipulator is obliged to give his constant attention during the boiling.

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

### Sugars Used.

Solutions for measurement of optical rotation were made in all cases by dissolving 5 g. of the sugar in distilled water and adjusting to 100 cc. at 25°. The solutions were allowed to stand until optical equilibrium had been attained before polarization.

A Schmidt and Haensch polarimeter, sensitive to 0.01° angular scale was employed, the solutions in 2 dcm. tubes being maintained at 25° ± 0.01°, by use of the thermostat described by Nelson and Beegle.<sup>7</sup>

Unpurified sodium flame, and the light of a mercury vapor lamp passed through Wratten No. 74 filter, giving a wave length,  $\lambda = 546\mu\mu$ ,<sup>8</sup> were used as sources of light.

**Sucrose.**—Bureau of Standards, Sample No. 17. Analysis showed: moisture 0.003%; ash, 0.003%. No reducing action was shown by 400 mg. of this sugar on Fehling solution at 80° during 30 minutes. Specific rotatory power,  $[\alpha]_{\lambda=546}^{26} = 78.200^\circ$ ;  $[\alpha]_D^{26} = 66.405^\circ$ .

**Dextrose.**—A specimen of the Corn Products Refining Company's c. p. Dextrose, was purified according to the Bureau of Standards method.<sup>9</sup> Analysis of this pure sample showed: moisture, 0.150%; ash, 0.003%. Specific rotatory power,  $[\alpha]_{\lambda=546}^{26} = 62.021^\circ$ ;  $[\alpha]_D^{26} = 52.480^\circ$ .

**Levulose.**—A white crystalline specimen kindly furnished by Prof. J. M. Nelson was recrystallized from conc. acetic acid solution as recommended by Hudson and Dale<sup>10</sup> for dextrose. The sample after drying *in vacuo* over saturated sodium hydroxide solution showed: moisture, 0.002%; ash, 0.060%. Specific rotatory power,  $[\alpha]_{\lambda=546}^{26} = -105.30^\circ$ ;  $[\alpha]_D^{26} = -89.40^\circ$ .

<sup>7</sup> Nelson and Beegle, *THIS JOURNAL*, **41**, 559 (1919).

<sup>8</sup> W. C. Vosburgh, *ibid.*, **42**, 1698 (1920).

<sup>9</sup> Bur. Standards, *Circ.*, **44**, 92 (1919).

<sup>10</sup> Hudson and Dale, *THIS JOURNAL*, **39**, 320 (1917).

**Invert Sugar.**—The invertase method of preparation was tried and found unsatisfactory for our purpose because of the formation of an insoluble copper compound when the residual invertase was heated with Fehling solution. This is the so-called "copper yeast gum" (Salkowski<sup>11</sup>), also mentioned by Nelson and Born.<sup>12</sup> For these reasons the invert sugar solution was prepared by mixing equal numbers of molecules of dextrose and levulose. Specific rotatory power,  $[\alpha]_{\lambda=546}^{25} = -21.50^{\circ}$ ;  $[\alpha]_{\text{D}}^{25} = -18.39^{\circ}$ .

**Lactose.**—Squibb's pure lactose was dissolved in boiling distilled water and when cold, a mixture of equal volumes of ether and alcohol was added. The lactose crystallized from this solution, washed with alcohol and ether, was dried *in vacuo* at 70°. This dried material was ground and dried further at 100° for 4 to 5 hours. Analysis showed it to contain: moisture (loss at 125° to 130°), 5.22%; ash, 0.03%. Specific rotatory power,  $[\alpha]_{\lambda=546}^{26} = 61.36^{\circ}$ ;  $[\alpha]_{\text{D}}^{25} = 52.90^{\circ}$ .

**Maltose.**—This sugar was prepared from Lintner<sup>13</sup> soluble starch by saccharification of the starch with highly purified malt amylase kindly furnished by Professor H. C. Sherman. The product of the saccharification was fermented by a pure culture of *Saccharomyces Ludwigi* to remove fermentable sugars other than maltose. Upon completion of fermentation, the solution was heated in an autoclave, cooled and the dextrins precipitated by alcohol. The filtrate was evaporated *in vacuo* to a thin syrup, which upon inoculation with a few maltose crystals yielded a crop of crystals which were recrystallized from hot 95% alcohol, using acid-purified cocoanut charcoal as decolorizing agent. This maltose was dried *in vacuo* at 60° for 4 hours, ground to powder and further dried at 90° for 10 hours. The final product contained: moisture, 5.05%; ash, 0.02%. Specific rotatory power,  $[\alpha]_{\lambda=546}^{25} = 153.75^{\circ}$ ;  $[\alpha]_{\text{D}}^{25} = 131.25^{\circ}$ .

### Atmospheric Pressure and Temperature of Boiling.

Irregularity of results due to the effect of fluctuations of atmospheric pressure on temperature of boiling has been reported by several investigators.<sup>14</sup> A series of experiments conducted in this laboratory at different times of the year under barometric pressures varying from 753 mm. to 760 mm. lend support to the above findings. The reducing power of 100 and of 150 mg. of dextrose determined by the 2-minute boiling over a flame and the 12-minute digestion in boiling water varied 2% between the atmospheric pressures mentioned.

It is apparent that variation in boiling point due to ordinary changes in atmospheric pressure and also due to location of laboratories at different altitudes may seriously affect the accuracy of results obtained by the reduction methods in common use.

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

<sup>11</sup> Salkowski, *Z. physiol. Chem.*, **31**, 305 (1900).

<sup>12</sup> Nelson and Born, *THIS JOURNAL*, **36**, 393 (1914).

<sup>13</sup> Lintner, *J. prakt. Chem.*, [2] **34**, 378 (1886).

<sup>14</sup> Traphagen and Cobleigh, *THIS JOURNAL*, **21**, 369 (1899). Rosenkranz, *Z. ver. Deut. Zuckerind.*, **61**, 426 (1911). Maquenne, *Compt. rend.*, **161**, 617 (1915); **162**, 145 (1916).

### Influence of Temperature and Time of Heating.

The results of a series of experiments to determine the most suitable temperature and length of time of heating for the oxidation of the sugars by a Soxhlet-Fehling solution are plotted in Figs. 1 and 2. The solution consisted of 50 cc. of mixed Soxhlet-Fehling<sup>16</sup> solution and 50 mg. of glucose, the volume being adjusted to 100 cc. with distilled water.

Upon critical examination of the curves in Figs. 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

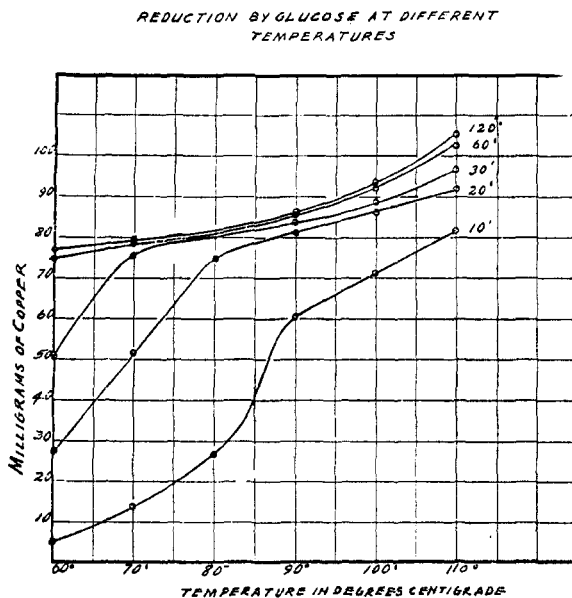


Fig. 1.

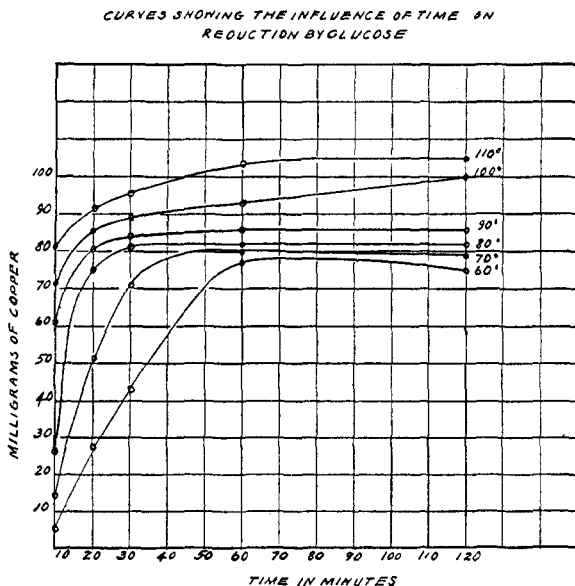


Fig. 2.

<sup>16</sup> Soxhlet's formula: (A) 34.639 g. of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in 500 cc. of solution; (B) 173 g. of Rochelle salt, and 51.6 g. of NaOH in 500 cc. of solution.

temperature and it appears complete at temperatures below  $90^\circ$  after an hour's heating. At temperatures above  $100^\circ$  there is an appreciable increase in reduction which may be due to auto-reduction of Fehling solution.

The curves in Fig. 3 show that Soxhlet-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° for periods of heating up to 30 minutes, and furthermore that at this temperature there is but slight formation of cuprous oxide even after heating for 1 hour.

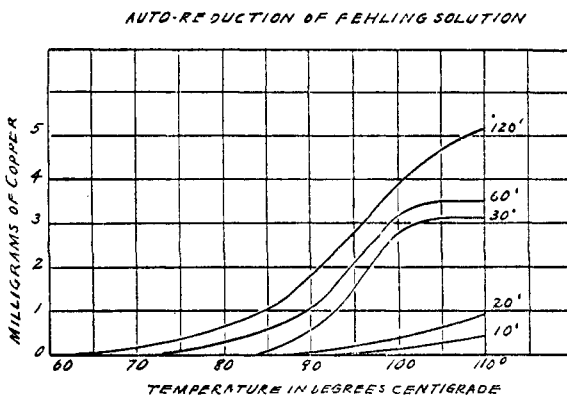


Fig. 3.

### Reducing Action of Sucrose on Fehling Solution.

A study of the effect of sucrose in the presence of reducing sugars has been made by Maquenne,<sup>16</sup> Pellet,<sup>17</sup> Saillard,<sup>18</sup> Browne<sup>19</sup> 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, when sucrose is present is considerable. Browne<sup>20</sup> gives an empirical formula for the correction of errors due to reducing action of sucrose on glucose. Meissl and Hiller<sup>21</sup> give tables of factors for calculating copper to invert sugar for different ratios of sucrose to invert sugar. Munson and Walker<sup>22</sup> 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 formulas and other unnecessary calculations involved, led to the following study of the comparative action of sucrose on Fehling solution with the view of establishing the best conditions by which the reducing action of sucrose could be entirely eliminated or reduced to a minimum.

<sup>16</sup> Maquenne, *Compt. rend.*, **162**, 207, 145 (1916); **161**, 617 (1915).

<sup>17</sup> Pellet, *Ann. Chem. Anal.*, **20**, 169 (1915); *Bull. Assoc. Chem. suc. dist.*, **31**, 183 (1913-14).

<sup>18</sup> Saillard, *Compt. rend.*, **161**, 591 (1915); *J. Fabr. suc.*, **56**, 1 (1915).

<sup>19</sup> Browne, *THIS JOURNAL*, **28**, 439 (1906).

<sup>20</sup> Browne, "Handbook of Sugar Analysis," **1912**, p. 428.

<sup>21</sup> Meissl and Hiller, *Z. Ver. Deut. Zuckerind.*, **39**, 735 (1889).

<sup>22</sup> Munson and Walker, *THIS JOURNAL*, **28**, 663 (1906).

From the curves in Fig. 4 it is apparent that the reducing action of sucrose is considerable when current methods are used. The amount of copper reduced mounts rapidly with increase in concentration of sucrose.

The reducing values plotted in the above figure were all corrected for "blank" reductions.

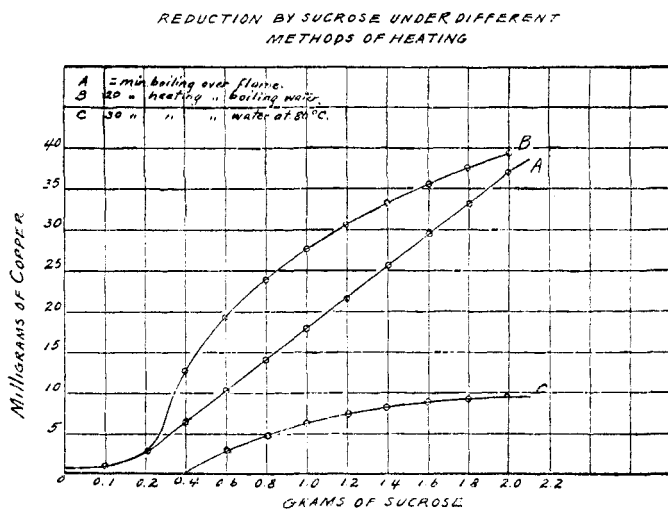


Fig. 4.

#### Selection of Conditions for Reduction.

If it were 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 when the time element is considered, it does not matter whether the terminal point is reached for the purpose of testing an analytical method. By selecting a set of standardized conditions such that the amount of reduction obtained is sharply defined at a selected point, that point may successfully be used as a terminal point. Upon critical study of the curves in Figs. 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 "terminal point." Temperatures other than 80° might have been selected, but a lower temperature would require a longer time of heating to give a reasonable amount of copper for quantitative determination, and at higher temperatures the difficulty of accurately maintaining the temperature of the bath interferes with the accuracy of short-time digestions.

Objection may be raised that 30 minutes' reduction involves too much time for the analysis, but experience has proved that when sets of 7 samples

are run under these conditions, the reducing power of a sample can be determined within 8 to 10 minutes from the time the sample is placed in the bath to the completed washing of the precipitated cuprous oxide.

A great advantage claimed for this method over the procedure of boiling over a flame is that continuous observation to determine the time boiling is not required. Furthermore, this method offers an important improvement over the current methods in the complete elimination of auto-reduction of Fehling solution; and as shown in Fig. 4, the presence of as much as 425 mg. of sucrose has no effect on the accuracy of the process. We find also that a long time of heating produces a coarse granular cuprous oxide which is easily washed and removed from the beaker without the aid of a rubber "policeman."

### Apparatus and Procedure.

**Thermostat.**—A 50-liter constant-temperature bath, similar to the one described by H. C. Sherman and A. W. Thomas,<sup>23</sup> and J. M. Nelson and F. M. Beegle<sup>7</sup> was constructed. The temperature was maintained at  $80^{\circ} \pm 0.1^{\circ}$ . Excessive evaporation of the water was prevented by the addition of 2 or 3 drops of colorless paraffin oil.

**Procedure.**—When the bath had attained proper temperature, a 350-cc. beaker containing the solution to be tested 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. Occasionally the beaker was shaken and the solution rotated to ensure thorough mixing. 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 cc. of 16 *N* nitric acid, and copper determined either by Beans and Stillman's<sup>24</sup> electrolytic method or E. C. Kendall's<sup>25</sup> modified iodide method.

### Fehling Reagent.

As a result of Nef's<sup>26</sup> work we know that the sugar molecule in the presence of Fehling solution undergoes oxidation, reduction, condensation or further decomposition giving rise to a great multiplicity of aldehydes and acids as end products, the kinds, as well as amounts of which, vary with concentration of sugar and of alkali used.

<sup>23</sup> Sherman and Thomas, *THIS JOURNAL*, **37**, 623 (1915).

<sup>24</sup> J. W. Stillman, "New Direct Method for the Electrolytic Determination of Copper," *Dissertation*, Columbia Univ., 1920.

<sup>25</sup> Kendall, *THIS JOURNAL*, **33**, 1947 (1911).

<sup>26</sup> Nef, *Ann.*, **357**, 214 (1907); **376**, 1 (1910); **403**, 204 (1914).

**Influence of the Nature of the Alkali.**—The nature and amount of alkali in Fehling solution influence both reduction and the physical character of the copper oxide formed. Sodium hydroxide and potassium hydroxide solutions of equivalent concentrations give practically the same reduction with dextrose and invert sugar. Glendinning<sup>27</sup> finds that maltose shows a higher reducing power when potassium hydroxide is substituted for sodium hydroxide in Fehling solution. Kjeldahl<sup>28</sup> studying the effect of different strengths of sodium hydroxide solution upon reduction, found that between limits of 0.5 to 2 *N* sodium hydroxide solution, the variation was not more than 1% with glucose, while with maltose and lactose there was a wide variation.

It is our experience that Fehling solution containing sodium hydroxide gives a more satisfactory precipitation than when potassium hydroxide or the carbonates are used. The curves in Fig. 5 illustrate the influence

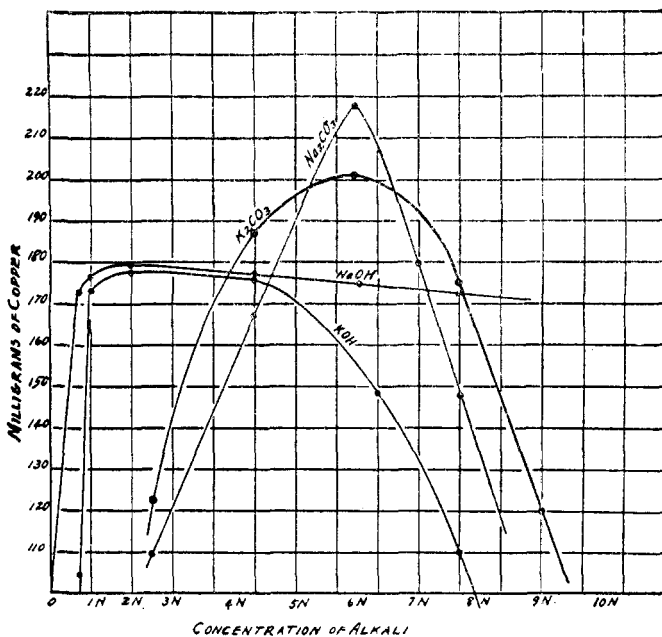


Fig. 5.—The influence of different alkalis in Fehling solution on the oxidation of glucose.

of kind and concentration of alkali upon the oxidation of glucose by Fehling solution.

**Influence of Concentration of Alkali.**—According to Fig. 6 the most effective alkalinity of Fehling solution is 1.6 *N* in terms of sodium hy-

<sup>27</sup> Glendinning, *J. Chem. Soc.*, 67, 999 (1895).

<sup>28</sup> Kjeldahl, *Compt. rend. trav. Laboratoire Carlsberg*, [1] 4, (1895).



dioxide. This alkalinity has also been found best by Kjeldahl<sup>28</sup> and Brown, Morris and Millar,<sup>29</sup> when reduction was carried on in a boiling-water-bath. Fehling solution, of this alkalinity corresponds to 65 g. of sodium hydroxide in 500 cc. of the alkaline tartrate solution.

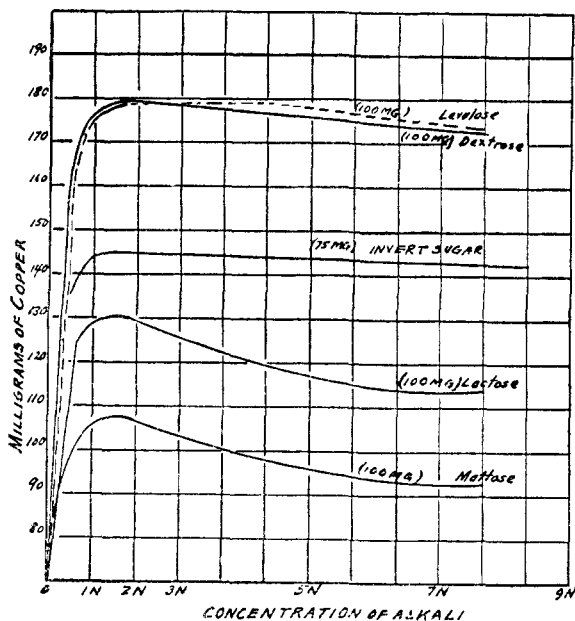


Fig. 6.—The influence of different concentrations of alkali in Fehling solution on the oxidation of sugars.

The curves show that the concentration of hydroxyl ions influences the rate and amount of oxidation of the sugars. The amount of copper reduced increases with increasing concentration of hydroxyl ions up to a certain point, where oxidation appears to be at its maximum, and from that point further increase in hydroxyl ions tends to decrease reduction. The most plausible explanation for this is that 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.

**The Effect of Varying the Amount of Copper Sulfate in Fehling Solution.**—To determine this effect a test solution consisting of 50 cc. of Fehling solution containing various amounts of copper sulfate, 3.2 g. of sodium hydroxide, 8.65 g. of the sodium potassium tartrate and 100 or 200 mg. of the sugar was used. The total volume of the solution was adjusted to 100 cc. with distilled water in each case.

<sup>29</sup> Brown, Morris and Millar, *J. Chem. Soc.*, 71, 96 (1897).

TABLE I.

OXIDATION OF REDUCING SUGARS BY FEHLING SOLUTION CONTAINING VARIOUS AMOUNTS OF COPPER SULFATE, AT 80° FOR 30 MINUTES.

Wt. of Cu in 50 cc. Mg.	Ratio of NaOH to Cu by weight.	Reduction in terms of mg. of Cu.						Blank.
		Dextrose.		Levulose.		Lactose.		
		100 mg.	200 mg.	100 mg.	200 mg.	100 mg.	200 mg.	
210	15.2:1	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 II.

OXIDATION OF DEXTROSE IN THE PRESENCE OF SUCROSE BY FEHLING SOLUTION CONTAINING VARIOUS AMOUNTS OF COPPER SULFATE.

Wt. of Cu in Fehling sol. Mg.	Reduction By Dextrose Alone.			Blank.
	Reduction in terms of mg. of Cu, Mg. of dextrose in 10 cc. 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

REDUCTION OF DEXTROSE IN THE PRESENCE OF SUCROSE.

	Reduction in terms mg. of Cu.		
	5 mg. Dextrose.	50 mg. Dextrose.	95 mg. 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 I and II, the most suitable ratio is seen to be from 5 to 6 of sodium hydroxide to 1 of copper by weight. In other words, with the alkalinity of 1.6 *N* (3.2 g. of sodium hydroxide per 50 cc. of mixed reagent), 0.525 to 0.630 g. of copper gives the most satisfactory reduction. It is also interesting to note that with increasing concentration of copper sulfate, total reduction is decreased, while auto-reduction of the solution is increased. Furthermore, Table II shows that in the presence of sucrose, increasing the concentration of copper sulfate 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.

**The Effect of Varying the Amount of Sodium Potassium Tartrate in the Presence of Constant Amounts of Alkali and Copper Sulfate.**

—The test solution consisted of 25 cc. of copper sulfate solution containing 525 mg. of copper (as cupric sulfate), 3.2 g. of sodium hydroxide, 100 mg. of the sugar and various amounts of tartrate. The volume of each solution was made up to 100 cc. with distilled water.

A saturated solution of sodium potassium tartrate was prepared from a commercial 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 washed with alcohol, and dried in an oven at 50° for 15 hours, and then at 60° to 65° for 10 hours. The salt as analyzed contained 4 molecules of water of crystallization.

TABLE III.  
INFLUENCE OF CONCENTRATION OF ROCHELLE SALT.

Weight of Rochelle salt.	Mg. of copper reduced.			
	Dextrose.	Levulose.	Lactose.	Maltose.
G.				
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 III shows that there is no material effect of increasing the concentration of the tartrate beyond that called for by the Soxhlet-Fehling formula. The formation of the complex cupric tartrate ion is assured by the presence of 8.65 g. of Rochelle salt in 50 cc. of the mixed Fehling solution. Less than this amount appears to give less reduction for lactose and maltose. The reduction by dextrose decreases with increasing concentration of the tartrate while with levulose there is no appreciable effect of increasing amounts of tartrate upon the reduction.

**Photosensitiveness and Keeping Quality.**—The photosensitiveness of Fehling solution as evidenced by the precipitation of cuprous oxide upon exposure of the solution to light was noticed by Fehling<sup>4</sup> and numerous other investigators<sup>30</sup> have confirmed this.

Our experiments show that 50 cc. of mixed Fehling solution exposed to direct sunlight will give traces of cuprous oxide precipitate within 10 to 20 hours, while if kept in the dark, or in opaque bottles, or in ray-filter solutions such as potassium dichromate, or acidified quinine sulfate, decomposition does not take place until after a month or longer. 50 cc. of mixed Fehling solution exposed to direct sunlight will be completely

<sup>30</sup> Eder, *Sitzungsber. Akad. Wiss., Wien*, **92**, 344 (1885); through *J. Phys. Chem.*, **17**, 204 (1913); Byk, *Z. physik. Chem.*, **49**, 659, 679 (1904); Leighton, *J. Phys. Chem.*, **17**, 204 (1913).

decolorized after 2 months, showing then only a slight amount of copper present.

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

Munson and Walker<sup>31</sup> 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 article<sup>22</sup> 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. The results varied from  $-0.8$  to  $+1.6$  mg. of cuprous oxide.

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 1520, showed no reducing power when heated at  $80^{\circ}$  for 30 minutes, *i. e.*, no "blank," after they had been kept in the dark for 5 months; but, if reduction was carried out according to Munson and Walker's method, the solutions gave a considerable precipitate, which varied from 1.5 to 4.0 mg. of copper.

### 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 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,  $\text{Cu}_2\text{O}$ , when in the colloidal state, are claimed by Svedberg<sup>32</sup> and Wo. Ostwald<sup>33</sup> to be nothing more than the color changes coincident with the gradual increase of the size of the particles, the red being the largest.

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 6 *N* alkalinity with the carbonates, while with the alkali hydroxides, the same dispersions are noticeable only at concentrations below 0.5 *N*. Reduction at high temperatures augments the production of the "colloidal" copper oxide. Low temperature and a fairly high con-

<sup>31</sup> Munson and Walker, *THIS JOURNAL*, 29, 541 (1907).

<sup>32</sup> Svedberg, "Herstellung Kolloider Lösungen," Steinkopff, 1920.

<sup>33</sup> Wo. Ostwald, *Kolloidchem. Beihefte*, 2, 409 (1911).

centration of alkali in Fehling solution, increase the size characteristic of the red cuprous oxide.

### Surface Oxidation.

The size and shape of receptacles in which the mixture of Fehling solution and sugars are heated have an effect on the amount of copper reduced. Also the nature of the material of which the receptacle is made has an influence upon the velocity of deposition of cuprous oxide.<sup>28,34,35</sup>

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

Table IV 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 mg. of copper with beakers in which the surface area of the liquid exposed to the air ranged from 43.7 to 85.6 <sup>bs</sup> cm. Heating for 20 minutes in boiling water gives a difference of 3.2 mg. and for 30 minutes in water at 80°, 2.9 mg. It should be observed that in all these three methods of heating 350 and 550 cc. 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 in order to study this relationship further. The solutions consisted of 100 mg. of dextrose and 50 cc. of Fehling solution, made up to 100 cc. 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 V shows that when 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

<sup>24</sup> Urecht, *Ber.*, **15**, 2687 (1882).

<sup>25</sup> Kendall, *THIS JOURNAL*, **34**, 317 (1912).

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 IV.  
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.  
(200 Mg. of Dextrose Used in Each Case.)

Receptacle.	Cc.	Diameter. Cm.	Height of column of liquid. Cm.	Lateral area of liquid exposed to glass. Sq. Cm.	Surface area of liquid exposed to air. Sq. Cm.	Methods of heating.		
						2 min. boiling over a flame.	20 min. heating in boiling water.	30 min. heating in water in 80°.
Beaker	750	10.0	2.0	65.2	85.6	305.0	304.7	302.9
"	550	9.5	2.7	79.5	70.1	305.8	306.4	304.6
"	350	8.5	3.5	92.2	56.8	305.7	306.7	304.7
"	250	7.5	4.0	93.0	43.7	310.8	307.0	305.8
Flask	250	9.8	3.0	89.4	70.4	306.7	310.2	304.5
Erlenmeyer								

The figures represent averages of duplicate determinations which varied not more than 0.7 mg. of Cu.

TABLE V.  
(100 Mg. of Dextrose Used in Each Case.)

Beaker.	Diameter.	Height of column of liquid. Cm.	Lateral area of liquid exposed to glass. Sq. Cm.	Surface area of liquid exposed to air. Sq. Cm.	(1)	(2)	(3)	Loss. (1)-(3).	
					Covered with toluene.	Covered with watch glass.	Uncovered.		
	Cc.	Cm.	Cm.	Sq. Cm.					
	1000	12.0	1.7	63.2	111.6	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

The 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 cc. of Fehling solution and making the total volume of the solution up to 100 cc. with distilled water, and transferring to 350 cc. beakers of same diameters. One cc. of toluene was added to each. After the mixtures had been heated for 30 minutes in water at 80°, the cuprous oxide was filtered off, washed and copper determined electrolytically.

	Covered with toluene. Mg.	Covered with watch glass. Mg.	Uncovered. Mg.
Weight of Cu <sub>2</sub> O used.....	147.9	234.2	230.6
" " " recovered.....	147.8	234.0	227.2
			239.6
			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. Kjeldahl<sup>28</sup> 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, the inconveniences attending such manipulation would preclude its general use. Covering the beaker with a watch glass approximates quite as well the true reduction as the other expedients suggested, and, of course, is preferable because of its simplicity.

### Mutual Effect of Sucrose and Reducing Sugars.

Earlier in this discussion it was noted that under the newly proposed conditions the presence of as much as 425 mg. of sucrose had no reducing action on the modified Fehling solution.

The reducing action of sucrose was further investigated by running reductions with different mixtures of sucrose and reducing sugars. The effects are shown in the tables following. In these tables the amounts of reducing sugar are to be found in the first column and the weights of sucrose mixed with the different amounts of reducing sugar are given in the top horizontal line. The figures given in the second column are the amounts of cuprous oxide precipitated by the respective amounts of reducing sugar, expressed in terms of milligrams of copper, while in the remaining columns is given excess reduction caused by the presence of the sucrose.

TABLE VI.  
REDUCTION BY MIXTURES OF SUCROSE AND DEXTROSE.

Dextrose Mg.	Sucrose in Mg.						
	0.	400.	600.	800.	1000.	1500.	2000.
0	0	0	3.0	3.4	6.2	8.7	9.3 mg. Cu
20	42.2	0	3.3	3.6	5.9	11.0	14.1
50	102.3	0	3.7	5.0	5.1	10.8	12.0
100	201.2	0	3.7	4.2	5.3	10.5	11.0
150	295.2	0	3.3	4.4	4.3	9.2	11.1
200	385.1	0	3.1	4.5	3.9	8.4	10.6
250	472.7	0	3.0	3.7	3.6	4.3	10.2

TABLE VII.  
REDUCTION BY MIXTURES OF SUCROSE AND LEVULOSE.

Levulose, Mg.	Sucrose in Mg.						
	0.	200.	300.	400.	500.	1000.	2000.
0	0	0	0	0	2.1	6.2	9.3 mg. Cu
10	19.0	0	1.2	2.3	3.3	9.3	13.8
40	75.5	0	2.6	3.7	4.6	9.5	12.5

TABLE VII.—(Continued).

Levulose Mg.	0.	200.	300.	400.	500.	1000.	2000.
80	150.5	0	1.2	2.5	4.2	6.6	11.2
120	220.2	0	1.6	3.3	5.1	7.9	12.3
160	291.8	0	0.2	1.5	3.8	6.8	11.5
200	360.6	0	0.2	0.5	2.8	4.9	10.9
240	428.1	0	0.1	0.8	2.0	4.3	11.0

TABLE VIII.

## REDUCTION BY MIXTURES OF SUCROSE AND INVERT SUGAR.

Invert Sugar. Mg.	Sucrose in mg.						
	0.	200.	300.	400.	500.	1000.	2000.
0	0	0	0	0	2.1	6.2	9.3 mg. Cu
10	20.0	0	1.7	2.5	3.1	11.4	19.2
20	39.1	0	1.9	2.3	3.2	11.1	19.1
40	79.5	0	1.0	0.4	2.4	8.3	16.4
80	154.6	0	0.5	0.6	2.0	7.5	16.3
120	229.3	0	1.1	0.5	1.7	6.6	16.1
160	301.5	0	0.4	1.1	2.1	5.6	16.6
200	373.0	0	0.0	0.0	0.1	3.9	13.0
240	441.5	0	0.2	0.0	0.0	4.0	11.2

TABLE IX.

## REDUCTION BY MIXTURES OF SUCROSE AND LACTOSE.

Lactose. Mg.	Sucrose in Mg.				
	0.	200.	500.	1000.	2000.
0	0	0	2.1	6.2	9.3 mg. Cu
10	12.2	0.8	1.5	7.2	16.3
50	61.5	1.4	3.4	9.0	18.6
100	123.0	1.5	3.4	9.3	18.6
150	184.5	1.5	3.5	9.7	18.9
200	246.0	1.6	3.2	9.9	18.4
250	307.1	0.6	3.4	8.3	17.7
300	368.0	0.3	3.2	8.3	16.5
350	429.5	0.3	3.2	7.1	16.2

TABLE X.

## REDUCTION BY MIXTURES OF SUCROSE AND MALTOSÉ.

Maltose. Mg.	Sucrose in Mg.					
	0.	50.	100.	200.	500.	1000.
0	0	0	0	0	2.1	6.2 mg. Cu
10	10.0	0	0	0.2	1.3	7.2
50	50.5	0	0.3	0.5	3.0	7.9
100	100.8	0	0.6	1.2	3.5	9.9
150	151.5	0	0.5	0.8	3.5	10.6
200	202.0	0	0.6	1.2	4.0	11.1
250	252.5	0	0.5	2.6	4.9	11.0
300	302.6	1.2	1.2	3.6	6.0	11.3
350	353.4	0.8	1.5	5.3	6.1	11.7



It is apparent that the reducing action of sucrose in the presence of dextrose is negligible at concentrations below 400 mg. 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 dextrose instead of acting as a protector, actually accelerates the decomposition of sucrose. This change 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.

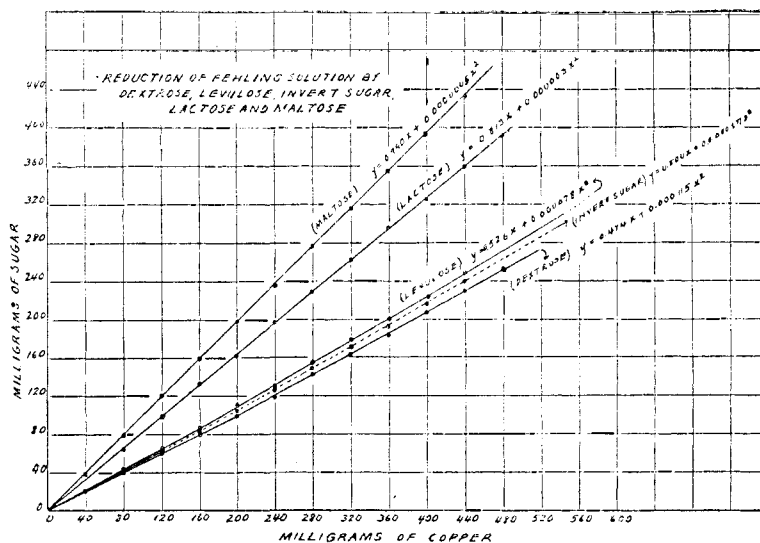


Fig. 7.

Table VII shows that the action of sucrose on levulose is negligible at concentrations below 300 mg. Levulose has an accelerating action upon the decomposition of sucrose, this action being greatest between 40

and 160 mg. of levulose, while in the presence of more than 160 mg. of levulose this phenomenon is not manifested, and as in the case of dextrose, when sufficient reducing sugar is present to precipitate the greater part of the copper, the reducing action of sucrose decreases.

These tables bring out certain effects of the various reducing sugars in the presence of sucrose which are difficult to interpret. The presence of as much as 400 mg. of sucrose does not affect the accuracy of determination of dextrose, while in the case of levulose the presence of 400 mg. of sucrose will cause no appreciable error provided 120 mg. or more of levulose is present. When less than this amount of levulose is present, then not more than 200 mg. of sucrose is permissible for accurate results. A protective action of invert sugar when present in large amounts is shown when mixed with 500 mg. of sucrose, and although this fails to a certain extent when from 1 to 2 g. of sucrose is present, yet the total reduction of the mixture of sugars is less than that of both taken separately.

Maltose and lactose show the odd effect of catalyzing the reduction by sucrose when this sugar is mixed with them. The action of maltose is more marked than that of lactose. It will be noted that in the presence of lactose, not more than 200 mg. of sucrose may be present if the true reducing power of the lactose is desired, and with maltose the amount of sucrose present must not exceed 100 mg. for analyses of high degree of accuracy.

#### Preparation of Proposed Modification of Fehling Solution.

1. **Copper Sulfate Solution.**—The crystals of C. P. copper sulfate 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 cc. of it contained 525 mg. of copper or 41.2 g. of cupric sulfate pentahydrate in 500 cc. 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 insoluble carbonates and other impurities had settled out. The clear solution was siphoned off and its alkalinity established by titration with standard acid.

*Crystallized Rochelle salt, 17.3 g.* (highest purity), was dissolved in water in a 500-cc. graduated flask and the calculated amount of sodium hydroxide solution added, so that 500 cc. of this alkaline tartrate solution contained exactly 65 g. of sodium hydroxide.

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.

### General Procedure.

Measure accurately 25 cc. each of the copper sulfate and alkaline tartrate solutions into a 400 cc. Pyrex or Bohemian glass beaker, the diameter of which is about 9 cm. Add 50 cc. of sugar<sup>36</sup> solution making a total volume of 100 cc. Cover the beaker with a watch glass and place the beaker in a water-bath which is maintained at 80°. After exactly 30 minutes' digestion, filter the cuprous oxide by suction through a mat of asbestos in a Gooch crucible. Wash the precipitate in the usual manner and determine the copper by one of the methods discussed at the end of this paper. From the weight of copper or cuprous oxide obtained, find the corresponding weight of reducing sugar from Table XIII or calculate it by means of the equations given below.

### Sugar Table.

Figures in the sugar table were calculated from the equation  $y = a + bx + cx^2$ , by method of least squares,<sup>37</sup> where  $y =$  mg. of sugar and  $x =$  mg. of copper.

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

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

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

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

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

$$\text{Maltose} \dots \dots y = 0.990x + 0.000005x^2$$

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

<sup>36</sup> Weights of the sugars to be taken for analysis. For dextrose, levulose and invert sugar, take 50 to 150 mg; for lactose and maltose, take 100 to 300 mg. These weights of sugars will reduce about 100 to 350 mg. of copper. In the presence of sucrose, the weight of sample to be taken for analysis can be judged by a preliminary test. In mixtures of sucrose and dextrose up to 400 mg. of sucrose will not influence the accuracy of the determination of dextrose. For mixtures of sucrose and levulose (or invert sugar) not more than 200 mg. of sucrose may be present, unless more than 160 mg. of reducing sugar is present, in which case as much as 400 mg. of sucrose will not affect the result. With sucrose-lactose mixtures the maximol permissible amount of sucrose is 200 mg. and 100 mg. in sucrose-maltose mixtures. In any case, where the composition of the sample is unknown, there will be no danger of error due to the presence of sucrose, if not more than 100 mg. of sample is taken. This allows for the possible presence of maltose.

Mellor, "Higher Mathematics for Students of Physics and Chemistry," Longmans, Green and Co., 1916, p. 329.

TABLE XI.

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

Copper. (Cu).	Cuprous oxide (Cu <sub>2</sub> O).	Dextrose. (d-glu- cose).	Levulose (d-fruc- tose).	Invert sugar.	Lactose. C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> .	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> .H <sub>2</sub> O.	C <sub>19</sub> H <sub>32</sub> O <sub>11</sub> .	Maltose. C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> .H <sub>2</sub> 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	325.1	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

TABLE XII.—REDUCING RATIOS.

Showing the Reducing Ratios of Levulose, Invert Sugar, Lactose and Maltose by the New Method.

Copper. Mg.	<u>Dextrose</u> <u>Levulose.</u>	<u>Dextrose.</u> <u>Invert</u> <u>sugar.</u>	<u>Dextrose.</u> <u>Lactose.</u>	<u>Dextrose.</u> <u>Lactose</u> <u>hydrate.</u>	<u>Dextrose.</u> <u>Maltose.</u>	<u>Dextrose.</u> <u>Maltose</u> <u>hydrate.</u>
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

These ratios were calculated from the sugar values corresponding to the several weights of copper in Table XI. The ratios for levulose and invert sugar are approximately equal to those obtained by Browne<sup>38</sup> using Allihn's method. The ratios for lactose and maltose are lower than those obtained by others.<sup>39,40</sup>

### Determination of Reduced Copper.

Three different methods were used (1) direct weighing of cuprous oxide, (2) E. C. Kendall's<sup>26</sup> modified iodide method, and (3) H. T. Beans and J. W. Stillman's<sup>24</sup> electrolytic method. For economy of space our extensive comparative tests of these three methods are omitted.

While the direct weighing of cuprous oxide is the quickest, it is liable to inaccuracy when solutions other than of pure sugars are used due to contamination of the precipitate with mineral and organic impurities. The extent of this error has been shown by Sherwood and Wiley.<sup>41</sup>

Kendall's modified iodide method is rapid but has the disadvantage of a poor end-point.

The reduced copper values given in the main sugar table No. XI and in most of the other tables in this paper were determined by the Beans and Stillman electrolytic method. This new electrolytic method is very rapid, and since the technique is at present available only in the original dissertation, it seems desirable to abstract it in brief.

**Electrolytic Method.**—The cuprous oxide is dissolved with 10 to 20 cc. of 16 *M* nitric acid, and the solution diluted to about 200 cc. with distilled water. Ten cc. of a 3% hydrogen peroxide is added. The solution is then electrolyzed at room temperature (18° to 25°) with a current of 1 ampere and an e. m. f. of from 2.5 to 3.5 volts. A weighed 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

<sup>38</sup> Browne, *op. cit.*, 1912, p. 421; THIS JOURNAL, 28, 436 (1906).

<sup>39</sup> Browne, *op. cit.*, p. 421.

<sup>40</sup> Sherman, "Organic Analysis," Macmillan and Co., 1918, p. 73.

<sup>41</sup> Sherwood and Wiley, *Bur. Chemistry, Bull.* 105, 120 (1907).

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° for a minute or two, then cooled in a desiccator, and weighed.

### Accuracy of Proposed New Method for Determination of Reducing Sugars.

Under the conditions for reduction, a series of experiments with dextrose and lactose was performed to study the degree of accuracy of the method.

TABLE XIII.

Reduction of Fehling Solution by Reducing Sugars.					
Wt. of sugar in mg.	Reduction in mg. of copper.				
	(1).	(2).	(3).	(4).	(5).
<b>Dextrose.</b>					
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
<b>Lactose.</b>	(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,  $m$  is the mean error of the mean;  $d$  the deviation from the mean, and  $n$  the number of determinations.

The mean error of the mean for dextrose and lactose under these conditions appears to be  $\pm 0.08$  and  $\pm 0.17$  mg. of copper respectively. The percentage error is approximately  $\pm 0.12\%$ . Besides the "mean error," in 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 XIV.  
The Effect of Temperature Deviations.

Wt. of sugar in mg.	Temperature, °C.				
	78.	79.	80.	81.	82.
<b>Dextrose.</b>					
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
<b>Lactose.</b>					
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  $\pm 0.23$  and  $\pm 0.44$  mg. of copper for dextrose and lactose respectively for every degree of temperature change around  $80^\circ$ , and  $\pm 0.50$  and  $\pm 0.94$  mg. of copper for dextrose and lactose for every  $2^\circ$ . This error is approximately equal to  $\pm 0.3\%$  for  $\pm 1^\circ$  and  $\pm 0.65\%$  for  $\pm 2^\circ$  around  $80^\circ$ . 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  $\pm 1^\circ$  does not materially affect the degree of accuracy required for most purposes. For high accuracy an automatically temperature controlled bath should be used.

### Summary.

The various sources of error in current methods for using Fehling solution have been discussed.

The interrelationship of temperature,  $60^\circ$  to  $110^\circ$ , and time of heating, 10 minutes to 2 hours, to determine the best time and optimum temperature for reduction has been investigated.

Fehling reagent has been studied from the standpoint of nature and concentration of alkali, concentration of copper sulfate and Rochelle salt to find the maximum and minimum concentrations of these constituents necessary to give the greatest yield of cuprous oxide and to ensure the formation of the complex cupric tartrate ion.

Auto-reduction of Fehling solution at different times and degrees of heating has been measured, revealing those conditions under which absolutely no "blank" reduction is obtained.

Conditions affecting the physical properties of the precipitated cuprous oxide, the photosensitiveness and keeping quality of Fehling solution have been recorded.

A study of surface oxidation involving different methods of heating and receptacles of various sizes has been carried out in order to determine

the amount of copper lost due to surface oxidation and how to avoid it. The catalytic effect of the walls of the container has been demonstrated.

A procedure for the proposed method with comparison of results for the determination of the reduced copper has been described, including a discussion of the degree of accuracy of the new method.

The reducing action of sucrose on Fehling solution, alone and when mixed with different proportions of reducing sugars, has been investigated with the view of elimination of the use of empirical formulas and factors for calculation of errors involved.

The new method is designed for use in the analysis of saccharine materials containing sucrose, dextrose, levulose, invert sugar, lactose and maltose. It is not claimed that this method can be used for the accurate quantitative determination of 0.1% or less of invert sugar in approximately pure sucrose.

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## VISCOSITY OF GELATIN SOLUTIONS.

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Owing to the divergence of results and conclusions arrived at regarding the various physical properties of gelatin solutions an investigation of the effects of different physical and chemical treatments of such solutions has been undertaken by this laboratory.

In this paper the investigation of the viscosity of gelatin solutions is described. Detailed results of the effects on viscosity of (1) method of effecting solution, (2) aging of solution, (3) concentration of hydrogen ion, (4) concentration of gelatin, (5) hydrolysis of gelatin are given for three different types of gelatins. Finally, a method is given whereby the viscosity of a gelatin may be determined with an accuracy well within 0.5%.

Previous work has shown that viscosity is affected by the age of the solution, concentration of the gelatin, the temperature employed for effecting solution and by the character of the gelatin. With the exception of the first two factors very few, if any, results of a definite nature have been published. Much work has been done on the effects of various salts, but Loeb<sup>1</sup> has shown the fallacy of the "Hofmeister Series" and has furnished adequate chemical data in explanation of such salt effects.

<sup>1</sup> Loeb, *J. Gen. Physiol.*, [III] 1, 85 (1920).