

## THE JOURNAL

OF THE

## American Chemical Society

---

[CONTRIBUTION FROM THE U. S. DEPARTMENT OF AGRICULTURE, BUREAU OF CHEMISTRY. SENT BY H. W. WILEY.]

## THE UNIFICATION OF REDUCING SUGAR METHODS.

BY L. S. MUNSON AND PERCY H. WALKER.

Received April 2, 1906.

SINCE Fehling<sup>1</sup> proposed the use of an alkaline copper tartrate solution for the quantitative estimation of reducing sugars, a large number of independent methods have been worked out having as their basis the power of these sugars to reduce such a copper solution. Among the most important and most generally used of these may be mentioned:

Allihn's method for *d*-glucose.<sup>2</sup>

Meissl's method for invert sugar alone, and in the presence of amounts of sucrose varying between 90 and 99 per cent.<sup>3</sup>

Hiller's method for invert sugar in presence of less than 90 per cent. sucrose.<sup>4</sup>

Herzfeld's method for 1 per cent. or less of invert sugar and a high percentage of sucrose.<sup>5</sup>

Soxhlet's method for lactose.<sup>6</sup>

Soxhlet's method for maltose.<sup>7</sup>

<sup>1</sup> Ann. 72, 106.

<sup>2</sup> J. pr. Chem. 22, 46 (1880).

<sup>3</sup> Z. Ver. Rübenzucker Ind. 29, 1034 (1879).

<sup>4</sup> Ibid. 39, 734 (1889).

<sup>5</sup> Ibid. 35, 985 (1885).

<sup>6</sup> J. pr. Chem. 21, 227 (1880).

<sup>7</sup> Allg. Brau. Hopf. Ztg., 1885.

Brown, Morris and Millar's method for *d*-glucose,<sup>1</sup> *δ*-fructose and invert sugar.<sup>2</sup>

Kjeldahl's method for *d*-glucose, *δ*-fructose, invert sugar, maltose, lactose and galactose.<sup>3</sup>

Defren's method for *d*-glucose, maltose and lactose.<sup>4</sup>

In all of the above methods the copper sulphate solution used has remained practically identical, *viz.*, 34.639 grams crystallized copper sulphate in 500 cc., which is very nearly the same concentration as was suggested by Fehling in 1850. The alkaline tartrate solutions and the methods of manipulation employed by the different analysts, however, have varied materially and as both of these factors have a marked effect upon the copper reducing powers of the different sugars, these variations constitute the principal differences in the methods above mentioned.

This work upon reducing sugars was taken up, therefore, for the purpose of so unifying the methods of analysis that a single set of solutions and a common method of manipulation might be used for all reducing sugars. This necessitated a consideration of the solutions to be employed with reference to their effect upon non-reducing sugars when present; the time required for making the determination; the accuracy and simplicity of the method, and the most satisfactory manner of conducting the reduction. It was also necessary that due consideration be given to methods that had long been in use, and consequently radical change from which might be made with reluctance.

The solutions of copper sulphate and of alkaline tartrate, commonly known as Soxhlet's solutions, were accepted as the most satisfactory for general use and these solutions have probably been more generally used in the past than any other set. Besides, these solutions are the ones employed almost exclusively, in this country at least, for volumetric work.

In the manner of making the reductions, two widely different general methods have been employed. Almost invariably the older workers have employed direct boiling for a short period, while the later workers, Brown, Morris and Millar, Kjeldahl, and

<sup>1</sup> The name which is accepted by international scientific usage is employed throughout this paper in place of "dextrose," which is more commonly used in American technical literature.

<sup>2</sup> J. Chem. Soc. 71, 275 (1897).

<sup>3</sup> Compt. rend. des travaux du laboratoire de Carlsberg, 1895, p. 1.

<sup>4</sup> This Journal, 18, 749 (1896).

Defren, have employed heating in a boiling water-bath, conducting the reduction for a longer period than where direct boiling is practised. Kjeldahl, in addition, made the reduction in an atmosphere of hydrogen, thus avoiding the effect of atmospheric oxidation, but this precaution makes the method too cumbersome to permit of its general use. In reference to the manner of conducting the reduction, advantages seemed to be largely in favor of direct boiling for a short period. In an investigation of this subject by one of us<sup>1</sup> it was shown that while somewhat higher results were obtained by heating in boiling water for a long period, the reduction even at the end of fifteen minutes was far from complete and that chances of surface oxidation were much greater than by direct boiling. The method of boiling over a free flame was therefore accepted.

#### PREPARATION OF SOLUTIONS AND METHOD OF MANIPULATION.

*Copper sulphate solution* must contain 34.639 grams of crystallized copper sulphate of highest purity in 500 cc. This salt should contain not more than mere traces of iron.

*Alkaline tartrate solution* must contain 173 grams of Rochelle salt and 50 grams of sodium hydroxide in 500 cc. The amount of alkali to be used is best obtained by weighing out a concentrated solution of sodium hydroxide, the strength of which has been determined by titration. It is not considered essential that this solution be prepared fresh each day, as many workers recommend. In case any precipitate separates out this is filtered off before using; otherwise our experience has been that this solution undergoes no material change upon standing for reasonable lengths of time.

*Manipulation.*—Transfer 25 cc. each of the copper and alkaline tartrate solutions to a 400 cc. Jena or Non-sol beaker and add 50 cc. of reducing sugar solution, or, if a smaller volume of sugar solution be used, add water to make the final volume 100 cc. Heat the beaker upon an asbestos gauze over a Bunsen burner, so regulate the flame that boiling begins in four minutes, and continue the boiling for exactly two minutes. Keep the beaker covered with a watch-glass throughout the entire time of heating. Without diluting, filter the cuprous oxide at once on an asbestos felt in a porcelain Gooch crucible, using suction. Wash the

<sup>1</sup> Proc. A. O. A. C., Bur. Chem., Bull. 73, p. 59.

cuprous oxide thoroughly with water at a temperature of about 60° C., then with 10 cc. of alcohol and finally with 10 cc. of ether. Dry for thirty minutes in a water oven at 100°, cool in a desiccator and weigh as cuprous oxide.

*Direct Weighing of Cuprous Oxide.*—This method of determining copper has been practised in the laboratories of the Bureau of Chemistry for a number of years and has given most excellent results. The method was checked several years ago<sup>1</sup> against the electrolytic method and later against Low's thio-sulphate method as described in this Journal, **24**, 1082 (1902), and the accuracy of the cuprous oxide method demonstrated.<sup>2</sup>

It is by far the most convenient method for the copper determination, the only precaution necessary in its use being the preparation of the asbestos. It has been the custom here to prepare the asbestos, which should be the amphibole variety, by first digesting with 1:3 hydrochloric acid for two or three days. Wash free from acid and digest for a similar period with soda solution, after which treat for a few hours with hot alkaline copper tartrate solution of the strength employed in sugar determinations. The asbestos is then washed free from alkali, finally digested with nitric acid for several hours, and after washing free from acid it is shaken up with water for use. In preparing the Gooch crucible load it with a film of asbestos one-fourth inch thick, wash this thoroughly with water to remove fine particles of asbestos; finally wash with alcohol and ether, dry for thirty minutes at 100°, cool in a desiccator and weigh.

It has been found most convenient to dissolve the cuprous oxide each time after weighing, with nitric acid, and use the same felts over and over again, as they improve with use. Twenty-

<sup>1</sup> Proc. A. O. A. C., Bur. Chem., Bull. 73, p. 59.

<sup>2</sup> For pure *d*-glucose and invert sugar the copper contained in the cuprous oxide corresponds within the limits of experimental error to the theoretical amount. The same is true with the mixture of invert sugar and sucrose when 400 milligrams of total sugar are used. For the mixture containing two grams total sugar, the copper content of the precipitate is slightly lower than theory indicates but the variation is not great enough to affect the results; besides, the results recorded are based on the weights of the precipitate and not on the content of copper. When as much as 10 grams total sugar are used, the variation from the theory may amount to several milligrams, but this variation is constant and would introduce no error when working with tables based on direct weighing of cuprous oxide.

six crucibles were thus used throughout this work and the average loss for an average of seventeen determinations with each crucible was but 0.39 mg. each determination. This loss is partly mechanical and results partly from the solvent action of the reagents used.

*Spontaneous Precipitation of Cuprous Oxide.*—When alkaline copper tartrate is boiled, a slight spontaneous precipitation of cuprous oxide takes place and the regular determinations should be corrected by the amount of cuprous oxide thus precipitated. In this work each one of us ran two blanks each day that determinations were made and the results of each worker are corrected by his blanks for that day. In this way proper correction is made for the cuprous oxide precipitated spontaneously as well as for any loss of asbestos, through solvent action of the alkaline liquid.

The following table shows the blanks obtained throughout this investigation. Attention is called to the rather wide range shown by some of these blanks.

Working in the manner above described the copper-reducing power of *d*-glucose, invert sugar, and two mixtures of invert sugar and sucrose was determined. All weights of sugar and of cuprous oxide are expressed in terms of brass weights in air. The flasks and pipettes used were accurately graduated by the Bureau of Standards and all were very close to their indicated volumes. In no case was the error introduced by their use sufficient to take into consideration.

Pure *d*-glucose was prepared by repeatedly recrystallizing the commercial product. The material finally used showed for  $(\alpha)_D^{20}$  a value of 53.17, with a concentration of 20 per cent., which value corresponds very closely to that calculated according to Tollen's formula.

Pure sucrose was prepared according to the method prescribed by the International Commission for Unifying Methods of Sugar Analysis, and the product used was shown to be of the highest purity. Invert sugar was prepared from this sucrose by hydrolyzing with fifth-normal hydrochloric acid, using 10 cc. of this acid for each 100 cc. final volume of invert sugar solution and heating upon the steam-bath for thirty minutes. The solution was then cooled rapidly, the acid barely neutralized with fifth-normal sodium hydroxide, and the volume completed at the

TABLE I.

Number of set.	Individual blanks.		Average.	
	Munson. Mgs.	Walker. Mgs.	Munson. Mgs.	Walker. Mgs.
1	0.4	1.4	0.0	0.8
	-0.4	0.2		
2	1.0	-0.6	0.1	0.0
	-0.7	0.5		
3	0.5	0.0	0.3	0.0
	0.2	0.0		
4	0.0	-0.4	-0.3	-0.1
	-0.6	0.2		
5	0.4	-0.4	0.0	-0.2
	-0.4	0.0		
6	1.6	0.8	1.0	0.1
	0.5	-0.5		
7	0.3	0.2	0.3	0.4
	0.3	0.6		
8	0.8	...	0.3	0.6
	-0.2	0.6		
9	0.3	0.2	0.5	0.4
	0.8	0.6		
10	0.6	0.4	0.5	1.2
	0.4	2.0		
11	-0.5	1.0	-0.5	1.3
	-0.5	1.6		
12	1.3	0.4	1.1	0.6
	0.8	0.8		
13	0.9	1.0	0.7	0.8
	0.5	0.6		
14	0.5	0.1	0.4	-0.2
	0.3	-0.4		
15	-0.4	0.1	-0.1	0.5
	0.2	1.0		
16	0.0	0.0	0.0	0.0
	-0.1	0.0		
17	-0.3	-1.3	-0.2	-1.2
	-0.1	-1.0		

temperature of graduation of the flask. Care was taken to retain this temperature until all portions were measured out. In all cases at least two different solutions were used in establishing each point and duplicate determinations were made by each worker. Points were determined 20 mgs. apart, beginning with 20 mgs. for each of the sugars and mixtures. With the invert sugar and sucrose mixtures, the requisite amount of cane-sugar was obtained by measuring with a burette a sucrose solution of known concentration. Results of all determinations for the four series are given in Table II. From the averages for each series a curve was drawn upon cross-section paper and by use of these curves the individual determinations that were sufficiently far from the curve to be considered among the less accurate of the determinations made were eliminated from the final averages. While all determinations made for each point are recorded, greatest weight was given to those lying nearest the curve. Those determinations not included in final averages are indicated by a star.

When working with either *d*-glucose or invert sugar alone, the results obtained were very uniform and agreed within reasonable limits of experimental error. With the mixtures of invert sugar and sucrose, however, results were less uniform, especially where large amounts of sucrose were present. Two sugar mixtures were worked upon, one containing a total of 0.400 gram invert sugar and sucrose and the other a total of 2,000 grams. By reference to the tables it is seen that the presence of sucrose materially increases the reduction, the amount of increase being influenced primarily by the amount of sucrose present and secondarily by the amount of invert sugar present. The amount of sucrose remaining the same, its influence will be less when a large amount of reducing sugar is present than when only a small amount is present.

In using a definite amount of total sugar, the authors have followed the plan carried out by Herzfeld in his method of determining 1 per cent. or less of invert sugar in the presence of large amounts of sucrose, and it is believed that this method of procedure will give much more satisfactory results with a less amount of analytical work than does the method of Meissl and Hiller, upon the basis of which tables have been worked out for definite percentages of invert sugar and sucrose.

From each series of average results as given in Table II was deduced, according to the method of least squares, the formula for calculating reducing sugar from any weight of cuprous oxide within limits of the method. In these formulas  $y$ =cuprous oxide and  $x$ =reducing sugar.

For *d*-glucose,

$$y = 0.5614 + 2.3484 x - 0.001209 x^2.$$

For invert sugar alone,

$$y = -0.2460 + 2.2747 x - 0.001077 x^2.$$

For invert sugar and sucrose, 0.400 gram total,

$$y = 6.3886 + 2.2279 x - 0.0009703 x^2.$$

For invert sugar and sucrose, 2.000 grams total,

$$y = 20.6600 + 2.2021 x - 0.0009030 x^2.$$

From these formulas the points of each series corresponding to the determined points were calculated, and in Table III are given the determined and the calculated values of each series together with the differences between these values. With dextrose, invert sugar, and the invert sugar and sucrose mixture of 0.400 gram total, these differences are very small and negligible. The 2,000 grams mixture showed less concordance, although satisfactory results were obtained.

Table IV gives the sugar values for each series of reducing sugars for each milligram of cuprous oxide between 10 and 490. The values for points 10 milligrams apart, beginning with 10 milligrams of cuprous oxide, were calculated from the formula and all intermediate points were determined by interpolation. The copper equivalents of cuprous oxide given in the second column of this table were obtained by multiplying cuprous oxide by 0.88827.

When this work was taken up it was hoped that factors might be worked out for all of the more important reducing sugars and for such mixtures as seemed necessary. To date, however, only the factors for *d*-glucose, invert sugar and two mixtures of invert sugar and sucrose have been determined. In continuing this investigation it will be necessary to do further work on other invert sugar and sucrose mixtures. Maltose and lactose are other important reducing sugars for which the present methods of determination are not entirely satisfactory, and the authors hope to take up the work on these within the next few months.





TABLE II—Continued.

Reducing sugar, Mgs.	<i>d</i> -Glucose.			Invert sugar.			Invert sugar and sucrose —0.400 gram total.			Invert sugar and sucrose —2.000 grams total.		
	Weight of Cu <sub>2</sub> O.		Average result. Mgs.	Weight of Cu <sub>2</sub> O.		Average result. Mgs.	Weight of Cu <sub>2</sub> O.		Average result. Mgs.	Weight of Cu <sub>2</sub> O.		Average result. Mgs.
	Munson. Mgs.	Walker. Mgs.		Munson. Mgs.	Walker. Mgs.		Munson. Mgs.	Walker. Mgs.		Munson. mgs.	Walker. Mgs.	
140	305.1	305.2	305.2	296.9	297.2	297.3	300.2	299.5	300.0	310.8	311.2	311.2
	305.2	305.2	...	297.0	298.2	...	299.7	300.5	...	310.6	312.2	...
160	343.8*	345.7	345.2	334.8	335.0	335.1	337.4	337.6	337.9	347.7*	349.4	349.4
	345.0	345.5	...	335.5	335.2	...	337.4	339.2	...	348.9	349.8	...
	344.9	344.7	...	...	...	...	...	337.7	...	...	...	...
180	384.7	383.7	384.3	370.0*	375.2	374.6	375.0	375.7	375.5	388.8	388.4	388.6
	385.1	383.7	...	373.8	374.2	...	373.9*	376.0	...	388.7	388.6	...
	...	...	...	374.9	374.8	...	374.5	376.4	...	...	...	...
	...	...	...	...	...	...	374.9	375.4	...	...	...	...
	...	...	...	...	...	...	...	375.9	...	...	...	...
200	422.1	421.5	421.7	411.8	411.6	411.4	413.5	414.2	413.7	422.6	424.0	423.6
	421.3	421.7	...	410.9	411.4	...	412.8	414.4	...	423.2	424.4	...
220	459.0	460.5	459.8	449.0	450.5*	449.1	450.4	449.6	449.8	461.9	462.4	462.2
	459.5	460.2	...	449.0	449.4	...	448.7	450.6	...	463.2	461.2	...
240	493.9	493.8	493.8	482.5	483.7	483.1	481.7*	484.4	484.8	...	...	...
	493.5	494.0	...	483.1	483.2	...	483.2*	484.4	...	...	...	...
	...	...	...	...	...	...	485.0	485.3	...	...	...	...

TABLE III.

Reducing sugar, Mgs.	<i>d</i> -Glucose.			Invert sugar.			Invert sugar and sucrose —0.400 gram total.			Invert sugar and sucrose —2.0000 grams total.		
	Determined Cu <sub>2</sub> O, Mgs.	Calculated Cu <sub>2</sub> O, Mgs.	Differ- ence, Mgs.	Determined Cu <sub>2</sub> O, Mgs.	Calculated Cu <sub>2</sub> O, Mgs.	Differ- ence, Mgs.	Determined Cu <sub>2</sub> O, Mgs.	Calculated Cu <sub>2</sub> O, Mgs.	Differ- ence, Mg.	Determined Cu <sub>2</sub> O, Mgs.	Calculated Cu <sub>2</sub> O, Mgs.	Differ- ence, Mgs.
20	47.1	47.0	0.1	44.9	44.8	0.1	50.4	50.6	—0.2	64.5	64.3	0.2
40	92.6	92.6	0.0	89.0	89.0	0.0	94.9	94.0	0.9	106.7	107.3	—0.6
60	137.0	137.1	—0.1	132.3	132.5	—0.2	135.9	136.6	—0.7	149.1	149.5	—0.4
80	180.6	180.7	—0.1	174.9	174.9	0.0	178.0	178.4	—0.4	193.0	191.1	1.9
100	223.7	223.3	0.4	216.7	216.4	0.3	219.4	219.5	—0.1	230.7	231.8	—1.1
120	264.9	265.0	—0.1	257.0	257.2	—0.2	259.6	259.8	—0.2	272.3	271.9	0.4
140	305.2	305.6	—0.4	297.3	297.1	0.2	300.0	299.3	0.7	311.2	311.3	—0.1
160	345.2	345.4	—0.2	335.1	336.1	—1.0	337.9	338.0	—0.1	349.4	349.9	—0.5
180	384.3	384.1	0.2	374.6	374.3	0.3	375.5	376.0	—0.5	388.6	387.8	0.8
200	421.7	421.9	—0.2	411.4	411.6	—0.2	413.7	413.2	0.5	423.6	425.0	—1.4
220	459.8	458.7	1.1	449.1	448.0	1.1	449.8	449.6	0.2	462.2	461.4	0.8
240	493.8	494.5	—0.7	483.1	483.6	—0.5	484.8	485.2	—0.4	...	...	...
		Aver,	0.30			0.34			0.41			0.75

TABLE IV.

Cuprous oxide (Cu <sub>2</sub> O). Mgs.	Copper (Cu). Mgs.	$\alpha$ -Glucose. Mgs.	Invert sugar. Mgs.	Invert sugar and sucrose, 0.400 gram. Total mgs.	Invert sugar and sucrose, 2.000 grams. Total mgs.
10	8.9	4.0	4.5	1.6	
11	9.8	4.5	5.0	2.1	
12	10.7	4.9	5.4	2.5	
13	11.5	5.3	5.8	3.0	
14	12.4	5.7	6.3	3.4	
15	13.3	6.2	6.7	3.9	
16	14.2	6.6	7.2	4.3	
17	15.1	7.0	7.6	4.8	
18	16.0	7.5	8.1	5.2	
19	16.9	7.9	8.5	5.7	
20	17.8	8.3	8.9	6.1	
21	18.7	8.7	9.4	6.6	
22	19.5	9.2	9.8	7.0	
23	20.4	9.6	10.3	7.5	
24	21.3	10.0	10.7	7.9	
25	22.2	10.5	11.2	8.4	
26	23.1	10.9	11.6	8.8	
27	24.0	11.3	12.0	9.3	
28	24.9	11.8	12.5	9.7	
29	25.8	12.2	12.9	10.2	
30	26.6	12.6	13.4	10.7	4.3
31	27.5	13.1	13.8	11.1	4.7
32	28.4	13.5	14.3	11.6	5.2
33	29.3	13.9	14.7	12.0	5.6
34	30.2	14.3	15.2	12.5	6.1
35	31.1	14.8	15.6	12.9	6.5
36	32.0	15.2	16.1	13.4	7.0
37	32.9	15.6	16.5	13.8	7.4
38	33.8	16.1	16.9	14.3	7.9
39	34.6	16.5	17.4	14.7	8.4
40	35.5	16.9	17.8	15.2	8.8
41	36.4	17.4	18.3	15.6	9.3
42	37.3	17.8	18.7	16.1	9.7
43	38.2	18.2	19.2	16.6	10.2
44	39.1	18.7	19.6	17.0	10.7
45	40.0	19.1	20.1	17.5	11.1
46	40.9	19.6	20.5	17.9	11.6
47	41.7	20.0	21.0	18.4	12.0

TABLE IV—Continued.

Cuprous oxide (Cu <sub>2</sub> O), Mgs.	Copper (Cu), Mgs.	d-Glucose, Mgs.	Invert sugar, Mgs.	Invert sugar and sucrose, 0.400 gram. Total mgs.	Invert sugar and sucrose, 2.000 grams. Total mgs.
48	42.6	20.4	21.4	18.8	12.5
49	43.5	20.9	21.9	19.3	12.9
50	44.4	21.3	22.3	19.7	13.4
51	45.3	21.7	22.8	20.2	13.9
52	46.2	22.2	23.2	20.7	14.3
53	47.1	22.6	23.7	21.1	14.8
54	48.0	23.0	24.1	21.6	15.2
55	48.9	23.5	24.6	22.0	15.7
56	49.7	23.9	25.0	22.5	16.2
57	50.6	24.3	25.5	22.9	16.6
58	51.5	24.8	25.9	23.4	17.1
59	52.4	25.2	26.4	23.9	17.5
60	53.3	25.6	26.8	24.3	18.0
61	54.2	26.1	27.3	24.8	18.5
62	55.1	26.5	27.7	25.2	18.9
63	56.0	27.0	28.2	25.7	19.4
64	56.8	27.4	28.6	26.2	19.8
65	57.7	27.8	29.1	26.6	20.3
66	58.6	28.3	29.5	27.1	20.8
67	59.5	28.7	30.0	27.5	21.2
68	60.4	29.2	30.4	28.0	21.7
69	61.3	29.6	30.9	28.5	22.2
70	62.2	30.0	31.3	28.9	22.6
71	63.1	30.5	31.8	29.4	23.1
72	64.0	30.9	32.3	29.8	23.5
73	64.8	31.4	32.7	30.3	24.0
74	65.7	31.8	33.2	30.8	24.5
75	66.6	32.2	33.6	31.2	24.9
76	67.5	32.7	34.1	31.7	25.4
77	68.4	33.1	34.5	32.1	25.9
78	69.3	33.6	35.0	32.6	26.3
79	70.2	34.0	35.4	33.1	26.8
80	71.1	34.4	35.9	33.5	27.3
81	71.9	34.9	36.3	34.0	27.7
82	72.8	35.3	36.8	34.5	28.2
83	73.7	35.8	37.3	34.9	28.6
84	74.6	36.2	37.7	35.4	29.1

TABLE IV—*Continued.*

Cuprous oxide (Cu <sub>2</sub> O). Mgs.	Copper (Cu). Mgs.	d-Glucose. Mgs.	Invert sugar. Mgs.	Invert sugar and sucrose. 0.400 gram. Total mgs.	Invert sugar and sucrose. 2.0000 grams. Total mgs.
85	75.5	36.7	38.2	35.8	29.6
86	76.4	37.1	38.6	36.3	30.0
87	77.3	37.5	39.1	36.8	30.5
88	78.2	38.0	39.5	37.2	31.0
89	79.1	38.4	40.0	37.7	31.4
90	79.9	38.9	40.4	38.2	31.9
91	80.8	39.3	40.9	38.6	32.4
92	81.7	39.8	41.4	39.1	32.8
93	82.6	40.2	41.8	39.6	33.3
94	83.5	40.6	42.3	40.0	33.8
95	84.4	41.1	42.7	40.5	34.2
96	85.3	41.5	43.2	41.0	34.7
97	86.2	42.0	43.7	41.4	35.2
98	87.1	42.4	44.1	41.9	35.6
99	87.9	42.9	44.6	42.4	36.1
100	88.8	43.3	45.0	42.8	36.6
101	89.7	43.8	45.5	43.3	37.0
102	90.6	44.2	46.0	43.8	37.5
103	91.5	44.7	46.4	44.2	38.0
104	92.4	45.1	46.9	44.7	38.5
105	93.3	45.5	47.3	45.2	38.9
106	94.2	46.0	47.8	45.6	39.4
107	95.0	46.4	48.3	46.1	39.9
108	95.9	46.9	48.7	46.6	40.3
109	96.8	47.3	49.2	47.0	40.8
110	97.7	47.8	49.6	47.5	41.3
111	98.6	48.2	50.1	48.0	41.7
112	99.5	48.7	50.6	48.4	42.2
113	100.4	49.1	51.0	48.9	42.7
114	101.3	49.6	51.5	49.4	43.2
115	102.2	50.0	51.9	49.8	43.6
116	103.0	50.5	52.4	50.3	44.1
117	103.9	50.9	52.9	50.8	44.6
118	104.8	51.4	53.3	51.2	45.0
119	105.7	51.8	53.8	51.7	45.5
120	106.6	52.3	54.3	52.2	46.0
121	107.5	52.7	54.7	52.7	46.5

TABLE IV—*Continued.*

Cuprous oxide (Cu <sub>2</sub> O), Mgs.	Copper (Cu), Mgs.	<i>d</i> -Glucose, Mgs.	Invert sugar, Mgs.	Invert sugar and sucrose, 0.400 gram, Total mgs.	Invert sugar and sucrose, 2.000 grams, Total mgs.
122	108.4	53.2	55.2	53.1	46.9
123	109.3	53.6	55.7	53.6	47.4
124	110.1	54.1	56.1	54.1	47.9
125	111.0	54.5	56.6	54.5	48.3
126	111.9	55.0	57.0	55.0	48.8
127	112.8	55.4	57.5	55.5	49.3
128	113.7	55.9	58.0	55.9	49.8
129	114.6	56.3	58.4	56.4	50.2
130	115.5	56.8	58.9	56.9	50.7
131	116.4	57.2	59.4	57.4	51.2
132	117.3	57.7	59.8	57.8	51.7
133	118.1	58.1	60.3	58.3	52.1
134	119.0	58.6	60.8	58.8	52.6
135	119.9	59.0	61.2	59.3	53.1
136	120.8	59.5	61.7	59.7	53.6
137	121.7	60.0	62.2	60.2	54.0
138	122.6	60.4	62.6	60.7	54.5
139	123.5	60.9	63.1	61.2	55.0
140	124.4	61.3	63.6	61.6	55.5
141	125.2	61.8	64.0	62.1	55.9
142	126.1	62.2	64.5	62.6	56.4
143	127.0	62.7	65.0	63.1	56.9
144	127.9	63.1	65.4	63.5	57.4
145	128.8	63.6	65.9	64.0	57.8
146	129.7	64.0	66.4	64.5	58.3
147	130.6	64.5	66.9	65.0	58.8
148	131.5	65.0	67.3	65.4	59.3
149	132.4	65.4	67.8	65.9	59.7
150	133.2	65.9	68.3	66.4	60.2
151	134.1	66.3	68.7	66.9	60.7
152	135.0	66.8	69.2	67.3	61.2
153	135.9	67.2	69.7	67.8	61.7
154	136.8	67.7	70.1	68.3	62.1
155	137.7	68.2	70.6	68.8	62.6
156	138.6	68.6	71.1	69.2	63.1
157	139.5	69.1	71.6	69.7	63.6
158	140.3	69.5	72.0	70.2	64.1

TABLE IV—Continued.

Cuprous oxide (Cu <sub>2</sub> O). Mgs.	Copper (Cu). Mgs.	d-Glucose. Mgs.	Invert sugar. Mgs.	Invert sugar and sucrose. 0.400 gram. Total mgs.	Invert sugar and sucrose. 2.000 grams. Total mgs.
159	141.2	70.0	72.5	70.7	64.5
160	142.1	70.4	73.0	71.2	65.0
161	143.0	70.9	73.4	71.6	65.5
162	143.9	71.4	73.9	72.1	66.0
163	144.8	71.8	74.4	72.6	66.5
164	145.7	72.3	74.9	73.1	66.9
165	146.6	72.8	75.3	73.6	67.4
166	147.5	73.2	75.8	74.0	67.9
167	148.3	73.7	76.3	74.5	68.4
168	149.2	74.1	76.8	75.0	68.9
169	150.1	74.6	77.2	75.5	69.3
170	151.0	75.1	77.7	76.0	69.8
171	151.9	75.5	78.2	76.4	70.3
172	152.8	76.0	78.7	76.9	70.8
173	153.7	76.4	79.1	77.4	71.3
174	154.6	76.9	79.6	77.9	71.7
175	155.5	77.4	80.1	78.4	72.2
176	156.3	77.8	80.6	78.8	72.7
177	157.2	78.3	81.0	79.3	73.2
178	158.1	78.8	81.5	79.8	73.7
179	159.0	79.2	82.0	80.3	74.2
180	159.9	79.7	82.5	80.8	74.6
181	160.8	80.1	82.9	81.3	75.1
182	161.7	80.6	83.4	81.7	75.6
183	162.6	81.1	83.9	82.2	76.1
184	163.4	81.5	84.4	82.7	76.6
185	164.3	82.0	84.9	83.2	77.1
186	165.2	82.5	85.3	83.7	77.6
187	166.1	82.9	85.8	84.2	78.0
188	167.0	83.4	86.3	84.6	78.5
189	167.9	83.9	86.8	85.1	79.0
190	168.8	84.3	87.2	85.6	79.5
191	169.7	84.8	87.7	86.1	80.0
192	170.5	85.3	88.2	86.6	80.5
193	171.4	85.7	88.7	87.1	81.0
194	172.3	86.2	89.2	87.6	81.4



TABLE IV—Continued.

Cuprous oxide. (Cu <sub>2</sub> O). Mgs.	Copper (Cu). Mgs.	<i>p</i> -Glucose. Mgs.	Invert sugar. Mgs.	Invert sugar and sucrose. 0.400 gram. Total mgs.	Invert sugar and sucrose. 2.0000 grams. Total mgs.
195	173.2	86.7	89.6	88.0	81.9
196	174.1	87.1	90.1	88.5	82.4
197	175.0	87.6	90.6	89.0	82.9
198	175.9	88.1	91.1	89.5	83.4
199	176.8	88.5	91.6	90.0	83.9
200	177.7	89.0	92.0	90.5	84.4
201	178.5	89.5	92.5	91.0	84.8
202	179.4	89.9	93.0	91.4	85.3
203	180.3	90.4	93.5	91.9	85.8
204	181.2	90.9	94.0	92.4	86.3
205	182.1	91.4	94.5	92.9	86.8
206	183.0	91.8	94.9	93.4	87.3
207	183.9	92.3	95.4	93.9	87.8
208	184.8	92.8	95.9	94.4	88.3
209	185.6	93.2	96.4	94.9	88.8
210	186.5	93.7	96.9	95.4	89.2
211	187.4	94.2	97.4	95.8	89.7
212	188.3	94.6	97.8	96.3	90.2
213	189.2	95.1	98.3	96.8	90.7
214	190.1	95.6	98.8	97.3	91.2
215	191.0	96.1	99.3	97.8	91.7
216	191.9	96.5	99.8	98.3	92.2
217	192.8	97.0	100.3	98.8	92.7
218	193.6	97.5	100.8	99.3	93.2
219	194.5	98.0	101.2	99.8	93.7
220	195.4	98.4	101.7	100.3	94.2
221	196.3	98.9	102.2	100.8	94.7
222	197.2	99.4	102.7	101.2	95.1
223	198.1	99.9	103.2	101.7	95.6
224	199.0	100.3	103.7	102.2	96.1
225	199.9	100.8	104.2	102.7	96.6
226	200.7	101.3	104.6	103.2	97.1
227	201.6	101.8	105.1	103.7	97.6
228	202.5	102.2	105.6	104.2	98.1
229	203.4	102.7	106.1	104.7	98.6
230	204.3	103.2	106.6	105.2	99.1
231	205.2	103.7	107.1	105.7	99.6

TABLE IV—Continued.

Cuprous oxide (Cu <sub>2</sub> O). Mgs.	Copper (Cu). Mgs.	d-Glucose. Mgs.	Invert sugar. Mgs.	Invert sugar and sucrose. 0.400 gram. Total mgs.	Invert sugar and sucrose. 2.0000 grams. Total mgs.
232	206.1	104.1	107.6	106.2	100.1
233	207.0	104.6	108.1	106.7	100.6
234	207.9	105.1	108.6	107.2	101.1
235	208.7	105.6	109.1	107.7	101.6
236	209.6	106.0	109.5	108.2	102.1
237	210.5	106.5	110.0	108.7	102.6
238	211.4	107.0	110.5	109.2	103.1
239	212.3	107.5	111.0	109.6	103.5
240	213.2	108.0	111.5	110.1	104.0
241	214.1	108.4	112.0	110.6	104.5
242	215.0	108.9	112.5	111.1	105.0
243	215.8	109.4	113.0	111.6	105.5
244	216.7	109.9	113.5	112.1	106.0
245	217.6	110.4	114.0	112.6	106.5
246	218.5	110.8	114.5	113.1	107.0
247	219.4	111.3	115.0	113.6	107.5
248	220.3	111.8	115.4	114.1	108.0
249	221.2	112.3	115.9	114.6	108.5
250	222.1	112.8	116.4	115.1	109.0
251	223.0	113.2	116.9	115.6	109.5
252	223.8	113.7	117.4	116.1	110.0
253	224.7	114.2	117.9	116.6	110.5
254	225.6	114.7	118.4	117.1	111.0
255	226.5	115.2	118.9	117.6	111.5
256	227.4	115.7	119.4	118.1	112.0
257	228.3	116.1	119.9	118.6	112.5
258	229.2	116.6	120.4	119.1	113.0
259	230.1	117.1	120.9	119.6	113.5
260	231.0	117.6	121.4	120.1	114.0
261	231.8	118.1	121.9	120.6	114.5
262	232.7	118.6	122.4	121.1	115.0
263	233.6	119.0	122.9	121.6	115.5
264	234.5	119.5	123.4	122.1	116.0
265	235.4	120.0	123.9	122.6	116.5
266	236.3	120.5	124.4	123.1	117.0
267	237.2	121.0	124.9	123.6	117.5
268	238.1	121.5	125.4	124.1	118.0

TABLE IV—*Continued.*

Cuprous oxide (Cu <sub>2</sub> O). Mgs.	Copper (Cu). Mgs.	d-Glucose. Mgs.	Invert sugar. Mgs.	Invert sugar and sucrose, 0.400 gram. Total mgs.	Invert sugar and sucrose, 2.0000 grams. Total mgs.
269	238.9	122.0	125.9	124.6	118.5
270	239.8	122.5	126.4	125.1	119.0
271	240.7	122.9	126.9	125.6	119.5
272	241.6	123.4	127.4	126.2	120.0
273	242.5	123.9	127.9	126.7	120.6
274	243.4	124.4	128.4	127.2	121.1
275	244.3	124.9	128.9	127.7	121.6
276	245.2	125.4	129.4	128.2	122.1
277	246.1	125.9	129.9	128.7	122.6
278	246.9	126.4	130.4	129.2	123.1
279	247.8	126.9	130.9	129.7	123.6
280	248.7	127.3	131.4	130.2	124.1
281	249.6	127.8	131.9	130.7	124.6
282	250.5	128.3	132.4	131.2	125.1
283	251.4	128.8	132.9	131.7	125.6
284	252.3	129.3	133.4	132.2	126.1
285	253.2	129.8	133.9	132.7	126.6
286	254.0	130.3	134.4	133.2	127.1
287	254.9	130.8	134.9	133.7	127.6
288	255.8	131.3	135.4	134.3	128.1
289	256.7	131.8	135.9	134.8	128.6
290	257.6	132.3	136.4	135.3	129.2
291	258.5	132.7	136.9	135.8	129.7
292	259.4	133.2	137.4	136.3	130.2
293	260.3	133.7	137.9	136.8	130.7
294	261.2	134.2	138.4	137.3	131.2
295	262.0	134.7	138.9	137.8	131.7
296	262.9	135.2	139.4	138.3	132.2
297	263.8	135.7	140.0	138.8	132.7
298	264.7	136.2	140.5	139.4	133.2
299	265.6	136.7	141.0	139.9	133.7
300	266.5	137.2	141.5	140.4	134.2
301	267.4	137.7	142.0	140.9	134.8
302	268.3	138.2	142.5	141.4	135.3
303	269.1	138.7	143.0	141.9	135.8
304	270.0	139.2	143.5	142.4	136.3
305	270.9	139.7	144.0	142.9	136.8

TABLE IV—*Continued.*

Cuprous oxide (Cu <sub>2</sub> O). Mgs.	Copper (Cu). Mgs.	d-Glucose. Mgs.	Invert sugar. Mgs.	Invert sugar and sucrose. 0.400 gram. Total mgs.	Invert sugar and sucrose. 2.000 grams. Total mgs.
306	271.8	140.2	144.5	143.4	137.3
307	272.7	140.7	145.0	144.0	137.8
308	273.6	141.2	145.5	144.5	138.3
309	274.5	141.7	146.1	145.0	138.8
310	275.4	142.2	146.6	145.5	139.4
311	276.3	142.7	147.1	146.0	139.9
312	277.1	143.2	147.6	146.5	140.4
313	278.0	143.7	148.1	147.0	140.9
314	278.9	144.2	148.6	147.6	141.4
315	279.8	144.7	149.1	148.1	141.9
316	280.7	145.2	149.6	148.6	142.4
317	281.6	145.7	150.1	149.1	143.0
318	282.5	146.2	150.7	149.6	143.5
319	283.4	146.7	151.2	150.1	144.0
320	284.2	147.2	151.7	150.7	144.5
321	285.1	147.7	152.2	151.2	145.0
322	286.0	148.2	152.7	151.7	145.5
323	286.9	148.7	153.2	152.2	146.0
324	287.8	149.2	153.7	152.7	146.6
325	288.7	149.7	154.3	153.2	147.1
326	289.6	150.2	154.8	153.8	147.6
327	290.5	150.7	155.3	154.3	148.1
328	291.4	151.2	155.8	154.8	148.6
329	292.2	151.7	156.3	155.3	149.1
330	293.1	152.2	156.8	155.8	149.7
331	294.0	152.7	157.3	156.4	150.2
332	294.9	153.2	157.9	156.9	150.7
333	295.8	153.7	158.4	157.4	151.2
334	296.7	154.2	158.9	157.9	151.7
335	297.6	154.7	159.4	158.4	152.3
336	298.5	155.2	159.9	159.0	152.8
337	299.3	155.8	160.5	159.5	153.3
338	300.2	156.3	161.0	160.0	153.8
339	301.1	156.8	161.5	160.5	154.3
340	302.0	157.3	162.0	161.0	154.8
341	302.9	157.8	162.5	161.6	155.4
342	303.8	158.3	163.1	162.1	155.9

TABLE IV—Continued.

Cuprous oxide (Cu <sub>2</sub> O). Mgs.	Copper (Cu). Mgs.	d-Glucose. Mgs.	Invert sugar. Mgs.	Invert sugar and sucrose. 0.400 gram. Total Mgs.	Invert sugar and sucrose. 2.0000 grams. Total mgs.
343	304.7	158.8	163.6	162.6	156.4
344	305.6	159.3	164.1	163.1	156.9
345	306.5	159.8	164.6	163.7	157.5
346	307.3	160.3	165.1	164.2	158.0
347	308.2	160.8	165.7	164.7	158.5
348	309.1	161.4	166.2	165.2	159.0
349	310.0	161.9	166.7	165.7	159.5
350	310.9	162.4	167.2	166.3	160.1
351	311.8	162.9	167.7	166.8	160.6
352	312.7	163.4	168.3	167.3	161.1
353	313.6	163.9	168.8	167.8	161.6
354	314.4	164.4	169.3	168.4	162.2
355	315.3	164.9	169.8	168.9	162.7
356	316.2	165.4	170.4	169.4	163.2
357	317.1	166.0	170.9	170.0	163.7
358	318.0	166.5	171.4	170.5	164.3
359	318.9	167.0	171.9	171.0	164.8
360	319.8	167.5	172.5	171.5	165.3
361	320.7	168.0	173.0	172.1	165.8
362	321.6	168.5	173.5	172.6	166.4
363	322.4	169.0	174.0	173.1	166.9
364	323.3	169.6	174.6	173.7	167.4
365	324.2	170.1	175.1	174.2	167.9
366	325.1	170.6	175.6	174.7	168.5
367	326.0	171.1	176.1	175.2	169.0
368	326.9	171.6	176.7	175.8	169.5
369	327.8	172.1	177.2	176.3	170.0
370	328.7	172.7	177.7	176.8	170.6
371	329.5	173.2	178.3	177.4	171.1
372	330.4	173.7	178.8	177.9	171.6
373	331.3	174.2	179.3	178.4	172.2
374	332.2	174.7	179.8	179.0	172.7
375	333.1	175.3	180.4	179.5	173.2
376	334.0	175.8	180.9	180.0	173.7
377	334.9	176.3	181.4	180.6	174.3
378	335.8	176.8	182.0	181.1	174.8
379	336.7	177.3	182.5	181.6	175.3

TABLE IV—*Continued.*

Cuprous oxide (Cu <sub>2</sub> O). Mgs.	Copper (Cu). Mgs.	d-Glucose. Mgs.	Invert sugar. Mgs.	Invert sugar and sucrose. 0.400 gram. Total mgs.	Invert sugar and sucrose. 2.000 grams. Total mgs.
380	337.5	177.9	183.0	182.1	175.9
381	338.4	178.4	183.6	182.7	176.4
382	339.3	178.9	184.1	183.2	176.9
383	340.2	179.4	184.6	183.8	177.5
384	341.1	180.0	185.2	184.3	178.0
385	342.0	180.5	185.7	184.8	178.5
386	342.9	181.0	186.2	185.4	179.1
387	343.8	181.5	186.8	185.9	179.6
388	344.6	182.0	187.3	186.4	180.1
389	345.5	182.6	187.8	187.0	180.6
390	346.4	183.1	188.4	187.5	181.2
391	347.3	183.6	188.9	188.0	181.7
392	348.2	184.1	189.4	188.6	182.3
393	349.1	184.7	190.0	189.1	182.8
394	350.0	185.2	190.5	189.7	183.3
395	350.9	185.7	191.0	190.2	183.9
396	351.8	186.2	191.6	190.7	184.4
397	352.6	186.8	192.1	191.3	184.9
398	353.5	187.3	192.7	191.8	185.5
399	354.4	187.8	193.2	192.3	186.0
400	355.3	188.4	193.7	192.9	186.5
401	356.2	188.9	194.3	193.4	187.1
402	357.1	189.4	194.8	194.0	187.6
403	358.0	189.9	195.4	194.5	188.1
404	358.9	190.5	195.9	195.0	188.7
405	359.7	191.0	196.4	195.6	189.2
406	360.6	191.5	197.0	196.1	189.8
407	361.5	192.1	197.5	196.7	190.3
408	362.4	192.6	198.1	197.2	190.8
409	363.3	193.1	198.6	197.7	191.4
410	364.2	193.7	199.1	198.3	191.9
411	365.1	194.2	199.7	198.8	192.5
412	366.0	194.7	200.2	199.4	193.0
413	366.9	195.2	200.8	199.9	193.5
414	367.7	195.8	201.3	200.5	194.1
415	368.6	196.3	201.8	201.0	194.6
416	369.5	196.8	202.4	201.6	195.2

TABLE IV—Continued.

Cuprous oxide (Cu <sub>2</sub> O). Mgs.	Copper (Cu). Mgs.	d-Glucose. Mgs.	Invert sugar. Mgs.	Invert sugar and sucrose. 0.400 gram. Total mgs.	Invert sugar and sucrose. 2.000 grams. Total mgs.
417	370.4	197.4	202.9	202.1	195.7
418	371.3	197.9	203.5	202.6	196.2
419	372.2	198.4	204.0	203.2	196.8
420	373.1	199.0	204.6	203.7	197.3
421	374.0	199.5	205.1	204.3	197.9
422	374.8	200.1	205.7	204.8	198.4
423	375.7	200.6	206.2	205.4	198.9
424	376.6	201.1	206.7	205.9	199.5
425	377.5	201.7	207.3	206.5	200.0
426	378.4	202.2	207.8	207.0	200.6
427	379.3	202.8	208.4	207.6	201.1
428	380.2	203.3	208.9	208.1	201.7
429	381.1	203.8	209.5	208.7	202.2
430	382.0	204.4	210.0	209.2	202.7
431	382.8	204.9	210.6	209.8	203.3
432	383.7	205.5	211.1	210.3	203.8
433	384.6	206.0	211.7	210.9	204.4
434	385.5	206.5	212.2	211.4	204.9
435	386.4	207.1	212.8	212.0	205.5
436	387.3	207.6	213.3	212.5	206.0
437	388.2	208.2	213.9	213.1	206.6
438	389.1	208.7	214.4	213.6	207.1
439	390.0	209.2	215.0	214.2	207.7
440	390.8	209.8	215.5	214.7	208.2
441	391.7	210.3	216.1	215.3	208.8
442	392.6	210.9	216.6	215.8	209.3
443	393.5	211.4	217.2	216.4	209.9
444	394.4	212.0	217.8	216.9	210.4
445	395.3	212.5	218.3	217.5	211.0
446	396.2	213.1	218.9	218.0	211.5
447	397.1	213.6	219.4	218.6	212.1
448	397.9	214.1	220.0	219.1	212.6
449	398.8	214.7	220.5	219.7	213.2
450	399.7	215.2	221.1	220.2	213.7
451	400.6	215.8	221.6	220.8	214.3
452	401.5	216.3	222.2	221.4	214.8
453	402.4	216.9	222.8	221.9	215.4

## REDUCING SUGAR METHODS.

TABLE IV—Continued.

Cuprous oxide (Cu <sub>2</sub> O). Mgs.	Copper (Cu). Mgs.	d-Glucose. Mgs.	Invert sugar. Mgs.	Invert sugar and sucrose. 0.400 gram. Total mgs.	Invert sugar and sucrose. 2,000 grams. Total mgs.
454	403.3	217.4	223.3	222.5	215.9
455	404.2	218.0	223.9	223.0	216.5
456	405.1	218.5	224.4	223.6	217.0
457	405.9	219.1	225.0	224.1	217.6
458	406.8	219.6	225.5	224.7	218.1
459	407.7	220.2	226.1	225.3	218.7
460	408.6	220.7	226.7	225.8	219.2
461	409.5	221.3	227.2	226.4	219.8
462	410.4	221.8	227.8	226.9	220.3
463	411.3	222.4	228.3	227.5	220.9
464	412.2	222.9	228.9	228.1	221.4
465	413.0	223.5	229.5	228.6	222.0
466	413.9	224.0	230.0	229.2	222.5
467	414.8	224.6	230.6	229.7	223.1
468	415.7	225.1	231.2	230.3	223.7
469	416.6	225.7	231.7	230.9	224.2
470	417.5	226.2	232.3	231.4	224.8
471	418.4	226.8	232.8	232.0	225.3
472	419.3	227.4	233.4	232.5	225.9
473	420.2	227.9	234.0	233.1	226.4
474	421.0	228.5	234.5	233.7	227.0
475	421.9	229.0	235.1	234.2	227.6
476	422.8	229.6	235.7	234.8	228.1
477	423.7	230.1	236.2	235.4	228.7
478	424.6	230.7	236.8	235.9	229.2
479	425.5	231.3	237.4	236.5	229.8
480	426.4	231.8	237.9	237.1	230.3
481	427.3	232.4	238.5	237.6	230.9
482	428.1	232.9	239.1	238.2	231.5
483	429.0	233.5	239.6	238.8	232.0
484	429.9	234.1	240.2	239.3	232.6
485	430.8	234.6	240.8	239.9	233.2
486	431.7	235.2	241.4	240.5	233.7
487	432.6	235.7	241.9	241.0	234.3
488	433.5	236.3	242.5	241.6	234.8
489	434.4	236.9	243.1	242.2	235.4
490	435.3	237.4	243.6	242.7	236.0