Diethylcyanoaceto-*p*-toluidide was prepared in the same manner as diethylcyanoacetanilide. Analysis of the salt gave the following result:

Calculated for $C_{14}H_{18}N_2O$:	C, 73.11; H, 7.88.
Found:	C, 72.97; H, 7.55.

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June, 1911.

[CONTRIBUTION FROM THE NUTRITION LABORATORY OF THE CARNEGIE INSTITUTION OF WASHINGTON, BOSTON, MASSACHUSETTS.]

A CRITICAL STUDY OF SUGAR ANALYSIS BY COPPER REDUCTION METHODS.

BY AMOS W. PETERS.

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I. Introduction.

The present examination of the processes for the determination of sugar by copper reduction methods had its origin in the needs of this laboratory for numerous daily estimations of sugar in urin. In consequence of the dual requirement of *rapidity and accuracy* none of the methods examined was found so highly satisfactory as to be adopted without modification. The object of the present work was not to devise a new method of sugar analysis but no less effort was finally required to adapt known procedures to both accuracy and speed and particularly to provide the controls that would guarantee the results obtained. The chief end accomplished, it is hoped, has been the rational selection and the detailed quantitative standardization of the conditions so that exact results can be obtained with rapidity and reliability and with practical freedom from the subjective factor.

The considerations and criticism resulting from an experimental examination of some current methods may be reviewed as follows. A first principle for the selection of a method that is to be rapid is that only volumetric methods can come into consideration. Therefore there remains on this point only the question of the relative accuracy of gravimetric and volumetric estimations of sugar.

Different investigators, *e. g.*, Munson and Walker,[1] pp. 667–668, and Pflüger,[2] pp. 470–471, have shown that the weight of the asbestos filtering apparatus used in the gravimetric method is not constant on account of solution of asbestos and self-reduction of reagents and requires a correction when this can be determined. Others[3] working on urin or other

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[1] Munson, L. S., and Walker, P. H., "The Unification of Reducing Sugar Methods," THIS JOURNAL, 28, 663–683 (1906).

[2] Pflüger, E., *Arch. ges. Physiol.*, 69, 399–471 (1898).

[3] Neubauer-Huppert, *Anal. des Harns*, 11 Aufl., Wiesbaden, 1910, pp. 411–12; also Pflüger, *Loc. cit.*

practical cases note the fact that the weighed precipitate may contain *undeterminable* amounts of material other than cuprous oxide. The superiority of the volumetric method is evident here if only its accuracy can be guaranteed and to the latter question the present work has been specially directed. The doubt regarding the volumetric procedure could pertain only to the measurement of the reduced copper for the method of reduction remains unchanged by the adoption of the volumetric method. An examination[1] of the volumetric estimations of the copper of alkalin tartrate solutions from sugar analyses and a comparison of the methods used for this purpose by various authors showed that the above doubt of accuracy or of agreement with gravimetric or electrolytic results might very reasonably be entertained. The consequent necessary preliminary of accurate volumetric copper determinations under the conditions of sugar analysis has already been dealt with in the paper cited, to which reference is made for details. In view of the facts there developed it was quite necessary to test for accuracy such methods of sugar analysis as were already volumetric or to adapt gravimetric methods which were accurate to volumetric procedure. The effort to do this also showed that the conditions of reduction in nearly all cases lacked such definit delimitation as to give assurance of constancy of reduction results. The experimental part of the present paper begins with a study of the conditions of reduction the accurate measure of which is based on the preceding paper on copper analysis. Thus the present work seemed justifiable from the standpoint of speed as the next and important requirement after accuracy, as well as the primary necessity of the latter in the volumetric procedure. The following observations, limited to the above point of view, made on several methods of sugar analysis may serve to make the present standpoint clearer.

The detailed investigation of Pflüger[2] on his accurately controlled method of sugar analysis very well exemplifies the principle of the above contention. The method is gravimetrically and volumetrically controlled using for the latter procedure the sulfocyanide method for the estimation of copper. The difficulties that escape unnoticed in the ready and superficial application of any method of copper analysis to the above purposes are here well developed. However, the method of Pflüger requires the heating of the reduction mixture for one-half hour on the boiling water bath and it was intended to be primarily a gravimetric method. Since the reduction process is practically never brought to completion, and in view of the length of time required by this method, it does not satisfy the requirement above made.

In view of the criticisms which Pflüger[3] has made on the Allihn method,

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[1] Peters, A. W., THIS JOURNAL, 34, 422 54 (1912).

[2] *Loc. cit.*

[3] *Loc. cit.*, pp. 399.

of which Pflüger's was a modification, and as the Allihn method is gravimetric, it will not be further considered. If Pflüger's criticisms are correct then the various methods for using the Allihn table volumetrically[1] do not remove these objections. Further criticisms will not be made on methods recommended for gravimetric use by their authors but which are stated to have been checked by volumetric procedure also.

Of independent volumetric methods that of Bertrand[2] exceeds all its predecessors in reliability. Like the gravimetric method of Munson and Walker it also has the advantage of using the same reagents and the same conditions for the different kinds of sugar. This excellent procedure is characterized by the solution of the filtered cuprous oxide by a ferric sulfate solution and the volumetric estimation of the reduced iron by permanganate. If the direct estimation of the reduced copper is correct it is difficult to see why the estimation of the unreduced copper in the filtrate (necessarily by a different method than the permanganate, *e. g.*, the iodide) is not equally accurate. The latter procedure which has been adopted in the present work is more rapid than the former by which it is first necessary to dissolve the precipitate. The residual method by estimation of the filtrate requires of course a blank determination on the reagents but this blank need be determined only at intervals. This determination is conducive to accuracy for it prevents the analyst from calculating the self-reduction of the reagents as sugar. Moreover in the Bertrand method a similar estimation for the self-reduction of the reagents is necessary for accuracy and certainty even though not required by the author of the method. All authors aiming at the highest accuracy have observed this precaution even though the error of self-reduction was very small. It is particularly unfortunate that in devising the Bertrand method a new set of reagents having a different quantitative composition from those in long established use should have been selected. All such reagents are empirical and there is no evidence of any adequate advantage due to the alterations in concentration adopted by Bertrand. Other methods of copper estimation can be applied with the use of the Bertrand reagents and *vice versa* the permanganate method of Bertrand could have been successfully applied with the customary Fehling or Allihn solutions. The custom of most authors of reporting the original data on which their tabular values are based was not observed by Bertrand, and the operator is thus deprived of judging by internal evidence of the probable limits of error of the procedure.

Some observations on the method of Kendall[3] by which larger amounts

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[1] U. S. Dept. Agr., Bur. Chem., *Bull.* 107, 51–53 (1907).

[2] Bertrand, M. G., "Le dosage des sucres réducteurs," *Rev. soc. hygiène aliment.*, 5, 781–793 (1908).

[3] E. C. Kendall, THIS JOURNAL, 34, 317–41 (1912).

of reduced copper are obtained and involving a heating period of twenty minutes will be made in connection with the discussion of the time of reduction on p. 940.

II. The Process of Reduction.

A comparison of the very numerous methods proposed for the determination of sugar by the reduction of alkalin copper solutions shows great variation in the time prescribed for heating the reduction mixture. This time ranges from that required to heat to boiling, to a period of thirty minutes heating in a water bath as in the method of Pflüger.[1] The favorite time seems to be two, three and five minutes for a given sugar, usually dextrose, and in most methods somewhat different time for other sugars. The reason for the selection of any given time is rarely if ever stated by any author and seems to have been absolutely arbitrary. The time, however, is almost always reckoned from the beginning of boiling. Experiment shows that it is practically impossible to determin by direct visual examination exactly when the reduction mixture begins to boil. Bubbles of gas escape at irregular intervals at first and at localized portions of the surface of the liquid before real boiling takes place. There is a time interval of easily measurable extent during which the observer cannot certainly decide at what point he shall begin to reckon the time. To substitute the criterion of "bubbling" instead of "boiling" only transfers the same difficulty. Only methods in which the time of heating is very long as, for instance, one-half hour in the method of Pflüger above cited, minimize this initial error so that it becomes *with certainty* negligible. These considerations of time and heating power become still more significant when it is recalled that the reduction process has thus far not been quantitatively defined by chemical equations and that it appears never to be absolutely complete for a given amount of sugar in the presence of an excess of copper. All these circumstances indicate that if accuracy and rapidity are sought it is necessary to examin the conditions of reduction to determin what their significance is or that they are negligible. In any case these conditions ought to be so standardized that different methods can be compared and so as to make the same method accurately reproducible by different operators.

Standardization of Heating Power.

In order to compare the heating powers used in the reduction process the indications of a mercury thermometer were taken as the sole indication of temperature conditions within the heated liquid regardless of ebullition of any nature. The mechanical arrangement which was adopted for the measurement of the heating power by means of a thermometer and a definit quantity of water is described below (p. 944).

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[1] E. Pflüger, *Pfluger's Arch.*, **89**, 344 (1898).

The criterion adopted for the measurement of any given heating power obtained with the described mechanical arrangements was *the time in seconds required to raise the temperature of 60 cc. of distilled water through the temperature interval extending from 35–95°*. For reduction experiments the standard heating power which was arbitrarily selected was such as to produce the above temperature change in *distilled water* in 120 seconds. When flasks of different size or form or other different conditions were to be compared *the product of grams of water (60) by time in seconds was divided by the area of the base of the vessel used in square centimeters*. By this method of calculation results were obtained which were proportional to the heating surface, since the approximate weight of water heated and the temperature interval were held constant. When the same size and form of flask were in continuous use no calculation was necessary, the heating powers then being simply inversely proportional to the number of seconds required to produce the temperature interval. The volume of 60 cc. was the same as was used in the presence of sugar and the tests of heating power were always made in this volume. Any other constant volume of from 50–100 cc. would have served equally well. The temperature interval was purposely selected to begin at a point somewhat above room temperature and to end below the boiling point, as it was the longest interval showing a very regular rise of the mercury thread with sharply demarcated beginning and end.

The members of a series of determinations made upon the same heating power differ from each other by 2–5 seconds with the use of a seconds hand, and only 1–2 seconds with a stop-watch. When the mechanical heating arrangements above described had once been set it was observed that the heating power varied but little from day to day, which of course would depend largely upon constancy, within undetermined limits, of the gas pressure or of the length of flame used. If, however, another operator assembled and set up the heating arrangement at random, *i. e.*, without quantitative direction, it was observed that repetitions of this procedure showed considerable variations in the heating powers obtained by this method or rather lack of method. This fact has a very evident significance for any one who devises a method of sugar analysis or makes a table of reduction values for the use of other operators or of other laboratories.

The numerous tests made on heating powers showed that it is fully practicable with ordinary laboratory facilities to produce and maintain a definit, measured heating power accurate to within a very small deviation from a given standard. The results of some experiments will now be given in which are compared the effects of a constant heating power upon 60 cc. of distilled water and upon the same value of alkalin tartrate solution containing dextrose. The latter was the reduction mixture regularly used in sugar analysis and consisted of 20 cc. of Allihn's alkalin tartrate solution, 20 cc. of Fehling's copper sulfate solution and 20 cc. of distilled water containing 100 mg. of pure dextrose. The tests were made with the previously described mechanical arrangements. In some experiments the times and temperatures were taken by two different observers working together.

60 cc. OF DISTILLED WATER.

	Time, seconds.		
Temp.	I.	II.	III.
35°	0	0	0
70°	63	61	62
95°	115	116	..
100°	...	...	11
102.5°	...	...	20
104°	...	...	40

The last preceding experiment was made principally to determin the conditions which prevailed at about 20 seconds subsequent to the time when a temperature of 95° is reached. This point is studied because it is of critical importance in the method of sugar analysis. It was frequently observed that *20 seconds beyond 95° very closely marked the maximum time during which the mercury thread rose regularly though rapidly.* Higher temperatures were reached subsequently but with much fluctuation up and down of the mercury, so that the thermometric indications no longer bore a proportional relation to the duration of heating. The temperature at the end of the 20 seconds and just before the fluctuation begins was frequently observed to be about 102.5° on the same thermometer. The rise in temperature was too rapid to be timed accurately. This rise above 100° and the fluctuations are of course related to the partial stoppering of the conical flask and perhaps to other features of the mechanical arrangement. The conditions in no way interfere with the maintenance of a standard heating power as previously defined. They do show, however, where the regularity of time and temperature as indices of heating effects ceases.

Similar experiments were now made with the reduction mixture above described with the following results. Experiment No. V was made on a standardized heating power which raised the temperature of 60 cc. of distilled water from 35–95° in 120 seconds.

100 MG. DEXTROSE, ETC.

	Time, seconds.			
Temp.	I.	II.	III.	
35°	0		0	
70°	53	57	56	
95°	95	100	102	
100°	..	...	111	
102.5°	..	...	*x*	Fluctuation begins.
104.5°	..	...	*x*	Maximum.

100 MG. DEXTROSE, ETC. (*continued*).

Temp.	Time, seconds. IV.		Time, seconds. V.	
35°	0		0	
70°	59		70	
95°	0		125	
102.5°	20		136	Fluctuation prevails.
103.5°	40		...	Fluctuation prevails.
104.0°	60	Fluctuation prevails.		
104.5°	120	Maximum.		

The Influence of Changes in Time and Temperature upon Reduction under a Standard Heating Power.

For the purpose of obtaining a more detailed knowledge of the process of reduction a series of experiments was made upon temperature and time as factors of reduction. The amounts of reduced copper were obtained which were produced by heating reduction mixtures of the same composition to different temperatures and for different lengths of time after 95° had been reached. These experiments were essentially sugar (dextrose) determinations made under varying conditions from among which were to be selected those of the method of estimation finally adopted. The same heating arrangement was used throughout and its heating power was examined at times during the experiments and adjusted when necessary to the standard of 120 seconds for heating 60 cc. of distilled water through the interval of 35–95°. The same operator made all these determinations. A constant amount of pure dextrose of 100 mg. was used in all the experiments. The reduction mixture consisted of the usual proportions of 20 cc. of Allihn's alkalin tartrate solution, 20 cc. of Fehling's copper sulfate solution and 20 cc. of distilled water containing 100 mg. of dextrose. The reduction mixture, which always had a volume of 60 cc., was placed in the usual 200 cc. Erlenmeyer flask under exactly the same conditions of stopper, thermometer and mechanical heating arrangements as are described on p. 944 below. *The mixture was allowed to stand on the heated asbestos gauze for exactly 20 seconds after the thermometer reached 95°.* Since the heating power of the arrangement has been standardized no attention need be given to the time until the mercury reaches the 95° mark. In some experiments the time as noted in the table below was varied from 20 seconds. *It is important to note that in this method of reduction, which was adopted for the regular method of sugar analysis also, all reckonings of time begin when the thermometer reads 95°.* This is a point of reference of evident superiority over the so-called "boiling" or "bubbling" or over any higher temperature that might be suggested as the mercury moves up very rapidly above this point. At the expiration of the desired time after 95° the flask was at once removed from the asbestos gauze and cooled with continual twirling in ice water. As was found later this low temperature is a source of a certain amount of error due to solution of some of the reduced cuprous oxide and the "temperature error" described on p. 948 is also easily committed under these circumstances. These errors were not recognized at the time when the data were obtained but their amount is not large enough to have any significant bearing on the use of the data for the comparative study of reductions at different times and temperatures. After the mixture had been cooled it was filtered through a thick asbestos filter in a Gooch crucible with the help of suction. After washing the precipitate the filtrate was transferred to a 100 cc. volumetric flask, filled to the mark, well mixed and then run into a buret. From this a portion of 20 cc. was taken for determination of the copper content. This gave a small volume in which to make the copper analysis which in presence of sufficient sulfuric acid and potassium iodide is highly conducive to accuracy when a dilute thiosulfate solution is used for titration. At a later date it was discovered that the total (and sometimes large) amount of copper in a much larger volume could be titrated at once with just as much accuracy. The latter procedure would be followed if the experiments were to be repeated. Since the absolute values play no important part for purposes of comparison the results directly obtained on the aliquot of 20 cc. are given in the table. To the aliquot of 20 cc. of alkalin liquid was added 1 cc. of concentrated sulfuric acid, and after cooling under the tap, 5 cc. of saturated aqueous solution of potassium iodide. The liberated iodine was titrated with a thiosulfate solution which had been standardized against a known copper solution. This thiosulfate had a value of 4.256 mg. of copper per cc. The copper value of a liquid of the same composition and volume as the reduction mixture except that it contained no dextrose, *i. e.*, of a blank experiment, was also determined. It was heated and subsequently treated exactly the same as the reduction mixture. The amount (aliquot of 20 cc.) of reduced copper was thus determined by the *residual* method when the copper value of the filtrate from a sugar determination was subtracted from the copper value of the blank. The duplicate values in the table below are repeated determinations of the copper in the filtrate of the *same* reduction mixture. They exhibit the close agreement of which duplicate copper determinations are capable and which is the theoretical limit which duplicate reductions approach when uniform. However, on account of errors of method above described these values are accurate only for purposes of comparison.

Column (4) shows the actual titrations obtained in cubic centimeters and if converted into milligrams of copper would represent the amount of unreduced copper. Columns (5), (8) and (9) represent the reduced copper which was the quantity sought in these experiments. This quantity has been stated in milligrams of copper in column (5) and has been expressed as a percentage in column (8). The base for these percentages was 38.7 mg., the value obtained (see observation No. 14) for reduction at 120 seconds after 95°. The copper value was taken at the nearest 0.1 mg. and the percentages are expressed to the nearest 0.1%. In column (9) are expressed the differences of percentages of reduction between the beginning and end of each temperature interval for observations Nos. 1–9 and for each time interval for observations Nos. 10–14.

TABLE I.—COPPER REDUCED BY 100 MG. DEXTROSE.

At intervals of temperature.

Blank for obs. Nos. 1–5, 14 = 71.03 mg. Cu. $Na_2S_2O_3$ = 4.256 mg. Cu/cc.
" " " " 6–9, 10–13 = 60.98 " ".

1. Observation No.	2. Interval.	3. Temperature.	4. Titrat. $Na_2S_2O_3$. cc.	5. Reduced copper, mg.	6. Absolute difference copper, mg.	7. Diff. per 5 degrees copper, mg.	8. Reduced Cu, per cent.	9. Diff. of per cent.
1.......		50°	15.57–15.57	4.76			12.4	
	1				+6.13	3.07		15.8
2.......		60°	14.15–14 10	10.89			28.2	
	2				+6.34	3.17		16.2
3.......		70°	12.64–12.64	17.23			44.4	
	3				+9.20	9.20		23.8
4.......		75°	10.48–1 .47	26.43			68.2	
	4				+2.72	2.72		7.3
5.......		80°	9.83– 9.85	29.15			75.5	
	5				+1.87	1.87		4.6
6.......		85°	9.39– 9.38	31.02			80.1	
	6				+4.08	4.08		10.6
7.......		90°	8.43– 8.43	35.10			90.7	
	7				+1.49	1.49		3.9
8.......		95°	8.08– 8.07	36.59			94.6	
	8				+1.19	1.19		3.0
9.......		100°	7.79– 7.80	37.78			97.5	
				38.7			100.0	

TABLE II.—COPPER REDUCED BY 100 MG. DEXTROSE.

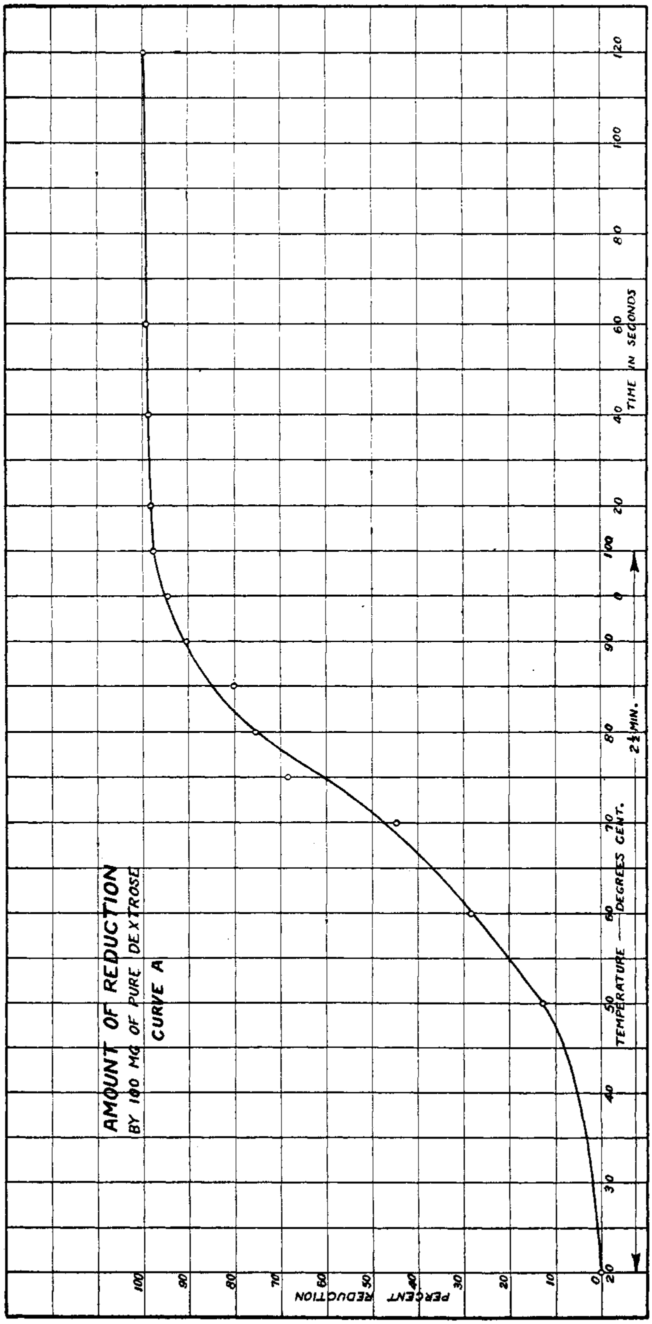
At intervals of time.

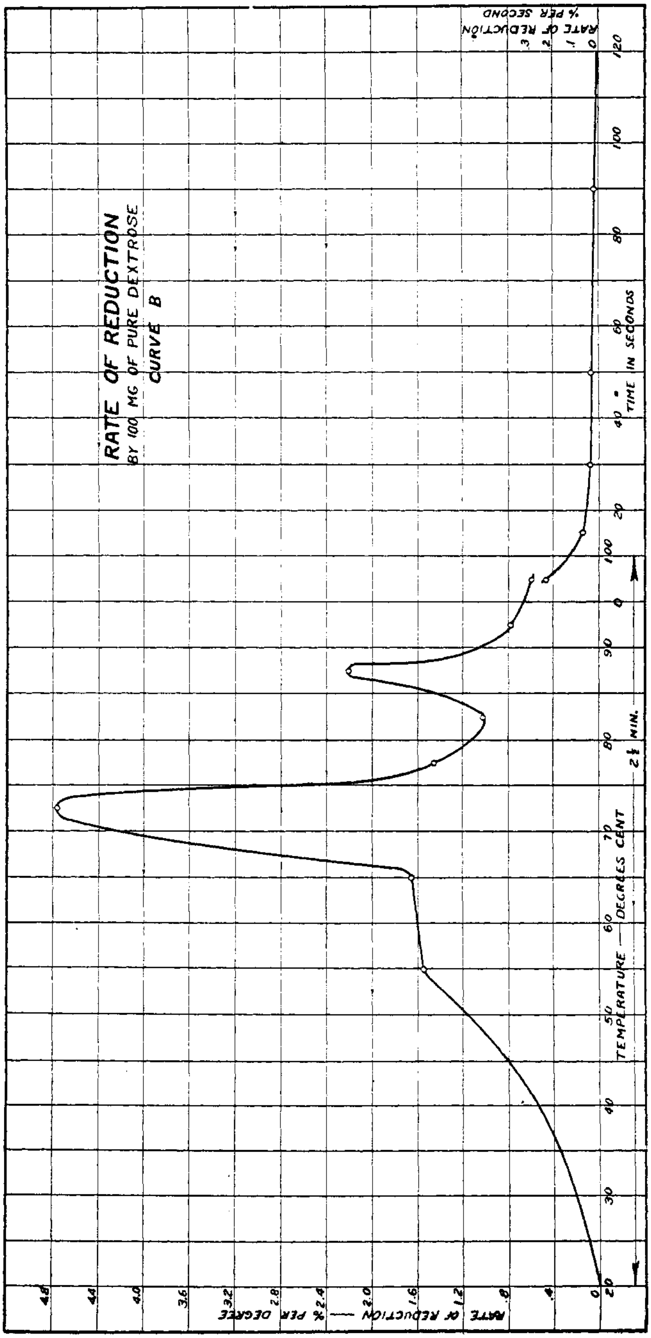
1. Observation No.	2. Interval.	3. Seconds after 95°.	4. Titrat. $Na_2S_2O_3$. cc.	5. Reduced copper. mg.	6. Difference copper. mg.	7. Difference per 5 sec. copper. mg.	8. Reduced Cu. per cent.	9. Diff. of per cent.
10.......		10	7.83–7.85	37.61			96.9	
	8				+0.22	0.11		0.7
11.......		20	7.79–7.79	37.83			97.6	
	9				+0.29	0.07		0.8
12.......		40	7.72–7.72	38.12			98.4	
	10				+0.17	0.04		0.6
13.......		60	7.68–7.68	38.29			99.0	
	11				+0.39	0.03		1.0
14.......		120	7.60–7.60	38.68			100.0	

On p. 933 the observation was recorded that at 20 seconds after 95° regularity in the rise of temperature ceases, so that the momentary relation between these two factors is no longer an index of heating effect. For the purpose of studying the course of the reduction process the heating was in these experiments continued longer, *i. e.*, the data of Table II are simply a continuation of those of Table I but the intervals between the determinations now represent time instead of temperature as in Table I. If then both sets of data are to be represented by one curve, which is highly desirable, the first values on the abscissa will represent temperatures and at some subsequent point the values for time intervals must begin.

By comparing the reduction values of column 5 of both tables with the intervals shown by column 2, it is evident that these data demarcate eleven different intervals of temperature and time the majority of which represent either 5° or 20 seconds. In curve A the ordinates represent the total amount of reduced copper expressed in percentages of the maximum amount which was observed. The asymptotic nature of the curve as a whole is at once apparent. It should, however, be noted that for the purpose of selecting the best conditions for a method of sugar analysis the final region of the curve which covers the higher temperatures and the time intervals is the portion of special significance. Thus the comportment of the reduction after, *e. g.*, 90° is of practical interest. From this point on the slope of the curve diminishes over each preceding interval without interruption. The relative amount of reduction which has been accomplished at 20 seconds after 95° is worthy of note as at this time the thermometer was found to begin to fluctuate. As here one observer made both the time and the temperature observations and made the time the primary object of attention, the temperature of 100° given for this time is much less certain than the accuracy of the time. The latter was made the criterion of when the reduction mixture was to be removed from the heating apparatus. Moreover the temperature at this point increases so fast that it would be difficult to make both time and temperature observations correctly by the same observer. A temperature of, for instance, 2–2.5° higher here would not alter the qualitative nature of these curves. Hence it may be observed that at 20 seconds after 95° 39/40 of the maximum reduction observed had been accomplished. At 60 seconds after 95° 99/100 of the maximum had occurred.

The real significance of these data for the selection of conditions for a method of sugar analysis appears best from a study of the *rate* of reduction as plotted in curve B. Here are plotted the *increments* in terms of percentage of the whole, which correspond to each interval. In other words the ordinates represent not the amount of reduction, but the *increase* of reduction of the end of the interval over that at its beginning. The values of these increments are represented by the ordinates which are


AMOUNT OF REDUCTION
BY 100 MG OF PURE DEXTROSE
CURVE A
PERCENT REDUCTION
100
90
80
70
60
50
40
30
20
10
0
TEMPERATURE — DEGREES CENT.
20
30
40
50
60
70
80
90
100
2½ MIN.
TIME IN SECONDS
20
40
60
80
100
120


RATE OF REDUCTION
BY 100 MG OF PURE DEXTROSE
CURVE B
RATE OF REDUCTION — % PER DEGREE
0
.4
.8
1.2
1.6
2.0
2.4
2.8
3.2
3.6
4.0
4.4
4.8
TEMPERATURE — DEGREES CENT
20
30
40
50
60
70
80
90
100
2½ MIN.
0
20
40
60
80
100
120
TIME IN SECONDS
RATE OF REDUCTION
% PER SECOND
0
.1
.2
.3

placed at the *middle* point of each interval. The curve B shows three peaks or rather two of marked prominence, one at 75° and one at 90°. The significance of these peaks is not of critical importance for the utilization of the terminal regions of the curve. The pronounced turbidity of these reduction mixtures at the low temperature of 75° when enough sugar is present attracts the attention of even the casual observer.

A study of the final region only of the curve has special significance for the selection of conditions for a method of sugar analysis. If it were feasible to determin when the process of reduction is complete that point would, other things being equal, be the ideal place at which to terminate reductions for analysis. Probably the selection of a very long time for reduction, *e. g.*, 30 minutes on the water bath in the method of Pflüger, was aimed at approaching this terminus to within negligible limits. Since, however, the reduction process is empirical and so long a time is a disadvantage from the standpoint of economy, it is preferable to cease the reduction at almost any earlier point where very definit and exact results can be obtained. It matters but little whether the amount of reduction is always 99% or 97.5% of x if only the conditions are so sharply defined that the amount of reduction obtained at a selected point is characterized by great constancy. This condition would preclude the selection of any point toward the left of the curve B *where the rate of reduction is rapidly changing* or even liable to fluctuation. Thus the terminal point must lie somewhere on the curve B beyond 95° where the rate of reduction has become very slow, and taking economy of time into consideration, the desired terminus does not lie beyond the point of 60 seconds reckoned after 95°.

In a recently published method by Kendall[1] the time required for heating the reduction mixture there described is 20 minutes. This protracted time is an unnecessary disadvantage if a copper reduction method is to be used, as the present experimental examination of the conditions of reduction with the ordinary reagents shows. The advantages of supposedly complete or even nearly complete reduction in relation to the accurate termination of the reduction after long heating are illusory. The reduction is never complete at any terminus thus far chosen and the accurate termination of the reduction with constant values can be made with the use of the ordinary Fehling and Allihn reagents by terminating the reduction under controlled conditions at a brief but properly selected time.

Several other considerations not apparent from the curves must be made before selecting the desired point for the cessation of the reduction process. It should be noted that a sugar analysis by the method of re-

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[1] Kendall, E. C., "A New Method for the Determination of the Reducing Sugars," THIS JOURNAL, 34, 317–341 (1912).

duction represents only the possible *reducing power* of all the substances in solution and not alone the reduction by sugar molecules. Other reducing substances than sugar are almost always present in small amount in all sugar analyses except those of pure sugar. Experiment indicates that by avoiding prolonged boiling the reduction by the easily attackable sugar is favored over that by the other substances which occur in small amount and the latter thus accomplish a smaller proportion of the whole reduction than they would after continued boiling. If this consideration has justifiable weight it leads to the selection of the shorter heating time within the limits of 95° to 60 seconds thereafter rather than the longer time.

Another consideration has reference to the fact that different sugars reduce alkalin copper solutions with different degrees of facility. If the selection of the short heating period which dextrose, levulose and invert sugar permit, should not be long enough for constant results with lactose, a different time would have to be selected for sugars of the latter type or a longer time selected for the former. The reducing power of both dextrose and lactose was therefore tested by exactly following the method of analysis which is described in Section III, p. 944, with the exception that data were obtained for both 20 seconds and 1 minute periods of heating after the 95° point had been reached. The results by this method are shown in the third column of the following Table III, and for comparison data compiled from the tables of other methods of sugar analysis are also shown.

TABLE III.—RELATIVE REDUCTION BY DEXTROSE AND LACTOSE UNDER DIFFERENT CONDITIONS.

	Sugar.	Time.	Reduced copper. mg.	Method.
1.	50.0 mg. dextrose	20 sec.	96.0	Peters
2.	50.0 mg. dextrose	60 sec.	96.8	Peters
3.	50.0 mg. dextrose	2 min.	102.2	Munson and Walker
4.	49.9 mg. dextrose	2 min.	98.0	Allihn
5.	50.0 mg. dextrose	20 min.	149.3	Kendall
6.	50.0 mg. dextrose	30 min.	110.8	Pflüger
7.	2.0 mg. dextrose	20 sec.	2.8	Peters
8.	50.0 mg. anhydrous lactose	20 sec.	63.6	Peters
9.	50.0 mg. anhydrous lactose	60 sec.	63.1	Peters
10.	50.3 mg. anhydrous lactose	2 min.	71.9	Munson and Walker
11.	50.0 mg. anhydrous lactose	20 min.	79.0	Kendall
12.	2.0 mg. anhydrous lactose	20 sec.	1.4	Peters

The above results show that the heating period of 20 seconds after 95° produces only a little less reduced copper by dextrose than 60 seconds time by the same method or two minutes time by the Allihn or the Munson and Walker methods. The small increase in reduced copper obtained by the Pflüger method is certainly not of sufficient advantage to counterbalance the expenditure of the thirty minutes required. By the method of Kendall there is obtained roughly one-half more reduced copper from dextrose and one-third more from lactose. In order to obtain this increased reduction it is necessary to heat for 20 minutes and also to weigh out in solid form for each analysis one of the reagents, salicylic acid. To offset these disadvantages it is recommended that four or more analyses be carried on together and the selection of the 20 minute period when experiment showed that 12 minutes was sufficient is explained by the following quotation.[1]

"When the time of heating is limited to 12 minutes it is impossible to filter one set while the following set is being heated, but if the time of heating be extended it is possible to give one's entire attention to filtering the reduced copper and hence there is no actual loss of time per determination." If this manner of reducing the time per analysis satisfactorily meets the requirement of rapidity then the older, well established gravimetric methods of sugar analysis, are also rapid enough for a sufficient number of analyses can be kept going simultaneously to occupy all of one's time. Furthermore, the values recorded in the table above show that with both rapidly and slowly reducing sugars (dextrose, lactose, respectively) the amounts of copper reduced by the customary methods are abundantly sufficient for very exact measurement by the iodide method. If the amount of reduction could be increased as in the method of Kendall, but without a considerable extension of the time requirement, then the factor of increased reduction values would have much more weight. What degree of accuracy the use of the short period of heating adopted in this paper permits can be seen by an inspection of the values of Table IV, p. 950.

The terminus which was selected for testing the constancy of the reduction values obtained with a constant amount of dextrose was that of 20 seconds after 95°. In addition to the various considerations above described this point has the presumed advantage of being the upward limit at which temperature and time changes run parallel and are both indications of the amount of reduction. Theoretically at least, when the temperature fluctuates as it does beyond this point, the chances of the exact repetition of these fluctuations seem unfavorable to the plan of continuing the reduction. It may be that the variability in these effects upon reduction is too small to be observable. However, beyond this point there is no good criterion of the progress of the reduction for, after a temperature fails as an indicator, a given time interval may or may not represent a constant amount of reduction. Previous to the point above selected both temperature and time were definitly related indicators of reduction. The regular recurrence of the same relation of time and temperature at

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[1] Kendall, E. C., THIS JOURNAL, 34, 322 (1912).

70° shown on p. 933 illustrates this fact. Owing to the regularity and easy determinability of all the conditions up to this point, it seemed highly probable that to this point at least constancy of reduction would be easily attained. Subsequent experiments confirmed this view. Since this also was the earliest time on the curve of reduction that could be favorably considered, the requirement of economy of time was satisfied. There must be good reasons adduced other than accident or arbitrary choice to justify the reduction for 2, 3, 5 or 30 minutes rather than any shorter time, when it is evident that reduction is a wholly empirical procedure. The fluctuations in temperature above referred to are probably and to some extent related to the Erlenmeyer form of flask and to its partial closure. The use of some other form of vessel, *e. g.*, the cylindrical beaker or of the open conical flask, with thermometer held by a clamp above, instead of in a stopper, would of course require separate investigation with regard to these same conditions. When the thermometer is carried by the flask the whole can be more quickly removed from the source of heat than if the thermometer must be removed first. Celerity of removal of the reduction mixture from the heating apparatus is of course related to promptness of termination of the reduction and consequent constancy of reduction values. The Erlenmeyer form of flask and its partial closure were adopted after some preliminary experiments had shown that regularity of conditions inside the flask, and consequently of thermometric indications, could be produced satisfactorily by these means. Open flasks and beakers were much more subject to irregularity.

Returning to curve B it should be noted that during the ninth interval there is a pronounced diminution in the rate of reduction. Here the curve reaches a low rate from which it does not again rise, which indicates the practical exhaustion of easily oxidizable material. This interval then may be regarded as the final well demarcated region of change in the curve. The significance of the curve for the determination of a method of sugar analysis may be best shown by dividing it into a region of rapidly rising rate of reduction, a next succeeding region of rapidly falling rate, and a final region of comparatively constant low rate. This derived curve would show three arms and its most significant place is the junction between the second and third arms, which no doubt occurs in the ninth interval of curve B. This triple form of curve for the rate of reduction was suspected long before any definit evidence of its nature was obtained. It was found experimentally that reduction experiments could be terminated sharply at this region of juncture, *i. e.*, with very concordant results for the amount of reduction by the same amount of sugar. As previously stated this result is due to the absolute slowness, at this point, of the rate of reduction coupled with its negligibly slow rate of change.

III. A Method of Standardized Conditions of Sugar Analysis.

The construction of the method of sugar analysis here proposed is based upon preceding developments for (1) the accurate volumetric determination of copper in alkalin tartrate liquids, (2) the standardization of the heating power used, and (3) the determination of constant and controllable conditions for the reduction. The procedure may be summarized under the following heads but for additional details reference is made to the preceding sections: A. The standardization of the heating power. B. The process of reduction. C. The determination of the reduced copper. D. The control of all the conditions by analytical results on pure or assayed dextrose.

The specifications are given at length, but when the conditions have once been established, including the determination of the blank value below described, a single estimation of sugar occupies from 12–15 minutes.

A. The Heating Power.

A heating apparatus is to be set up of such power that it will heat 60 cc. of distilled water under described conditions through the temperature interval of 35–95° in 120 seconds ± 5 seconds. The water is placed in an Erlenmeyer flask (best of Jena glass to withstand rapid changes of temperature) of about 200 cc. capacity and of about 6 cm. basal diameter, so that the depth of the liquid will be about 18 mm. The flask is partially closed with a two-hole stopper, one hole of which carries a thermometer. The thermometer should not have a greater error at the boiling point of water than ± 0.5° when the barometric pressure is taken into consideration. The lower end of the thermometer should be placed at a distance of about 2 mm. from the bottom of the flask and the region of the stem above 35° should extend above the upper surface of the stopper so as to be plainly visible. The advantages of the arrangement consisting of a partially closed Erlenmeyer flask, especially when used as further described, are little or no fluctuation in the rise of temperature during the heating and the practical exclusion of air by steam when a reduction mixture is heated to boiling in this flask. The flask is to be placed in the center of a plate of asbestos gauze carried by a ring stand so that it can be raised or lowered. The asbestos gauze should not be perforated, which prevents the direct flame from touching the flask and retains and evenly distributes the heat. The lower surface of the asbestos gauze should at first be placed at a distance of 4 cm. from the upper edge of the Bunsen or Méker burner standing beneath the center of the asbestos plate. The distance between burner and asbestos should always fall within the limits of 3–5 cm., thus avoiding a long flame which would be easily affected by air currents. Room temperature and freedom from draughts should prevail. If the latter condition cannot be provided the heating apparatus may be wholly or partially protected by a cylindrical hood of sheet metal or asbestos. The thermometer, the flask and its contents should all be at room temperature at the beginning of each test. The time may be taken with the seconds hand of a watch or with a stop-watch. In the former case the duplicate tests may vary from 2–5 seconds, in the latter from 1–2 seconds. The standardization is begun by making a test of the heating power with the ordinary Bunsen flame of about 15 cm. length when the burner is removed from under the asbestos gauze and the flame viewed against a dark background. With a burner of the Méker type the full flame is used at once. When the apparatus with the burner in place has become well heated the flask as above described with 60 cc. of distilled water is placed on the asbestos and the time observed that is required for the mercury to rise from 35–95°. The rough adjustment to 120 seconds is made by regulating the flow of gas so as to change the power of the flame with the position of the asbestos constant at 4 cm. The finer adjustment is made by moving the asbestos plate towards or away from the burner within the limits of 3–5 cm. The conditions are not properly adjusted until repeated determinations of the time give constant results within limits of error of only a few seconds. *At 120 seconds the standard heating power here adopted has been established.*

B. The Process of Reduction.

The reduction mixtures are to be placed in the same kind of flasks with the same stopper, thermometer, etc., under the same conditions of heating power as were used for the standardization of the latter. The sole difference is the presence in the reduction flask of the alkalin tartrate-copper-sugar mixture. The volume of the reduction mixture is always 60 cc. except for such small constant *variations* as the use of approximately correct automatic pipets might introduce. The reduction mixture consists of 20 cc. of Allihn's alkalin tartrate solution, 20 cc. of Fehling's copper sulfate solution and 20 cc. of distilled water for a blank determination or, in the case of sugar analysis, of an aqueous sugar solution with enough distilled water added to make a final volume of 60 cc.

The Fehling-Soxhlet solution contains 34.639 grams of pure crystallized copper sulfate in a volume of 500 cc., but the purity of the copper sulfate is more important than its exact weight. Since its correct *copper* content cannot with reliability be deduced from the formula $CuSO_4.5H_2O$, this value is found by means of standardized thiosulfate as described on p. 947 for the "basis titration." The Allihn alkalin tartrate solution contains 173 grams of sodium potassium tartrate (Rochelle salt) and 125 grams of potassium hydroxide in a volume of 500 cc.

For a determination of sugar the flask containing the reduction mixture is placed on the heating apparatus and the time is observed, but in this case *the reckoning begins only when the thermometer reaches 95°*. The flask is to stand undisturbed in the center of the asbestos gauze *until the* *expiration of 20 seconds after the temperature* of 95° has been reached. Then it is promptly removed with stopper and thermometer still in place and given a few seconds twirling under the tap in order to cool the mixture rapidly *to only a few degrees under the boiling point, for the mixture is to be filtered hot without delay.* The cooling to just below the boiling point is sufficient to diminish the reduction to an imperceptible rate under the conditions then prevailing (see curves of reduction) and the hot filtration is to prevent resolution of cuprous oxide in the alkalin liquid, especially in the presence of dissolved oxygen. A Gooch crucible should have been previously prepared with a very heavy filter so that suction will draw water through it only slowly. A very close and satisfactory filter may be made by placing a layer of pure talcum powder between two layers of asbestos in the Gooch crucible. A good filter may also be made by substituting for the Gooch a calcium chloride drying-tube carried by a one-hole rubber stopper. This is packed first with glass wool and upon this is made, in the bulb of the tube, a very heavy mat of asbestos, which may also be made as close as desired by means of talcum. The asbestos used is ordinary acid washed of exactly the same good quality as for Gooch filters. This filter must by no means be identified with the Allihn tubes which are laboriously made of selected asbestos fiber. The filter should be so operated that the liquid runs through it under a given suction in rapidly succeeding drops not quite sufficient to produce a continuous stream. The same filter is used many times, the precipitate being removed from it by nitric acid (sp. gr. 1.42) diluted with one-half of its volume of distilled water. This requires less time than making a new filter for each determination. The suction flask should have a capacity of about 200 cc. so that a titration on the filtrate can be performed in the suction flask, thus avoiding a transfer of the liquid. After cooling for a few seconds to just under the boiling point, the very hot reduction mixture is at once filtered, using the thermometer stem as a glass rod. The precipitate, none of which should be lost, need not be quantitatively transferred, but both flask and precipitate should be washed rapidly to prevent resolution of cuprous oxide, which occurs readily. The washing may be done with a fine stream of either cold or hot water. Not more than 15–20 cc. of wash water should be used as it all remains as part of the final volume at the end of titration. This volume should not exceed 120 cc. to which volume the concentration of the reagents used for the determination of copper has been adjusted. For the inexperienced it is recommended to make a rough measurement of the final volume of the liquid obtained at the end of titration.

C. The Determination of Reduced Copper.

The amount of copper that has been reduced under the above conditions is a quantitative index of the amount of sugar originally present. The reduced copper may be determined directly upon the precipitated cuprous oxide or residually by the determination of the amount of copper still remaining unreduced in the alkalin filtrate above obtained. Both the direct and the residual methods should give concordant results in pure solutions of sugar and in such solutions they may be checked against each other. In any case the precipitate of cuprous oxide obtained by reduction and filtration may, and in most practical cases does, contain other contaminating substances. This fact renders the direct gravimetric method the least reliable of all the available procedures (see p. 928 and references). By the direct volumetric method the precipitate may be dissolved on the asbestos filter in nitric acid to which half its volume of water has been added and the solution thus obtained may be treated by the nitric acid-talcum method previously described[1] and finally determined by the volumetric iodide method. The direct electrolytic method[2] may also be applied after first approximately neutralizing the nitric acid solution above obtained. When the amount of copper in a given precipitate has been determined by the direct volumetric method or by the direct electrolytic method the result should be accurately concordant with that obtained from a residual determination on the alkalin copper filtrate of the same reduction experiment. This is an excellent method of control on the accuracy of the copper measurements. The amount of copper in the filtrate may be determined either electrolytically for control or volumetrically by the iodide method. For rapidity combined with accuracy the iodide method is recommended, but only when it has first been controlled for the conditions under which it is to be used. For the alkalin tartrate copper solutions which are here used (Allihn's alkalin tartrate, etc.), this control has been satisfactorily made. The residual method for the determination of the reduced copper may therefore be regarded as certainly the most rapid as well as accurate when the iodide method is applied as has been previously described[3] and under the following provisions.

(1) The thiosulfate solution should have a value between 12.5 and 13.0 mg. of copper per cubic centimeter. It should be standardized preferably against the same stock solution of copper which is to be used in the sugar analyses and the true copper value of this solution should be determined electrolytically. If, however, the stock solution is made of chemically pure copper sulfate there will be no appreciable difference between the standard value of the thiosulfate determined by this electrolytically controlled solution and by the nitric acid-talcum procedure above referred to using pure metallic copper, which procedure may then

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[1] Peters, A. W., THIS JOURNAL, 34, 430–433 (1912).

[2] *Ibid.*, 34, 426–430 (1912).

[3] *Ibid.*, 34, 422–454 (1912).

be substituted for the electrolytic determination on the stock solution. The standardization may appropriately be made on 20 cc. of the stock solution which is the same volume as will be used in sugar determinations, and with a final volume at the end of titration in no case exceeding 120 cc. This volume should contain 20 cc. of concentrated acetic acid and about 7 cc. of saturated solution of pure potassium iodide. The thiosulfate value of 20 cc. of the stock solution of copper sulfate determined in simple acetic acid solution is designated as the "basis titration" and is of fundamental importance for subsequently testing the constancy of solutions and of conditions even when it is not used for the purpose of standardization. A solution of pure copper sulfate will remain at a constant standard value, but the thiosulfate will change somewhat if much exposed to air and especially to light.

(2) The alkalin filtrates from sugar determinations, whose volume must not exceed 75 cc. are to be acidified with concentrated sulfuric acid. The amount of acid used is to be such that the thiosulfate value of a blank determination in which the unheated reduction mixture of 60 cc. above described containing all the reagents but no sugar, will be identical with the "basis titration." This amount has been found by experiment to be 4 cc. of concentrated sulfuric acid but should be verified or changed if necessary.[1]

The "temperature error" which is made by conducting the iodide reaction at some other temperature than that at which the standardization of the thiosulfate solution has been made, *e. g.*, 20°, must be avoided in all the copper determinations. The thermometer placed in the liquid is the only safe guide especially with the use of suction flasks which consist of heavy glass. Observation has shown that inattention to this detail in winter, when the tap water used for cooling is quite cold, may deprive the operator of the very close uniformity of results of which this method is capable.

After acidification and cooling to 20°, potassium iodide is added to the amount of 6–7 cc. of an aqueous solution that is saturated at room temperature. The iodine which is evolved is titrated at once with thiosulfate using a solution of soluble starch as indicator near the close of the titration. The end point should be determined by the "spot test," especially in the presence of urinary or other coloring matter and may be verified by immediate back titration with a drop of iodine solution of about the same concentration as the thiosulfate.

(3) For the residual method a blank determination is always necessary. The assumption that there is no self-reduction of reagents may lead to error, although by the present method pure and especially freshly prepared reagents show a negligibly small self-reduction. The blank value

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[1] Peters, A. W., THIS JOURNAL, 34, 440 (1912).

is obtained with the reduction mixture of 60 cc. above described but without the presence of sugar. This is heated and filtered and treated in all respects as when a determination of sugar is made. From the thiosulfate value of the copper thus found unreduced is subtracted the same value found in the same way in a subsequent sugar analysis. This *thiosulfate difference* evidently shows the amount of reduced copper due to the action of the sugar. This difference may be converted into milligrams of copper by means of the standard value of the thiosulfate above obtained and the corresponding amount of sugar may be found from a table of reduction values.

D. The Control of all the Conditions.

All the conditions of this method and the technic of the operator can be controlled by the use of a standard solution of pure dextrose. The results thus obtained are a standard of comparison to determin whether in a given case the conditions have been correctly adjusted for the use of the tabular values on dextrose which are below recorded. The dextrose here used was the purest obtainable from Kahlbaum, had been pulverized and long dried in a desiccator so as to contain no water of hydration and a weighed amount was made into a 10% stock solution. This solution was effectively preserved under a layer of toluene about 1 cm. in depth and portions free from toluene were obtained by means of a half-liter separating funnel with glass stopcock at its lower extremity. From portions thus taken quantitative dilutions were made to 10–20 volumes for stock solutions which were likewise preserved under toluene. Calibrated measuring utensils were used throughout.

The polarization value of this concentrated solution after it had stood a week was determined with a Schmidt and Haensch triple field instrument and agreed very closely with that required by the weight of substance in solution. Furthermore the observed heat of combustion[1] of this same dextrose agreed with the theoretical value. However, it was found by experiment that a dextrose of the highest purity as that described above is not absolutely necessary for testing whether the operator has established conditions comparable with those represented by the tabular values of this method. If the weights of powdered and well dried specimens of nearly pure dextrose, *e. g.*, 99.7–99.9%, which are easily obtained, be checked by means of the polarimeter the percentage of purity based on the polarization value may be taken as a factor to represent with close approximation the reducing power of this solution regarded as dextrose. The operator must be familiar with all the precautions of polarimetric technic.

From these controls the values for 50 and 100 mg. obtained from a 0.5%

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[1] Emery, A. G., and Benedict, F. G., "The Heat of Combustion of Compounds of Physiological Importance," *Am. J. Physiol.*, **28**, 301–307.

or 1.0% standard solution of dextrose were usually selected for the first tests. The quantities to be compared should be the dextrose/copper ratios as the limits of error of these are evident from the table.

IV. Tabular Values for Reduction by Dextrose.

Table IV.—Reduction Values for Dextrose.

1. Dextrose. mg.	2. $Na_2S_2O_3$. cc.	3. Copper. mg.	4. Av. copper. mg.	5. $\frac{\text{Dex.}}{\text{Cu}}$ Ratio —	6. Average.
1	0.08 × 12.62 =	1.00			
1	0.10 × 12.62 =	1.26	1.2 ± 0.13	0.833	
1	0.10 × 12.62 =	1.26			
1	0.11 × 12.69 =	1.40			
2	0.22 × 12.62 =	2.8			
2	0.21 × 12.62 =	2.7	2.8 ± 0.1	0.714	
2	0.23 × 12.62 =	2.9			
2	0.20 ⎫ × 12.69 =	2.7			
2	0.22 ⎭				
5	0.65 × 12.69 =	8.3			
5	0.66 × 12.69 =	8.4			
5	0.63 × 12.62 =	8.0			
5	0.64 × 12.62 =	8.1	8.2 ± 0.14	0.610	
5	0.64 × 12.62 =	8.1			
8	1.10 × 12.62 =	13.9			
8	1.08 × 12.69 =	13.7			
8	1.09 × 12.69 =	13.8			
8	1.09 × 12.62 =	13.8	13.8 ± 0.1	0.580	
8	1.11 × 12.62 =	14.0			
10	1.34 × 12.62 =	16.9			
10	1.40 × 12.62 =	17.7	17.4 ± 0.3	0.575	
10	1.36 × 12.62 =	17.2			
10	1.41 × 12.62 =	17.8			
10	1.37 × 12.69 =	17.4			
15	2.15 × 12.62 =	27.1			
15	2.15 × 12.62 =	27.1			
15	2.20 × 12.62 =	27.8	27.7 ± 0.5	0.542	
15	2.24 ⎫ × 12.69 =	28.4			
15	2.23 ⎭				
15	2.21 × 12.69 =	28.0			
20	2.91 × 12.62 =	36.7			
20	2.93 × 12.62 =	37.0	37.1 ± 0.2	0.539	
20	2.95 × 12.62 =	37.2			
20	2.94 × 12.69 =	37.3			
25	3.85 × 12.62 =	48.6			
25	3.84 × 12.62 =	48.5	48.1 ± 0.4	0.520 –	0.522
25	3.82 ⎫ × 12.62 =	48.1			
25	3.79 ⎭				
25	3.73 × 12.69 =	47.3			

TABLE IV (*continued*).

1. Dextrose. mg.	2. $Na_2S_2O_3$. cc.	3. Copper. mg.	4. Av. copper. mg.	5. $\frac{Dex.}{Cu}$ Ratio	6. Average.
30	4.54	× 12.62 = 57.3	57.3 ± 0.05	0.524	0.522
30	4.53	× 12.62 = 57.2			
30	4.53	× 12.62 = 57.3			
30	4.55				
30	4.55	× 12.62 = 57.4			
35	5.36	× 12.62 = 67.6	67.6 ± 0.13	0.518	0.522
35	5.36	× 12.62 = 67.8			
35	5.38				
35	5.36	× 12.62 = 67.6			
35	5.35	× 12.62 = 67.5			
35	5.37	× 12.62 = 67.8			
35	5.33	× 12.62 = 67.3			
40	6.02	× 12.62 = 76.0	76.2 ± 0.1	0.525	0.522
40	6.04	× 12.62 = 76.2			
40	6.06	× 12.62 = 76.4			
40	6.04				
40	6.04	× 12.62 = 76.2			
45	6.80	× 12.62 = 85.8	86.0 ± 0.1	0.523	0.522
45	6.83	× 12.62 = 86.1			
45	6.81				
45	6.78	× 12.69 = 86.0			
50	7.48	× 12.69 = 94.9	96.0 ± 0.5	0.521	0.522
50	7.58	× 12.62 = 95.7			
50	7.65	× 12.62 = 96.5			
50	7.63	× 12.62 = 96.3			
50	7.63	× 12.62 = 96.4			
60	9.12	× 12.69 = 115.7	115.5	0.520	0.522
60	9.14	× 12.62 = 115.3			
70	10.60	× 12.69 = 134.5	134.4	0.521	0.522
70	10.64	× 12.62 = 134.3			
80	12.05	× 12.69 = 152.9	152.9	0.523	0.522
80	12.11	× 12.62 = 152.8			
90	13.45	× 12.69 = 170.7	171.0	0.514	
90	13.57	× 12.62 = 171.3			
100	15.12	× 12.62 = 190.8	191.6 ± 0.35	0.522	0.522
100	15.19	× 12.62 = 191.7			
100	15.16	× 12.62 = 191.3			
100	15.19	× 12.62 = 191.7			
100	15.10	× 12.69 = 191.5			
100	15.07				
100	15.15	× 12.69 = 192.3			

TABLE IV (*continued*).

1. Dextrose. mg.	2. $Na_2S_2O_3$. cc.	3. Copper. mg.	4. Av. copper. mg.	5. $\frac{\text{Dex.}}{\text{Cu.}}$ Ratio	6. Average.
110	16.46	× 12.69 = 208.9			
110	16.45	× 12.69 = 208.7	208.9 ± 0.15	0.527	
110	16.58	× 12.62 = 209.2			
110	16.46 }	× 12.69 = 209.0			
110	16.48 }				
120	18.00	× 12.69 = 228.4	228.1	0.526	
120	18.04	× 12.62 = 227.7			
135	20.10	× 12.69 = 255.1	255.0	0.529	
135	20.08	× 12.69 = 254.8			
150	22.32	× 12.62 = 281.7			
150	22.26	× 12.62 = 280.9	280.8 ± 0.6	0.534	
150	22.06	× 12.69 = 279.9			
165	24.10	× 12.69 = 305.8	306.8	0.538	
165	24.39	× 12.62 = 307.8			
180	26.19	× 12.62 = 330.5			
180	26.03	× 12.69 = 330.3	330.5 ± 0.1	0.545	
180	26.05	× 12.69 = 330.6			
200	27.70	× 12.62 = 349.6			
200	27.55	× 12.69 = 349.6	349.6	0.572	
200	27.56	× 12.69 = 349.7			

A series of tabular values obtained with pure dextrose by the method described in the preceding section is presented in Table IV. These values are intended for the determination of all amounts of dextrose from 1–200 mg. when contained in a volume of 20 cc. or less. The method below described of using this table by means of the dextrose/copper ratios of column 5 makes it unnecessary to present values for each milligram of dextrose or of copper. Furthermore, the values actually obtained are recorded with the intentional omission of any mathematical procedure for the distribution of errors, as the values themselves as expressed in columns 4 and 5 are sufficiently uniform for all practical purposes. The determination of these values was made to extend through a period of many days involving renewed standardizations of conditions in order that the results might correctly express the accidental variations to which subsequent operators would be liable in their use of this method. Only those pairs of values which are connected by braces were done in immediate succession, all others representing determinations done on different days and in some cases by different persons. The exceedingly close agreement of the paired values was commonly obtained not only on pure solutions of dextrose but also on urins or other uniform material when the analyses were made in immediate succession. These paired results are treated as only one analysis in calculating the average deviation from the mean recorded in column 4.

The important relation between the amounts of dextrose and the corresponding amounts of reduced copper can be expressed for any table by the ratio of dextrose/copper, each expressed in milligrams, or by the reverse ratio of copper/dextrose. The former ratio is preferred because the experimentally found quantity is copper and this can be converted into its equivalent in sugar by multiplication by the ratio F = dextrose/copper. In other words, the quantities in column 5 of Table IV are factors for use with the corresponding amounts of copper shown in column 4. The most important property of these ratios from a practical standpoint is *that they change their value slowly* in comparison with the amounts of reduced copper to which they pertain. Thus an inspection of the tabular ratios shows that by rough estimation and excepting the extreme ends of the table the amount of dextrose is always approximately a little more than half the numerical value of the reduced copper. More exact examination shows that the ratios in the region of the table included between 25 and 100 mg. dextrose simply fluctuate about a mean value of 0.522 with an average deviation of 1.7 parts in 522 or about $\pm\ ^1/_3\%$. In other words, for amounts of reduced copper extending from 48–192 mg. no table is necessary for the calculation of results since the common factor of 0.522 applies throughout this range. In the other regions of the table both below and above the limits described it should be observed that the slow change of the ratios will enable the operator to interpolate mentally the required ratio for any amount of reduced copper lying between any two adjacent values of column 4. This can be done easily to within one or two units of the third decimal place, noting that for the small amounts of dextrose at the beginning of the table the third decimal place evidently has no practical significance.

It is worthy of observation that this method shows a high degree of certainty and consistency with very small quantities of dextrose. The above tabular values on 2 mg. were obtained on a volume of 20 cc. of solution to be analyzed, *i. e.*, in a dilution of one part in ten thousand. The reduced copper thus obtained was plainly perceptible on the white asbestos filter used. It appears safe to state that for those who have experience in quantitative technic a maximum absolute error lying within a few tenths of a milligram may be expected on any amounts of dextrose represented in the table whether large or small. The small range of variation in the results is due to definit delimitation or standardization of conditions.

The above method of standardized conditions has been applied to the determination of the reducing power of both diabetic and normal urins. It has been found applicable to the determination of the reducing power of these materials with ease of technic and with very concordant results without the necessity of any preliminary preparation of the urin. From 1–10 cc. of urin have been used in a determination, the usual amount being 2 cc. in the case of the urin of diabetes. An advantage of the method is the use of small volumes of urin consistently with accuracy. A quantity of 50–100 mg. of pure dextrose when added to diabetic urin was always found determinable either exactly or to within a fraction of 1% of the total amount present. It should be noted that reduction methods show reducing power only, which is not always an exact index of the sugar present, especially in urin. In order to guard against erroneous conclusions the power of normal urin, and to a less extent that of diabetic urin, to hold curpous oxide in solution with or without oxidation must be remembered.

V. Summary of Results.

In consequence of the need for the greatest rapidity compatible with the highest accuracy a procedure for the determination of sugar has been developed from the results of a study of the conditions of reduction and of the accurate measurement of copper in sugar analysis. The procedure is characterized primarily by the quantitative standardization in detail of the conditions of reduction, and of the volumetric estimation by the iodide method of the copper in alkalin tartrate solutions. A set of tabular values has been presented with the data of the original determinations by means of which the probable limits of error can be ascertained. This method has been applied with good results to the determination of the reducing power of urine and of other physiological materials.

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NOTE.

Determination of Melting Points with the Aid of the Microscope.—In toxicological investigations it sometimes happens that a method of determining an approximate melting point under the microscope is of service where the amount of available material is too small to be introduced into a capillary tube. The apparatus described below does not make possible the determination of melting points accurately, but it is of service where an approximation within a few degrees or so is helpful.