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A Method Employing a Carbonate Buffered Cupritartrate Reagent of Low *p*H for Estimating Macro Quantities of Reducing Sugars

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At the Ninth Session of the International Commission for Uniform Methods of Sugar Analysis held in London in 1936 the Committee on the Determination of Reducing Sugars reported that "the type of method most likely to command general confidence as an international standard method is one in which Fehling solution is replaced by a copper reagent of low alkalinity, containing sodium carbonate instead of hydroxide and having only a slight action on sucrose."[1] Twenty-nine common methods were reviewed at this meeting but none of them employ a copper reagent at a *p*H below 9.2 except perhaps the method revised by F. W. Zerban and L. Sattler[2] that employs an alkaline copper acetate reagent.

The present study deals with the adaptation of the method of P. A. Shaffer, A. F. Hartmann and M. Somogyi to the determination of macro quantities of reducing sugars. The best results have been obtained by using a carbonate buffered cupritartrate reagent at *p*H 8.7. Special attention has been paid to the effects of small changes in the variables upon the yield of cuprous oxide, and to the application of the method to dextrose and levulose, and mixtures of these sugars and sucrose as they occur in invert sugar and partly inverted sucrose.

The reactions involved in the method are

(1) Cupric tartrate complex in mildly alkaline solution + sugar + heat = $Cu_2O(s)$ + oxidized sugar

(2) IO_3^- in the copper reagent $+ 8I^- + 6H^+ = 3I_3^- + 3H_2O$

(3) $Cu_2O(s) + I_3^-$ + excess oxalate + acid = cupric oxalate complex in acid $+ 3I^- + H_2O$

(4) I_3^- in acid $+ 2S_2O_3^= = 3I^- + S_4O_6^=$

Part of the sugar, instead of directly reducing the copper, is converted into substances some of which in turn reduce the copper but for the most part less efficiently than the parent sugar. The amounts of cuprous oxide produced directly and indirectly by the sugar, and the ratio of these amounts depend upon the *p*H, the heat treatment and the nature of the sugar. There is also a small amount of cuprous oxide lost by oxidation by the air.

The amount of cuprous oxide produced less the amount lost is calculated by means of the equation

$$\text{Moles } Cu_2O = (M/2000)\ (b - r)$$

M represents the moles of $S_2O_3^=$ per liter of its solution, and b and r represent the ml. of this solution required to titrate the iodine in the blanks and sugar solutions made up of equal volumes of the copper reagent.

Materials.—The dextrose, levulose and sucrose were parts of the same carefully purified materials used in our earlier work[3] on the micro method. The melting points and specific rotations in water solution agreed with the accepted values. The following commercial materials also had the same melting points and specific rotations in water, gave the same yields of cuprous oxide within 0.2%, and contained no detectable amount of 5-hydroxymethylfurfural: Squibb, U.S.P. XII, anhydrous dextrous equal or superior to that commonly known as C.P.; Pfanstiehl, levulose, C.P. special crystals of D-fructose, Lot 1378; Domino cane sugar, adant cut loaf.

The sugars were stored in the dark in an evacuated desiccator over "Anhydrone" and were used only when the finely ground crystals did not adhere to each other or to the walls of the containers.

Two tests were made for 5-hydroxymethylfurfural. One was the well known qualitative color test made with resorcinol and hydrochloric acid. The other test was much more sensitive, and was carried out with a polarograph using a procedure based on the work of S. M. Cantor and Q. P. Peniston.[4] Both tests were negative and the test employing the polarograph set the amount of 5-hydroxymethylfurfural in the sugars at less than 0.01% by weight.

All of the other reagents were of analytical reagent grade or C.P. quality.

The water was taken from the laboratory supply of chloride-free distilled water. Small amounts of chloride do not interfere with the method, but unless the water is free from chloride it usually contains enough organic material to deposit cuprous oxide.

Apparatus and Technique.—Most of the apparatus and technique employed in our work on the micro method were also used in this research except that the analysis made with 50 ml. of the copper reagent required test-tubes 30 × 200 mm. and these were capped with short stem funnels.

The glassware was cleaned until it drained freely without drop formation on the walls. All the filtering was done through fine sintered glass filters and care was taken to prevent the solutions

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[1] Report of Committee on Determination of Reducing Sugars, Supplement to International Sugar Journal, Vol. 39, Jan. (1937), pp. 9a-16a.

[2] F. W. Zerban and L. Sattler, *Ind. Eng. Chem., Anal. Ed.*, **10**, 669 (1938).

[3] L. J. Heidt, F. W. Southam, J. D. Benedict and M. E. Smith, THIS JOURNAL, **71**, 2190 (1949).

[4] S. M. Cantor and Q. P. Peniston, *ibid.*, **62**, 2113 (1940).

from contacting oxidizable material of any kind in order to avoid the production of cuprous oxide by anything except the sugars. The *p*H values were measured within 0.01 unit by means of a *p*H meter employing a glass electrode.[5]

Composition of Solutions and Analytical Procedure.—The composition of the copper reagent that was found to be best suited to the macro method is given in Table I. All the solutes in the reagent except the sodium carbonate and bicarbonate are five times more concentrated than in the micro reagent.[3] The concentration of copper is also five times greater than in Ofner's reagent[1]; it is 0.1 *M* compared to 0.07 *M* with citrate instead of tartrate in the Luff–Schoorl method[1]; it is the same as in the reagent with citrate instead of tartrate employed by R. F. Jackson and E. J. McDonald,[6] but it is less, 0.1 *M* compared to 0.14 *M*, than in the tartrate reagents employed in the Berlin Institute,[1] Munson–Walker and Lane–Eynon Methods.[7]

TABLE I

COMPOSITION AT 25° OF ONE LITER OF THE COPPER REAGENT EMPLOYED TO ESTIMATE MACRO QUANTITIES OF REDUCING SUGARS

A. 25 g. (0.1 mole) $CuSO_4 \cdot 5H_2O$ in *ca.* 100 ml. of water.
B. 85 g. (0.3 mole) Rochelle salt and 25 g. (0.24 mole) Na_2CO_3 in *ca.* 500 ml. water. Add solution A to B slowly through a separatory funnel with exit well immersed in solution B and with adequate stirring of B.
C. Add 3.50 g. (0.0165 mole) KIO_3, 5.0 g. (0.03 mole) KI and water to bring the total volume to 980 ± 10 ml.
D. Add enough $NaHCO_3$ to bring *p*H to desired value and dilute to one liter. The amounts of $NaHCO_3$ needed to bring the *p*H to 8.6, 8.7, 8.8, 8.9, 9.0 and 9.3 are roughly 40, 34, 27, 20, 15 and 0 g. per liter, respectively.

The reagent was allowed to age in the dark in Pyrex at least three days at room temperature before it was filtered through a fine sintered glass filter. The *p*H of the filtered solution was always checked and rarely differed more than 0.01 from its initial value unless a precipitate appeared. The reagent does not deposit a cupric precipitate when it stands quietly in the dark for several months if its *p*H is 8.7 or greater provided no more than 25 g. of sodium carbonate is in one liter.

Analyses were carried out using 5 and 50 ml. of the macro copper reagent. It was easier and more economical to use 5 instead of 50 ml.; also the range in the amount of sugar that could be determined with the 5-ml. sample overlapped the range covered by the micro method. Volumes were measured out in the manner previously described.[3]

In the analyses employing 5 ml. of the copper reagent, this volume was reproduced to ±0.001 ml. and to this was added up to 5 ml. of the sugar solution measured to ±0.002 ml. and enough water to bring the sum of the volumes in each analysis up to 10±0.02 ml. The mixture was then thoroughly mixed and subsequently heat treated.

The liberation of the iodine and the oxidation of the cuprous oxide was brought about by adding to the cooled heat-treated solution 5 ± 0.1 ml. of a solution containing 129 g. (0.7 mole) of $K_2C_2O_4 \cdot H_2O$ and 70 g. (0.42 mole) of potassium iodide per liter, followed by 3 ± 0.1 ml. of 2.5 *N* sulfuric acid. The resulting mixture was thoroughly mixed until a clear solution of I_3^- was obtained, whereupon the I_3^- was titrated immediately by the addition of a fresh slightly alkaline solution of 0.01 *M* sodium thiosulfate whose volume was measured to ± 0.01 ml. The 0.01 *M* thiosulfate was made up on the day it was used by diluting the proper amount of a stock solution 1 *M* in thio and 0.01 *M* in sodium hydroxide with distilled water. The concentrations of the thio solutions were determined within 0.05% by using fine crystals of potassium iodate as the standard and these in turn were occasionally checked against freshly dried arsenious oxide. The end-point of the titration was determined with the aid of 2 drops of a fresh 1% solution of water soluble starch.

In the analyses employing 50 ml of the copper reagent this volume was reproduced to ±0.01 ml. and to this was added up to 5 ml. of the sugar solution measured to ±0.002 ml. and enough water to bring the total volume up to 55 ± 0.02 ml. The heat treatment was carried out after adjusting the water levels in the baths to about one inch above the surface of the solutions in the test-tubes.

The liberation of the iodine and the oxidation of the cuprous oxide was brought about by adding 3 ± 0.1 g. of potassium iodide,[8] Merck Reagent Crystals, followed by 10 ± 0.1 ml. of a solution containing 216 g. (1.17 moles) of $K_2C_2O_4 \cdot H_2O$ per liter.[9] The test-tube was swirled until the potassium iodide had all dissolved and the cuprous oxide was uniformly suspended in the liquid, whereupon the slurry was dumped into an empty clean 250-ml. erlenmeyer flask. The inside of the test-tube was rinsed once or twice with the supernatant liquid in the erlenmeyer flask. The mixture in the flask was then acidified slowly with 20 ± 0.1 ml. of 2.5 *N* sulfuric acid. This was done by letting the acid run freely from the pipet down the side of the flask while the latter was swirled in order to reduce local excess of acid and prevent the formation of a white precipitate which could not be redissolved.

The iodine was titrated with a solution about 0.1 *M* in $Na_2S_2O_3$ and 0.001 *M* in sodium hydroxide until a dark green color was produced about 4 ml. from the end-point. The test-tube was rinsed again with the liquid in the flask and the titration resumed until a blue-green color was obtained. Again the test-tube was rinsed with the liquid and the titration resumed until the end-point was reached when the solution changed sharply from a blue-green to a royal blue. The end-point was reproduced within 0.02 ml. of the 0.1 *M* thio without the use of starch. Care was taken to avoid loss of material when transferring it back and forth between the test-tube and the erlenmeyer flask.

Variations in the Yield of Cuprous Oxide Produced by Changes in the Environment

In order to conserve space, the letters D, L, S and I indicate that the sugar was, respectively, dextrose, levulose, sucrose and invert sugar, an equimolar mixture of dextrose and levulose. The

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(5) The *p*H readings were reproduced within 0.005 *p*H unit when employing the Cambridge Research Model Electron-Ray *p*H Meter fitted with the shielded dipping glass electrode assembly.

(6) R. F. Jackson and E. J. McDonald, *J. Research Natl. Bur. Standards*, **34**, 213 (1945); *J. A. O. A. C.*, **28**, 371 (1945).

(7) "Official and Tentative Methods of Analysis," the Association of Official Agricultural Chemists, Sixth Edition, 1945, Sugars and Sugar Products, pp. 570–594.

(8) Two grams of potassium iodide are insufficient to prevent loss of iodine. The iodide could not be included in the oxalate solution because solutions containing four or more moles of potassium iodide per liter were oxidized slowly to iodine by air even when sodium bicarbonate was present.

(9) Nine ml. of the oxalate solution are insufficient to prevent the formation of a cloudy solution. The oxalate solution should be discarded for a fresh one when the end point cannot be reproduced even though it remains sharp.

letter W indicates that water but no sugar was used in the experiment. The number immediately following the letter gives the ml. of the copper reagent used in each analysis. This number may be followed by the *p*H of the reagent, and this in turn by the net yield of cuprous oxide in moles per mole of hexose or sucrose whichever is employed, and then the length in minutes of the heat treatment at 100° may be given. In Fig. 1 the results were obtained using sufficient sugar to reduce between 47 and 50% of the copper when the plateau was reached. All *p*H values were obtained at 26 ± 1°.

Variations in the heat treatment changed the yields of cuprous oxide as shown in the middle part of Fig. 1. The yields produced by the reducing sugars are seen to rise eventually to a plateau where they change less than 1% over a period of ten minutes at 100°. The length of the heat treatment required to reach the plateau increased less than 10% as the *p*H of the copper reagent was lowered from 9.3 to 8.7; it was over twice as long for dextrose as for levulose and the same within 10% for invert sugar as for dextrose alone. The yield produced by invert sugar when the plateau was reached was equal to the sum of the yields produced by dextrose and levulose when alone.

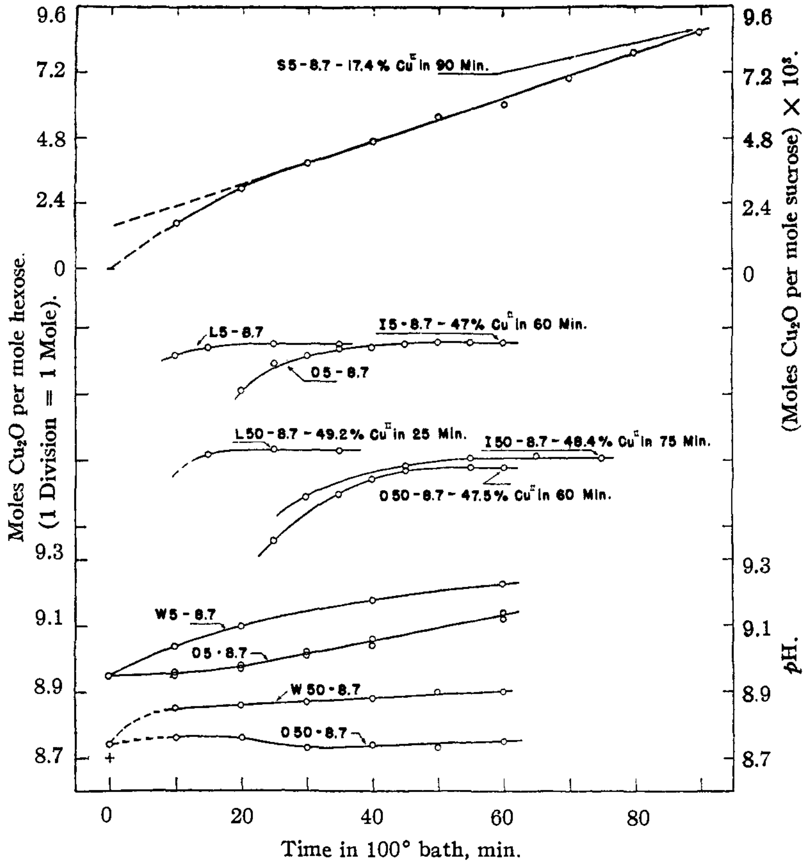

Fig. 1.—Variation in *p*H and yield of cuprous oxide with the extent of the heat treatment.

The yield of cuprous oxide obtained when the plateau was reached increased as the *p*H was decreased from 9.3 to 8.7. The increase in the yield was greater in the case of levulose than in the case of dextrose, and their yields became equal when the *p*H of the mixtures of copper reagent and sugar was between 8.9 and 8.95 at 25° before they were heated. The yields changed less than 2% when 50 instead of 5 ml. of the appropriate copper reagent was employed provided the initial values of the *p*H of the resulting mixtures of copper reagent and sugar were the same. A change in this *p*H of 0.1 unit, however, changed the yield up to 0.15 mole. In order to reproduce the yield within 0.2% it was, therefore, necessary to reproduce the *p*H of the copper reagent within 0.01 unit and the dilution of the reagent within 1%.

The amount of cuprous oxide produced by sucrose does not reach a plateau of nearly constant yield as the heat treatment is prolonged for any reasonable time. This is shown by the top line of Fig. 1. The results are similar to those obtained by E. J. McDonald and A. L. Turcotte[10] using Ofner's method and support their implied belief that the cuprous oxide is not necessarily produced by invert sugar or other reducing substances as impurities in the sucrose, but by sucrose itself or the products of its decomposition produced by the heat treatment. It may well be that the sucrose is hydrolyzed by the heat treatment, *i. e.*, that hydroxyl as well as hydrogen ions catalyze the hydrolysis because the yield of cuprous oxide produced by sucrose increases with the *p*H of the alkaline heat treated mixture whereas the yield produced by invert and other simple reducing sugars decreases. As a consequence of this, the rate of hydrolysis of sucrose should reach a minimum at some *p*H nearer seven than the isomerization, mutarotation, of dextrose because the rate of the latter reaction is catalyzed to a markedly lesser extent by hydrogen than hydroxyl ions whereas the catalytic effects of these ions upon sucrose appear to be more nearly equal.

The increases in *p*H brought about by the dilution and subsequent heat treatment of the copper reagent are shown in the lower part of Fig. 1. Dilution of 5 and 50 ml. of the copper reagent with

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[10] E. J. McDonald and A. L. Turcotte, *J. A. O. A. C.*, **30**, 124 (1947).

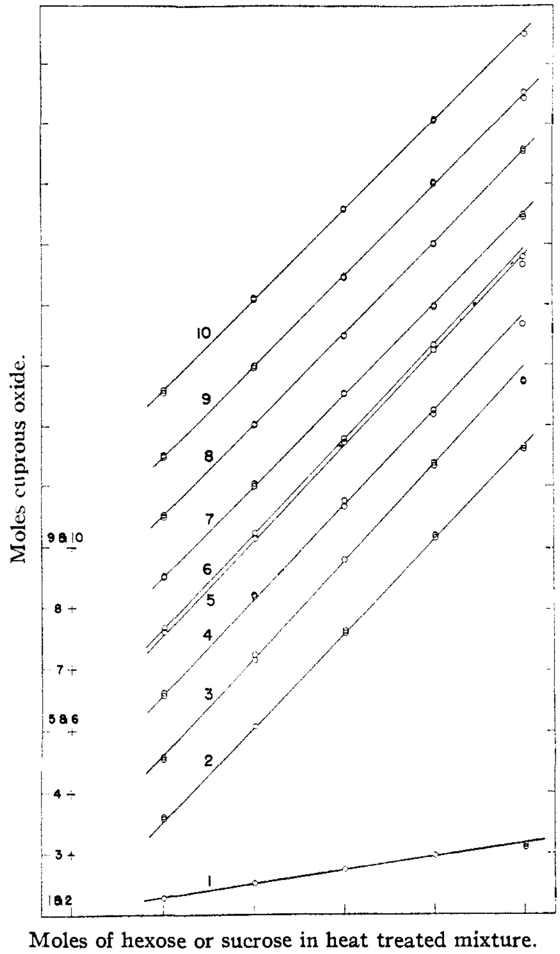

Fig. 2.—The cuprous oxide produced by selected amounts of the sugars. The + signs along the ordinate indicate the origins of the respective lines.

Line 1 represents S 5 − 8.7 − "0.0058 ± 0.0003" in fifty minutes. One division along the ordinate represents 2.5×10^{-5} mole cuprous oxide and along the abscissa 10^{-3} mole of sucrose. Parentheses indicate that the yield is based on the assumption that the sucrose was pure. Actually the sucrose contained 0.017 ± 0.001 wt. % of invert sugar.

Line 2 represents D 50 − 8.7 − 4.90 ± 0.02 in sixty minutes; line 3, L 50 − 8.7 − 5.12 ± 0.02 in twenty-five minutes; line 4, I 50 − 8.7 − 4.97 ± 0.04 in sixty minutes; line 5, I 50 − 8.7 − 5.00 ± 0.02 in sixty minutes, and line 6, (1 + S) 50 − 8.7 for sixty minutes. In the case of these lines, one division along the ordinate represents 2.5×10^{-4} mole of cuprous oxide, and along the abscissa 7.824×10^{-5} mole of hexose. In the case of line 6, every mixture also contained 5×10^{-3} mole of sucrose. Line 7 represents D 5 − 8.7 − 4.84 ± 0.02 in fifty minutes; line 8, L 5 − 8.7 − 4.80 ± 0.02 in twenty-five minutes; line 9, I 5 − 8.7 − 4.82 ± 0.01 in fifty minutes, and line 10, (I + S) 5 − 8.7 for fifty minutes. In the case of lines 7, 8, 9 and 10, one division along the ordinate represents 2.5×10^{-5} mole of cuprous oxide, and along the abscissa 7.824×10^{-6} mole of hexose. In the case of line 10, every mixture also contained 5×10^{-3} mole of sucrose.

5 ml. of water or sugar solution increases the *p*H by 0.25 and 0.04, respectively. The *p*H is increased further by the heat treatment, but this increase is seen to be less in the solutions producing cuprous oxide. The amount of base removed by the production of the cuprous oxide is for the most part obscured by the buffer in the solution. The removal of this base produces a smaller increase in *p*H during the heat treatment when 50 instead of 5 ml. of the copper reagent is employed and the same fraction of the copper is reduced.

The effect of changes in the concentration of tartrate upon the yield of cuprous oxide and the stability of the copper reagent and heat treated mixtures was determined over a range of *p*H from 8.6 to 9.3 inclusive in steps of 0.1 unit for the copper reagent and over a range of tartrate concentration corresponding to ratios of tartrate to copper from 1.5 to 3.0 inclusive. The yield of cuprous oxide increased about 10% as the ratio was raised from 1.5 to 3.0, but the increase was less than 2% from 2.3 to 3.0. No precipitate appeared in the copper reagents, nor was any precipitate other than cuprous oxide produced by the heat treatment, nor did aging of the copper reagent produce any change in the yields of cuprous oxide when the formal concentration of tartrate was more than 2.8 times that of the copper and the copper reagent was at *p*H 8.7 or higher. The value of the ratio is three in the case of the micro method. In view of this and the observations cited in this paragraph, we chose to make the value of the ratio equal to three in the macro reagent.

The cuprous oxide produced by selected amounts of the sugars is given in Fig. 2. The heat treatment was sufficient in the case of the reducing sugars to reach the plateau of nearly constant yield when less than 60% of the copper was reduced. Under these conditions the amount of cuprous oxide produced depends linearly upon the amount of sugar producing it. This was proved by a statistical analysis of the data. The points representing up to 60% reduction of the copper fall on straight lines within the limits of error which was less than 0.5%. The slope of a line gives the yield of cuprous oxide and the negative of the value of its intercept on the cuprous oxide axis representing no sugar gives the average amount of cuprous oxide lost. The amount of cuprous oxide lost was about 10^{-6} mole in every case and is equivalent to a difference of 0.2 ml. between 0.01 *M* thio titers for the blanks and sugar solutions. When more than 60% of the copper was reduced, lower yields of cuprous oxide were obtained; this is shown in Fig. 2 by the points that fall below the upper ends of the straight lines which were drawn to represent the results up to 60% reduction.

The amount of cuprous oxide produced by sucrose was found to depend linearly upon the amount of sucrose put into the heat treated mixture when the heat treatment was fifty minutes in the 100°-bath and only a small fraction of the copper was reduced. This is shown by line 1 in Fig. 2. The percentage error in the results represented by line 1 is greater than in the rest of the work because the differences between the thio titers for the blanks and sucrose solutions were about one-tenth as large. The yield of cuprous oxide given by line 1 for sucrose is less than 0.0006 of the yield produced by invert sugar. The amount of cuprous oxide produced by a mixture of invert sugar and sucrose is the same, however, after correcting for the oxide lost, as the sum of the amounts produced by the invert sugar and sucrose when each is alone in the solution. Lines 6 and 10 in Fig. 2 were drawn on this basis and represent the data up to 60% reduction of the copper. The amount of cuprous oxide produced by sucrose in these experiments would have been less than 1% of the total if the mole ratio of invert to sucrose had been 0.06 or greater in the sample analyzed. It should be emphasized, however, that when significant amounts of cuprous oxide were produced by sucrose the heat treatment was reproduced within 0.5%.

Discussion

The observed additivity of the reducing powers of invert sugar and sucrose in the same solution under certain conditions makes it possible to calculate by means of linear equations the amounts of these sugars in a sample containing a mixture of them from the total amount of cuprous oxide they produce under these conditions. In the analysis of a known weight, g. of the sample, the equation is

$$A + a = Y_1(g - s)/360 + Y_2(s/342) \qquad (1)$$

and s is calculated from the values of the other quantities. In the analysis of a known volume of the sample the equation is

$$A + a = Y_1(T - S^\circ) + Y_2S^\circ \qquad (2)$$

and S° is calculated from the values of the other quantities.

In these equations, A represents the moles of cuprous oxide produced by the sugars as obtained from the thio difference and a represents the moles of cuprous oxide lost.[11]

Y_1 and Y_2 represent the moles of cuprous oxide produced per mole of invert sugar and sucrose, respectively. The values are obtained from calibration plots like those labelled I and S in Fig. 2 where Y_1 is about 10 and Y_2 is about 0.004 for pure sucrose with a heat treatment of fifty minutes in the 100°-bath.

T and S° represent total moles of invert sugar plus sucrose and moles of sucrose, respectively, and s represents the weight of sucrose in the sample analyzed. The value of T may be obtained by any of the official methods of the Association of Official Agricultural Chemists.[5]

The above method of determining the amounts of the components in a mixture of sugars is not limited to mixtures of invert sugar and sucrose. One can use it for other mixtures of sugars and glycosides like those encountered in determining the rate of hydrolysis of these substances from the increase with time in the reducing power of the solutions.

The amount of invert sugar in refined sucrose can be estimated in the following way. The slope of the straight line portion of the top line in Fig. 1 beyond fifty minutes is produced entirely by the decomposition of sucrose and equals 7.98×10^{-5} mole of cuprous oxide per mole of sucrose per minute at the bath temperature of 100°. In terms of the sucrose in the heat treated mixture this slope was calculated from an increase of 0.0795 ml. per minute in the 0.01 M thio difference. Thus at the end of fifty minutes, the observed thio difference would have been $(0.0795 \times 50 = 3.98)$–(the cuprous oxide lost $\simeq$ 0.20 ml.) = 3.78 ml. if sucrose alone had been present. The actual smoothed value was 5.25 ml.; so 1.47 ml. was produced by the invert sugar; hence $1.47 \times 10^{-5} \times 0.5 \times 360/(4.82 \times 2) = 2.76 \times 10^{-4}$ g. of invert was in the heated mixture. The wt. per cent. of invert in the sample of refined sucrose, therefore, was 2.76×10^{-4} g. × 100/1.702 g. = 0.016%. The value is 0.018% if one allows three minutes for the mixture originally at 25° to reach 100° after it was placed in the 100°-bath; hence there were less than 2 g. of invert sugar in 10 kg. of the refined sucrose.

In the case where all but a negligible amount of the cuprous oxide is produced by a single reducing sugar or a mixture consisting of a constant ratio of these sugars such as invert sugar, the moles of sugar producing the oxide even when more than 60% of the copper is reduced can be read off the appropriate calibration plot[12] from the observed thio difference. In most cases a negligible amount of

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[11] The value of A should fall within the following ranges: (4 to 16) 10^{-6} mole cuprous oxide when employing 5 ml. of the micro copper reagent[3] at pH 9.00 and the heat treated mixture is made up to 10 ml., (2 to 8) 10^{-5} mole cuprous oxide when employing 5 ml. of the macro reagent at pH 8.70 and the heat treated mixture is made up to 10 ml., (2 to 8) 10^{-4} mole cuprous oxide when employing 50 ml. of the macro reagent at pH 8.70 and the heat treated mixture is made up to 55 ml. The value of a is about 10^{-6} mole cuprous oxide in all cases. Our best results have been obtained when employing 5 ml. of the macro reagent at pH 8.7 and a total volume of 10 ml. The range in the values of A given above corresponds to the determination in a single analysis of 10^{-6} to 3×10^{-4} mole (0.2 to 50 mg.) of dextrose or its equivalent in reducing power, and to concentrations ranging from 0.0002 to 0.3 mole of dextrose or its equivalent per liter or from 3.6 to 5000 mg. per 100 ml. of solution when aliquots ranging from 1 to 5 ml. of the sugar solution are employed in the analyses. In order to determine the invert sugar produced by the complete inversion of a saturated solution of sucrose, 1.5 g. (1.2 ml. at 20 to 30°) or less of it would need to be made up with water to 100 ml.

[12] The calibration plot can usually be constructed with sufficient accuracy if 1 cm. of the graph paper represents 0.05 ml. of the thio difference and along the other axis (abscissa) moles of reducing sugar in the heat treated mixture. The best straight line is then passed by inspection through the points representing the results up to 60% reduction of the copper. The line can be extrapolated back to zero thio difference but to pass through the points representing greater than 60% reduction of copper it usually needs to be bent downward. Only the linear portion of the curve should be used with equations 1 and 2.

cuprous oxide is produced by non-reducing sugars and glycosides at concentrations less than 0.05 molar in the heat treated mixtures.

Before using the method with confidence one should, of course, determine (1) the defecation treatment, if any, which should be applied to the raw sample, (2) the accuracy required for the purpose at hand especially in the absolute and relative amounts of invert sugar and sucrose, and (3) the concordance obtainable by different chemists.

In order to obtain a precision of 0.2% the following recommendations should be observed aside from the measurement of volumes.

1. Run blanks and duplicates in all cases.
2. Reproduce the *p*H of the copper reagent within 0.01 unit as determined with a *p*H meter, and its formal composition within 1%. The best value of the *p*H is 8.70 for the macro reagent.
3. Keep the dilution of the copper reagent constant within 1% when making up the mixture of copper reagent and sugar.
4. Heat the mixtures long enough to reach the plateau of constant yield of cuprous oxide produced by the reducing sugar. It is then sufficient to reproduce the length of the heat treatment within two minutes when the mole ratio of sucrose to invert sugar is less than 0.06; otherwise it should be reproduced within 1%. The minimum length of the heat treatment differs with changes in the configuration and structure of the reducing sugar. Adequate heat treatment at the lowest *p*H is sufficient at higher *p*H values.
5. Use sufficient sugar to reduce between 10 and 60% of the copper when the plateau is reached; the amounts of cuprous oxide and sugar then depend linearly upon each other and estimation of the sugars is simplified.
6. Calibrate the method with pure sugar in the same environment that prevails in the samples to be analyzed, at least as regards *p*H and buffer capacity toward the copper reagent. In most cases the sugar solutions to be analyzed are not buffered and do not contain other reducing substances so water alone is usually adequate to bring the final volume up to the desired value.
7. It is usually necessary to correct for the difference between actual volumes employed and those marked on the glassware especially if the temperature is not at 20°. The uncertainty in the volumes delivered by transfer pipets is apt to produce large errors unless extreme precautions are taken.

Summary

1. The analytical method devised by P. A. Shaffer and co-workers employing a carbonate buffered cupritartrate reagent of low *p*H has been adapted to the determination of macro quantities of reducing sugars ranging from 4×10^{-6} to 2×10^{-4} mole of dextrose or its equivalent in reducing power in a single analysis with a precision of a few tenths of one per cent.

2. The effect of several variables upon the yield of cuprous oxide has been determined. (a) Aside from the measurement of volumes the *p*H needs to be reproduced within 0.01 in order to obtain a precision of a few tenths of 1%. (b) The yields produced by levulose, dextrose and invert sugar rise to plateaus of nearly constant value as the heat treatment is prolonged, but the yield produced by sucrose continues to increase. (c) The yields obtained when the plateau has been reached increase with decreasing *p*H but the increase is larger for levulose than for dextrose, and their yields become equal when the mixtures of copper reagent and sugar before the heat treatment are at *p*H 8.9 at 25°. Under these conditions about ten moles of cuprous oxide are produced per mole of invert sugar.

3. Under certain prescribed conditions the amount of cuprous oxide produced depends linearly upon the amount of sugar in the heat-treated mixture, and the yields produced by dextrose, levulose and sucrose alone are not altered when they are together in the same mixture. Also the amount of cuprous oxide lost is the same in every analysis. This simplifies the estimation of the sugars in raw mixtures.

4. The yield of cuprous oxide produced by sucrose is less than one-thousandth of the yield produced by invert sugar and varies with changes in the environment in a way suggesting that most of the cuprous oxide is produced by invert sugar resulting from hydrolysis of sucrose during the heat treatment.

5. A method is given for estimating the invert sugar in refined sucrose. The sucrose employed contained between 0.016 and 0.018 wt. per cent. of invert sugar.

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