A SEMI-MICRO METHOD FOR THE DETERMINATION OF REDUCING SUGARS

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(WITH THREE FIGURES)

Introduction

The MUNSON-WALKER method (**3**) with the modification of BERTRAND (**1**) has long been used for the determination of reducing sugars in plant extracts. The large amounts of solutions required by this method, however, make necessary the frequent preparation and standardization of solutions, which is inconvenient when large numbers of determinations are being made over an extended period of time. For plant materials containing not more than 10 to 12 per cent. sugar, it seems unnecessary to use the amounts of Fehling's solutions originally specified. ILJIN (**2**) in adapting the MUNSON-WALKER method to a micro scale, used 3-ml. portions of Fehling's solutions A and B. Where more than one per cent. is encountered, however, the dilutions made necessary by this modification in order to keep within his range of 1 to 25 mg. of sugar, result in too great a dilution factor in the final value. An additional advantage of reducing the amounts of reagents used would be in making possible carrying out the reduction and subsequent washing of the copper oxide precipitate directly in centrifuge tubes, thus eliminating the tedious and inconvenient procedure of filtering the precipitate on a Gooch crucible. The use of the centrifuge for micro-sugar analysis has been reported by ILJIN (**2**) and PHILLIPS (**4**).

Another objection to the MUNSON-WALKER method is the practice of boiling the sugar solutions over an open flame. QUISUMBING and THOMAS (**5**) have shown that this practice is subject to error, since at 100° C. the end-point of the copper reduction is taken on a continuously ascending curve, so that small changes in time or temperature will result in appreciable error. They pointed out the desirability of using a temperature at which the reduction curve would eventually become level, at which point the end-point could be taken. A temperature of 80° C. was found best for this purpose. SHAFFER and SOMOGYI (**8**) have also stressed the importance of taking the end-point of reduction on the level portion of the curve.

The use of standard potassium permanganate solution for titrating the reduced cuprous oxide according to the BERTRAND modification, has the disadvantages of instability of solution, sensitiveness to foreign organic matter and poor end-point, especially when the solutions cannot be thoroughly cleared. In view of the recent development of an inside indicator for potassium dichromate (**7**), it would appear desirable to use this solution instead of potassium permanganate for titration of the reduced copper oxide.

With the above considerations in mind, the MUNSON-WALKER-BERTRAND method has been modified to a semi-micro scale by incorporating the following changes:

(1) Using 20 ml. of Fehling's solution instead of 50 ml.
(2) Carrying out the sugar-copper reactions in an 80° C. water bath instead of boiling over an open flame.
(3) Centrifuging the Cu_2O precipitate instead of filtering on a Gooch crucible.
(4) Using potassium dichromate with sodium diphenylamine sulphonate inside indicator as a standard oxidant instead of potassium permanganate.

Preliminary experimentation

PREPARATION OF REAGENTS.—Fehling's solutions A and B were prepared according to the modification proposed by QUISUMBING and THOMAS (5) as follows:

Solution A was prepared by dissolving 69.28 gm. of c.p. $CuSO_4 \cdot 5H_2O$ in warm water and then diluting to 1 liter in a volumetric flask. After standing for several hours, the solution was filtered and transferred to a Pyrex reagent bottle.

Solution B was prepared by dissolving 346 gm. of c.p. sodium potassium tartrate in warm water and then transferring to a 1-liter volumetric flask. A saturated solution of sodium hydroxide was then digested on a steam bath until insoluble carbonates had settled out. The exact alkalinity of the solution was then determined and the amount containing exactly 130 gm. of sodium hydroxide added to the sodium potassium tartrate solution and made to 1 liter.

Ferric ammonium sulphate solution was made by dissolving 240.9 gm. of $Fe(NH_4)(SO_4)_2 \cdot 12\ H_2O$ in warm water, then adding 200 ml. of concentrated H_2SO_4. After cooling, the solution was diluted to 1 liter and filtered.

A 0.1000-N solution of potassium dichromate was prepared by dissolving 4.9037 gm. of highest purity $K_2Cr_2O_7$ (previously dried) in distilled water and then diluting to 1 liter in a volumetric flask.

Sodium diphenylamine sulphonate indicator was made by dissolving 0.32 gm. of barium diphenylamine sulphonate in 100 ml. of water, then adding 1.0 gm. of sodium sulphate and allowing the precipitate of barium sulphate to digest for one hour. The solution of sodium diphenylamine sulphonate was then filtered through a heavy filter paper.

DETERMINATION OF SUGAR-COPPER REDUCTION TIME.—QUISUMBING and THOMAS (5) have shown that 100 ml. of sugar-copper solutions held in an 80° C. water bath required 30 min. for complete reduction. Since smaller quantities of solutions were to be used, the possibility of reducing the time for reduction established by these investigators was investigated. Three

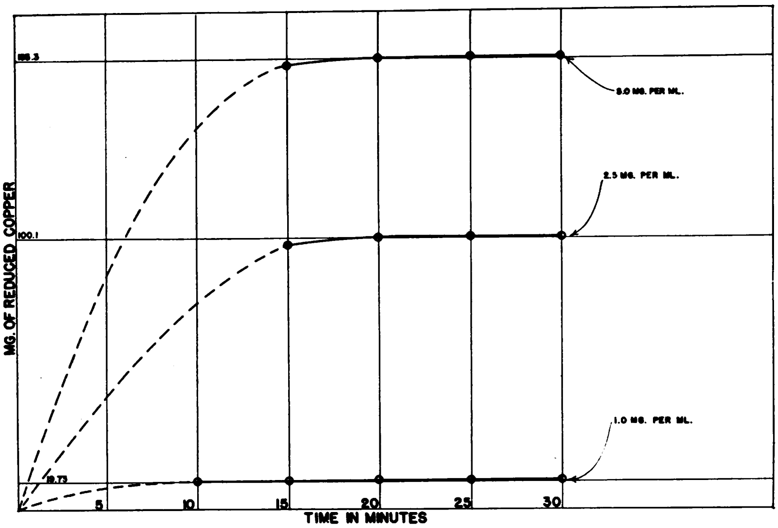

FIG. 1. Time required for digestion of varying concentrations of glucose and an alkaline copper tartrate solution in an 80° C. water bath.

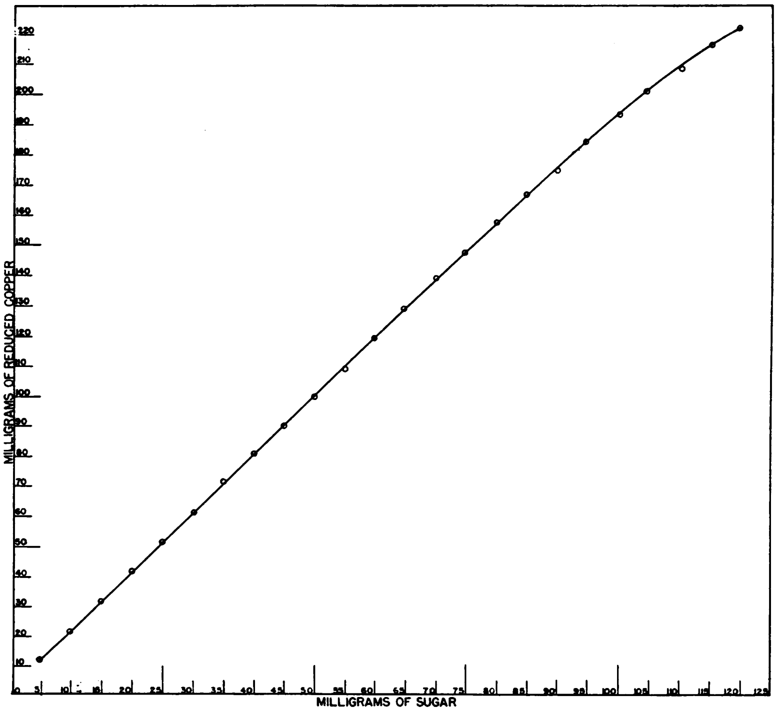

FIG. 2. Copper-glucose curve, determined by experiment.

glucose solutions were prepared containing 1 mg., 2.5 mg., and 5 mg. respectively per ml., representing the maximum, minimum, and median range of the sugar curve. After mixing with 20 ml. of Fehling's solutions, duplicate samples of the three sets were subjected to time intervals of 10, 15, 20, 25 and 30 minutes in an 80° C. water bath regulated to 1° C. by two bunsen burners. In this case, the results show (fig. 1) that the reduction curves became level shortly after 15 min., remained so at 20 to 25 min., and only slightly ascended in 30 min. In order to preserve uniformity, the time of 20 min. was arbitrarily accepted for further experimentation.

Arrangement of the centrifuge.—For these investigations an International type 1C centrifuge with an 8-place head carrying 50 ml. Pyrex tubes was used. To facilitate the rapid balancing of the centrifuge, the metal carriers were weighed and balanced to within 0.05 gm. by placing small pieces of sheet copper beneath the rubber cushions. The weight of the heaviest glass centrifuge tube was then determined, and the deficiencies in weight of the other seven, varying from 0.20 to 2.40 gm., recorded on the

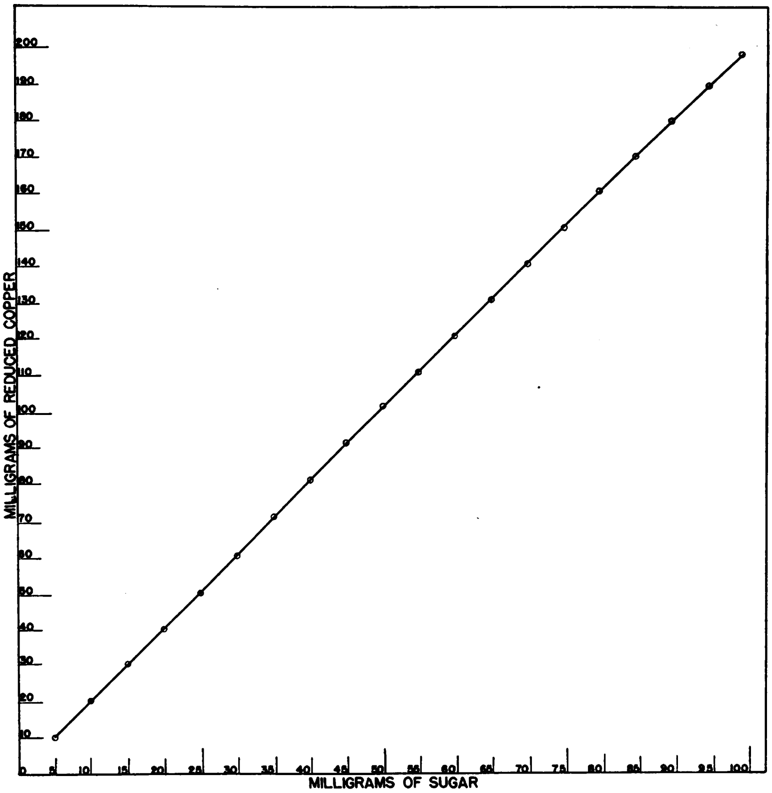

Fig. 3. Copper-sucrose curve, determined by experiment.

tubes with a glass-marking pencil. Hence, the centrifuge was rapidly balanced by the simple expedient of adding the same amount of water (measured from a burette set up for the purpose) in milliliters as recorded in grams on the centrifuge tubes. Since the centrifuge was designed to accommodate eight tubes at one time, two sets of eight tubes were prepared.

Method of analysis

As a result of preliminary experimentation, the following analytical procedure was adopted. To 20 ml. of sugar solution contained in a 50-ml. Pyrex centrifuge tube, measure from a burette 10 ml. each of Fehling's solutions A and B. Place in a water bath held at 80° C. (± 1° C.) for 20 min. While the first set of eight tubes is digesting, another set may be prepared to be placed in the water bath as soon as the first set is removed for centrifuging, etc. If this procedure is followed, 16 determinations may be made

TABLE I

GLUCOSE* $y = 0.4650x + 0.00028x^2$

COPPER	SUGAR	0.1000N $K_2Cr_2O_7$	COPPER	SUGAR	0.1000N $K_2Cr_2O_7$	COPPER	SUGAR	0.1000N $K_2Cr_2O_7$
mg.	*mg.*	*ml.*	*ml.*	*mg.*	*ml.*	*mg.*	*mg.*	*ml.*
5.0	2.33	0.78	85.0	41.55	13.31	165.0	84.37	25.85
10.0	4.67	1.57	90.0	44.12	14.10	170.0	87.04	26.68
15.0	7.04	2.38	95.0	46.71	14.90	175.0	89.94	27.25
20.0	9.41	3.14	100.0	49.30	15.70	180.0	92.78	28.21
25.0	11.80	3.93	105.0	51.92	16.49	185.0	95.65	29.00
30.0	14.20	4.71	110.0	54.55	17.25	190.0	98.48	29.80
35.0	16.62	5.50	115.0	57.18	18.05	193.3†	100.0	30.40
40.0	19.06	6.28	120.0	59.84	18.84	199.6	105.0	31.42
45.0	21.49	7.07	125.0	62.51	19.60	208.5	110.0	32.80
50.0	23.96	7.85	130.0	65.18	20.38	216.2	115.0	34.00
55.0	26.43	8.62	135.0	67.87	21.19	222.5	120.0	35.0
60.0	28.91	9.40	140.0	70.59	21.98			
65.0	31.41	10.20	145.0	73.33	22.75			
70.0	33.93	10.80	150.0	76.06	23.50			
75.0	36.46	11.74	155.0	78.80	24.25			
80.0	38.99	12.51	160.0	81.57	25.08			

* Sugar values for both glucose and sucrose were calculated from experimentally determined data (see fig. 2 and fig. 3) by the method of least squares according to the equations:

(1) $y = a + bx + cx^2$

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

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

The mean eror, calculated from the equation $d = \sqrt{\dfrac{\Sigma d^2}{n}}$ is 0.251 mg. for glucose and 0.228 mg. for sucrose.

† Values determined experimentally.

in approximately one hour. Remove the tubes and cool for 2 to 3 minutes in cold water. Equalize the weight of the tubes by adding water to each in accordance with its deficit in weight, and centrifuge at not less than 1000 r.p.m. for 4 min. If some light flakes of Cu_2O persist in floating after centrifuging, add 2 to 4 drops of n-butyl alcohol to reduce the surface tension. Decant the excess Fehling's solution from the Cu_2O precipitate with a stream of water from a wash bottle, add 5 to 10 ml. of ferric ammonium sulphate solution, depending upon the amount of cuprous oxide present. Transfer the solution quantitatively to a 150-ml. beaker, add 6 to 9 drops of sodium diphenylamine indicator and titrate with standard potassium dichromate solution. At the end point, the color of the solution changes from an emerald green to an intense purple, and in practice it is not difficult to detect this change with $\frac{1}{2}$ drop of potassium dichromate. Duplicate titrations should check within 0.05 ml.

Determination of sugar-copper ratios

Using the procedure outlined, the ratios of copper to glucose and invert sugar were determined. For this purpose stock solutions containing 5 mg. of sugar per milliliter were prepared from Pfanstiehl's highest purity glucose and sucrose, dried for 2 hours in a vacuum oven at 70° C. previous to weighing. Stock solutions of sucrose were hydrolyzed at pH 5.0 with 2 ml. of Difco invertase solution for 4 hours. The experimental curves showing the copper equivalents to glucose and invert sugar and glucose are shown

TABLE II

INVERT SUGAR* $y = 0.4594x + 0.00028x^2$

COPPER	SUGAR	0.1000N $K_2Cr_2O_7$	COPPER	SUGAR	0.1000N $K_2Cr_2O_7$	COPPER	SUGAR	0.1000N $K_2Cr_2O_7$
mg.	*mg.*	*ml.*	*mg.*	*mg.*	*ml.*	*mg.*	*mg.*	*ml.*
5.0	2.30	0.77	85.0	41.06	13.31	165.0	83.43	25.85
10.0	4.66	1.57	90.0	43.61	14.10	170.0	86.18	26.68
15.0	6.90	2.38	95.0	46.17	14.90	175.0	88.96	27.25
20.0	9.30	3.14	100.0	48.74	15.70	180.0	91.76	28.21
25.0	11.64	3.93	105.0	51.32	16.49	185.0	94.61	29.00
30.0	14.03	4.71	110.0	53.93	17.25	190.0	97.41	29.80
35.0	16.42	5.50	115.0	56.53	18.05	195.0	101.2	30.62
40.0	18.83	6.28	120.0	59.16	18.84	200.0	113.1	31.42
45.0	20.24	7.07	125.0	61.80	19.60			
50.0	23.67	7.85	130.0	64.44	20.38			
55.0	26.11	8.62	135.0	67.11	21.19			
60.0	28.58	9.40	140.0	69.80	21.98			
65.0	31.04	10.20	145.0	72.61	22.75			
70.0	33.53	10.80	150.0	75.22	23.50			
75.0	36.03	11.74	155.0	77.93	24.25			
80.0	38.56	12.51	160.0	80.67	25.08			

* See footnote at the bottom of table I.

in figures 2 and 3. The sugar values for both invert sugar and glucose were calculated from the experimental data by the method of least squares and are shown in tables I and II.

Summary

1. A semi-micro modification of the MUNSON-WALKER-BERTRAND method for determining reducing sugars has been presented.

2. The method described uses smaller quantities of reagents, substitutes a water bath for digestion, centrifuges the cuprous oxide precipitate, and uses potassium dichromate as a standard oxidant.

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