*Determination of Reducing Sugars by Means of Back Titration against Alkaline Copper Solution. I**

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(Received May 23, 1959)

Since the determination by means of Fehling solution has become used so generally for sugar analysis, different chemists have devised, in consequence of its importance in both practice and research, so many modifications that there is probably no method in analytical chemistry which suffers more from variety of procedure. Numerous modifications have been described which differ in analytical details. Fehling solution is rather unstable, so efforts have been made to improve its stability, and to establish a simpler and more practical procedure.

The amount of copper which is reduced by various sugars has been found to vary according to the alkalinity, the temperature, the rate of ebullition, the time of heating, the type of tartrate, the amount of contact with air, etc. Among them the most important variables are the temperature, the time of heating, and the rate of ebullition. Consequently some methods have devised for heating because it is difficult to standardize the temperature and the rate of ebullition. The indirect heating is frequently employed as in the case of Main's pot method where the temperature is regulated by a boiling water bath and the reduction is carried out in a test tube provided with floats. But this is not a fine procedure, because it requires a complicated operation and demands skilfulness in analyst. Then, the results should be corrected according to the variation of variables rather than a strict standardization of the reacting condition is demanded. Blank titration with a standard sugar solution, which checks the variation of conditions and corrects the results, is most suitable for the present purpose.

Numerous procedures already devised for the determination of the quantity of reduced copper can briefly be divided into a partial reduction method and a total reduction method. In the former a certain volume of Fehling solution is brought into a reaction with a given amount of sugar which does not reduce all the copper, and the cuprous oxide produced is weighed directly or estimated by means of redox titration. The other is a method which, in principle, involves the determination of the volume of sugar solution required to reduce completely a measured volume of alkaline copper solution, the end point being indicated by a spot test or an internal indicator. This is indeed one of the earliest procedures for the determination of the reducing power. Soxhlet heated a known volume of Fehling solution to boiling in an open dish and added the sugar solution until the copper was completely reduced. No great accuracy was possible by this procedure, even in highly skilled hand. The degree of accuracy of this method was considerably increased by various devices, namely, by boiling the solution in a flask instead of an open dish, to diminish back-oxidation, and by using a suitable indicator. Lane and Eynon¹⁾ found that the end point was internally indicated by the reduction of methylene blue to methylene white by minute excess of reducing sugar and determined the weight of each sugar required to reduce the copper completely. These weights, which varied with nature of sugar and with its concentration, constituted their table for analysis. This method, described as "the most convenient, most expeditious, and frequently the most accurate method"²⁾, has been adopted for industrial and scientific purposes, but seems still to be a complicated and time-consuming procedure with some disadvantages. For instance, it consumes a large volume of sugar solution, and is not available for the determination of a small quantity of sugar, etc.

In general, the total reduction method,

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* Partly presented at the Annual Meeting of the Chemical Society of Japan, Tokyo, April, 1957.

1) J. H. Lane and L. Eynon, *J. Soc. Chem. Ind.* (*London*), **42**, 32T, 143T (1923); **44**, 150T (1925); **46**, 434T (1927).

2) F. J. Bates et al., "Polarimetry, Saccharimetry and the Sugars", U. S. Government Printing Office, Washington (1942), p. 185.

represented by Lane and Eynon's method, is simpler, more practical, and more accurate than the partial reduction method, represented by the gravimetric method and Bertrand's method. In view of this advantageous quality, the author has endeavored to improve the former procedure and to establish a more convenient method of procedure with a high accuracy on such a way as to confer the maximum possible simplicity of procedure and to eliminate the personal factor as far as possible. To this end a back titration method has been devised.

Experimental

Materials.—The following substances were used for the present investigation. An anhydrous glucose was obtained by recrystallizing a commercial glucose (monohydrate, the Japanese Pharmacopoeia) from water and acetic acid. A pure commercial lactose was recrystallized from water. A pure commercial galactose was recrystallized from water and alcohol. Crystalline copper sulfate ($CuSO_4 \cdot 5H_2O$) of reagent grade was recrystallized from water. A 50% solution of sodium hydroxide (reagent grade) was prepared and allowed to stand until a precipitate of sodium carbonate appeared. By filtering it through asbestos a clear solution was obtained. Benzoic acid, Rochelle salt and methylene blue were all of reagent grade.

Reagents. — *I) Standard sugar solution.* — One gram of crystalline benzoic acid and 2.046 g. of crystalline glucose were dissolved in water and made up 1 l. with water.

II) Copper solution.—Sixty-six grams of caystalline copper sulfate was dissolved in water and made up to 1 l. with water.

III) Alkaline solution.—The calculated amount of sodium hydroxide solution containing 110 g. of sodium hydroxide and 345 g. of Rochelle salt were dissolved in water and made up to 1 l. with water.

IV) Indicator.—Half a gram of methylene blue was dissolved in 100 ml. of water.

Procedure. — In Soxhlet's method, Lane and Eynon's method and others, a measured volume of the boiling alkaline copper solution is titrated with a sugar solution, the end point being indicated by a spot test or an internal indicator, and the concentration of the sugar solution is estimated from the volume of the sugar solution required to reduce the alkaline copper solution completely. On the other hand, in this back titration method a boiling mixture of a measured volume of the alkaline copper solution and a measured volume of the sugar solution is back-titrated with the standard sugar solution, and the concentration of the sugar solution is estimated from the volume of the standard sugar solution required to reduce the mixture completely.

It is well known, for example, that if the sugar solution is added in portions 5 ml. and the liquid boiled for 10 sec. after each addition, the total volume required will not be exactly the same as if the whole of the sugar solution is added at once. Now in this method nearly the whole of the standard sugar solution is added at once and after boiling the rest quantity is added (normal titration). As in the Lane and Eynon's method, a preliminary titration system is adopted, by which the required volume of the standard sugar solution is found approximately in order that almost the whole of it may be added at one time simultaneously with heating. In the case of the blank titration the required volume is known approximately, so that a preliminary titration is unnecessary.

In a blank titration, a mixture of 20 ml. of water and 10 ml. of an alkaline copper solution is titrated with the standard sugar solution. In the normal titration, the volume of the standard sugar solution can be obtained, which is required to reduce a mixture of the alkaline copper solution (10 ml.) and 20 ml. of the sugar solution. The difference between the two quantities obtained thus represents the concentration of the sugar solution.

a) Blank titration.—Equal volumes of solutions II and III are mixed thoroughly. Ten milliliters of the mixed solution and 20 ml. of distilled water are measured into a 100∼300 ml. flask and heated over an asbestos wiregauze with a 300∼500 W. electric heater charged full voltage. About 23.5 ml. of the standard sugar solution delivered from a 25 ml. burette is added into the flask as soon as possible. For this purpose an automatic burette is very convenient, the outlet of which is connected by melting with a glass tube (about 10 cm.) bent twice at right angles so that the solution in the burette is not heated by the reaction mixture. After the liquid has begun to boil vigorously, it is kept in moderate ebullition by lowering the working voltage. After boiling for two minutes, 3 to 6 drops of the methylene blue indicator are added, and the standard sugar solution is added in small quantities, that is to say, several drops or less at a time, and after the commencement of decolorization, drop by drop. The liquid is boiled for about ten to fifteen seconds between successive addition, and the titration is continued until the color of the indicator is discharged. The titration is completed in about one minute, so that the reaction liquid is boiled altogether for 3 min. without interruption. The decolorization of the methylene blue is easily detected, since the blue color of copper ion has already vanished. The rate of heating is regulated carefully so as to make the precipitate as much as possible. The end point can be determined with accuracy of less than one drop of the standard sugar solution. At the end point the blue color of the methylene blue vanishes suddenly, and the faint purple tint is still observable. The methylene white is rapidly reoxidized by air, particularly at the high alkalinity of this reaction mixture. Hence it is essential that air is excluded during the titration.

This is accomplished by making an uninterrupted current of steam issue from the neck of the flask on which the analysis is performed.

So long as solutions II and III are mixed correctly, the titer of the blank titration is constant; so that the blank titration is unnecessary at each time of mixing. When the sample solution contains materials other than sugars to be determined, a blank titration should be made against the solution containing these materials.

b) *Preliminary titration.*—Ten milliliters of the mixed copper solution is boiled in a 100~300 ml. flask after adding 20 ml. of the sample sugar solution. Usually after ten seconds of ebullition the reduction of the copper solution is nearly completed, which can easily be seen from the disappearance of the blue color of cupric ion and the bright red color imparted to the boiling liquid by the suspended cuprous oxide. If it is judged that nearly all the copper has been reduced, boiling is continued for about one minute from the beginning of ebullition, 3 to 5 drops of the methylene blue indicator are added, and then the standard sugar solution is added in small quanties, that is to say, 0.2 ml. or less at a time at regular intervals, the liquid being allowed to boil, until the color of the indicator is discharged. When unreduced copper is still found to be there in large quantity after boiling a mixture of 20 ml. of the sample solution and the copper solution about ten seconds, 3 ml. of the standard sugar solution is added and the whole is allowed to boil for ten seconds and so on, until it is considered unsafe to add a further large increment of the standard sugar solution. Boiling is continued further thirty seconds, after which the indicator is added and the titration is completed by small additions of the standard sugar solution as already mentioned. The indicator should not be added until the end point is nearly reached.

When maximum accuracy is not required, a single preliminary titration gives satisfactory results; the error will, as a rule, not exceed 0.6 mg. per 20 ml. of the sample solution.

c) *Normal titration.* — Ten milliliters of the mixed copper solution and 20 ml. of a sample sugar solution are measured into a 100~300 ml. flask, and, as soon as the mixture is heated, almost the whole of the standard sugar solution is added, which required to reduce all the copper, so that if possible not more than 1 ml. is required later to complete the titration. The approximate volume of the standard sugar solution required is estimated by a preliminary titration. After the liquid has begun to boil, it is kept in moderate ebullition for 2 min., and then 3 to 6 drops of the methylene blue indicator are added, and the titration is then completed in 1 min.

Results

The author has measured the volume of the standard sugar solution required to reduce a mixture of 10 ml. of the alkaline copper solution and 20 ml. of water (*blank titration*), and that of the standard sugar solution required to reduce a mixture of 10 ml. of the alkaline copper solution and 20 ml. of the solution of sugars of various concentrations (*normal titration*). The relationship among the weight (W) of sugar in mg. in 20 ml. of sugar solution, the volume (B) in ml. of the standard sugar solution required in the blank titration, and the volume (m) of the standard sugar solution in the normal titration, has been found to be;

$$W=2(B-m)tf$$

where t is a correction term, and f is a factor of the standard sugar solution.

For the purpose of calculating the weight of sugar from difference ($B-m$), a correction term t has been introduced, which varies with the nature of the sugar and the term ($B-m$). The term t is 1.000 over widely different concentrations, viz. from 1 to 48 mg. per 20 ml. in the case of glucose, but in case of lactose and galactose it varies with the difference ($B-m$), the values of which are shown in Table I.

In the titrations on which Table I is based, the volume of the standard sugar solution added to the reaction mixture after heating was about 0.3~0.5 ml. less than the total volume required, and during the last third minute of ebullition, this complementary quantity was added, two

TABLE I. "t" OF LACTOSE AND GALACTOSE

$B-m$, ml.	Lactose	Galactose
1	1.32	1.03
2	1.32	1.03
3	1.32	1.03
4	1.32	1.03
5	1.32	1.03
6	1.32	1.03
7	1.318	1.030
8	1.315	1.031
9	1.314	1.031
10	1.312	1.032
11	1.310	1.032
12	1.309	1.032
13	1.308	1.032
14	1.307	1.033
15	1.307	1.033
16	1.307	1.033
17	1.306	1.034
18	1.306	1.034
19	1.036	1.034
20	1.306	1.034
21	1.306	1.034
22	1.305	1.035
23	1.305	1.035
24	1.305	1.035

TABLE II. EXAMPLES

Reducing sugar contained	Weight of reducing sugar in solution mg./20 ml.	Material mixed	B (ml.) required in blank titration	m (ml.) required in normal titration	f factor	t correction term	$2(B-m)ft$ weight of reducing sugar determined mg./20 ml.
Glucose	44.64	no	24.30	2.45	1.021	1.000	44.62
Lactose	45.66	no	23.98	7.30	1.047	1.307	45.64
Glucose	38.18	Sucrose 20 g./100 ml.	20.60	1.90	1.021	1.000	38.18
Glucose	42.24	Sucrose 5 g./100 ml.	23.55	1.26	0.948	1.000	42.22
Glucose	21.12	Sucrose 5 g./100 ml.	23.55	12.40	0.948	1.000	21.10

drops at a time at intervals of about 10～15 sec. until the end point was reached.

Operating in this way consistent results have been attained with all the reducing sugars tested, viz. glucose, lactose and galactose. On the basis of these results Table I was prepared. Over the whole of these ranges it is possible to attain a higher degree of precision, and the consistent results may be obtained, the error being within 0.06 ml., namely, 0.12 mg. in the case of glucose.

In the case of the sugar solutions containing other materials whose influence can not be overlooked, blank titration was made against the mixture of 10 ml. of the alkaline copper solution and 20 ml. of a solutions containing all materials of the same concentrations except for reducing sugars to be determined.

Some examples of glucose in solutions containing 5 and 20 g. of sucrose per 100 ml. are shown in Table II. It is important that, in all cases which have been investigated, the correction term t is not affected noticeably by contamination with other materials.

The experimental factor of the standard sugar solution should be 1.000, but a small deviation is inevitable according to the variations of the experimental conditions and personal differences in judging the end point. It is advisable to standardize the standard sugar solution, after its preparation, by titrating against a weighed pure glucose. If the deviation be large, adjustment of the standard sugar solution should be made so as to make the value of the factor approximately equal to one by adding glucose or water to the standard sugar solution.

On the other hand, it is possible to estimate the sugar content in a solution of unknown concentration. The quantity $2(B-m)tf$ gives the weight in mg. of the sugar in 20 ml. of the solution.

Some remarks should be made. Very stable and reproducible heating is possible by use of an electric heater. A reagent grade preparation of sodium hydroxide ordinally containing carbonate may be used, although the decolorization of methylene blue is somewhat slower than it is in the presence of carbonate free sodium hydroxide.

Summary

A new volumetric method determining reducing sugars has been developed by titrating a boiling mixture of modified Fehling solution and sugar solution with a standard sugar solution. By the method the concentration of sugar solution can be determined rapidly with high degree of precision.

Errors caused by variables of analytical conditions automatically being corrected, the maximum degree of accuracy will be possible by this procedure even in an unskilled hand.

There are several advantages over Lane and Eynon's method and other methods; the method is applicable over a wide range of sugar concentrations, especialy dilute solution, and in addition the difficulty in changing or washing a burette for a new solution, etc., is eliminated.

The author wishes to express his sincere thanks to Professor Y. Tsuzuki of Tokyo College of Science for his kind advice, and to Professors S. Kusunoki and T. Kinugasa of Kobe University for their encouragement throughout this work.

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