THE DETERMINATION OF REDUCING SUGARS.

A VOLUMETRIC METHOD FOR DETERMINING CUPROUS OXIDE WITHOUT REMOVAL FROM FEHLING'S SOLUTION.\(^1\)

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INTRODUCTION.

In the course of an investigation, still in progress, of the physiological activity of a group of soil organisms, it became necessary to determine the quantity of dextrose in a series of solutions. It was desirable to have a method as accurate as the Fehling gravimetric one but which would yield results about as quickly as by direct titration. The reducing sugar present in the 150 cc. of each of these solutions varied from nearly zero to about 550 mg. A definite quantity of a copper salt had to be added to each solution. Direct titration could not be used unless this salt was first removed and the solutions of small sugar content were concentrated, a procedure which would make this method a very long one. There were no such difficulties if the gravimetric method was employed, as less copper sulphate solution could be used in mixing the Fehling's reagents and the sugar solution could then be added directly.

The new volumetric method was devised for the determination of the cuprous oxide in the Fehling's solution. It consists in converting the oxide into cuprous chloride and then pipetting a definite volume into a known quantity of iodine solution. The iodine which is not reduced is titrated with sodium thiosulphate solution.

Since this method was completed the author's attention has been called to the work of Rupp and Lehmann\(^2\) who also de-

\(^1\) Published by the permission of the Secretary of Agriculture.

scribe a procedure for determining cuprous oxide in Fehling's solution. Their method is entirely different in principle and is not nearly as accurate as the proposed one. Among other faults it necessitates the use of the whole Fehling's solution so that duplicate titrations cannot be made.

The time required for a single determination by the new method is not over twenty minutes; that is, it takes ten minutes more than the time required for the whole Munson and Walker procedure up to the filtration of the cuprous oxide. When a series of determinations is to be made, the technique can be modified so that the time required for a single one will be much less.

When dextrose, maltose, lactose, or invert sugar are oxidized by Fehling's solution, the acids formed are without action on iodine, so that these sugars and no doubt the other reducing ones may also be determined by this method.

Method.

Apparatus.—While no special apparatus is necessary for this method a slight modification is made in the usual form of a 200 cc. volumetric flask. This flask, marked to contain, is cut off 20 mm. above the mark. The edge is polished and flared a little so that a rubber stopper may be inserted and pressed down to within 12 or 14 mm. of the capacity mark.

As it is not always convenient to time a pipette for the delivery of a definite volume the author has substituted the method of calibrating a 50 cc. pipette against the 200 cc. volumetric flask and then placing a small file mark near the tip of the pipette at the point where the solution stops. Four times the volume contained between the mark on the stem and the tip of the pipette should exactly fill the flask.

A rubber stopper, with one hole, that will fit a 500 cc. Erlenmeyer flask is slipped over the tip of the 50 cc. pipette into a position on the stem so that when the stopper is in the flask the tip of the pipette will be about 1 cm. below the surface of the 275 cc. of solution which the flask contains. A small V-shaped vent should be cut in the side of the stopper. As two flasks are used,

\[1\] In the determination of 24.2, 27.3, 48.6, and 117. mg. of dextrose their error was 2., 2.0, 1.4, and 0.8 per cent respectively.
it is well to pick out two with mouths of about the same diameter. Rubber stoppers without vents are placed in each of these flasks.

A 25 cc. volumetric pipette is also marked on the tip at the point where the solution stops, so that exactly the same volume may be measured each time; the absolute volume in this case is of no importance.

Procedure.—The procedure for making a determination is comparatively simple and lends itself well to routine work.

The Fehling's solutions⁴ are mixed in a 400 cc. Jena beaker, in the proportions recommended by Munson and Walker,⁵ the sugar solution is poured in, and the volume made up to 100 cc. with distilled water. The beaker is covered with a watch glass and placed on an asbestos mat over a Bunsen burner with the flame so regulated that boiling begins in four minutes. The boiling is continued for two minutes.

As soon as the Fehling's solution is placed over the flame, approximately 250 cc. of distilled water are poured into each of the 500 cc. Erlenmeyer flasks. 25 cc. of iodine⁶ are then pipetted into each flask. The iodine must be very accurately measured so that exactly the same volume that was used for the standardization will be obtained. The 25 cc. pipette with the mark on the tip acts as a check to prevent any mistakes in this desired accuracy. When the iodine is in the flasks they are sealed with a rubber stopper.

A 75 mm. funnel with a long stem is placed in the 200 cc. volumetric flask and then 15 cc. of concentrated HCl are poured into it.

Approximately 15 cc. of water (between 80° and 95°) are added to the acid in the volumetric flask just before the boiling of the Fehling's solution is completed. The tip of the funnel should be below the surface of this solution.

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⁴ The modified Fehling's solutions recommended by Soxhlet were used. Solution A contains 34.639 gm. of CuSO₄·5H₂O in 500 cc. of water. Solution B contains 173 gm. of Rochelle salts and 50 gm. of NaOH in 500 cc. of water.


⁶ The 2% iodine solution is prepared by dissolving 6.5 gm. of resublimed I and 9 gm. of KI in 1 liter of distilled water.
When the Fehling's solution has boiled two minutes the beaker is removed from the flame, the solution agitated to bring the precipitate into suspension, and then rapidly poured onto the funnel. The beaker, watch glass, and funnel are washed with hot water and the volumetric flask is then filled almost to the mark. A few cc. of water from the wash bottle are run into the beaker and then by means of a 1 cc. pipette the solution in the flask is brought up to the mark. The rubber stopper is immediately inserted and the flask covered with a towel while it is tilted back and forth five or six times or sufficiently to give the contents an even distribution. As a slight pressure develops in the flask it is well to place the finger over the stopper to prevent its being forced out. When the acid solution is satisfactorily mixed the stopper is withdrawn and a 50 cc. quantity of the solution immediately pipetted into each of the flasks containing iodine solution. The stem of the pipette is placed in the flask and the rubber stopper pressed down so that the pipette is held steady and its contents are delivered about 1 cm. below the surface of the solution. The Erlenmeyer flask is agitated occasionally to distribute the solution and prevent the formation of a precipitate by an excess of the acid solution accumulating in one place. As this solution drains down the rubber stopper is drawn out and the pipette tilted so that its contents will run out more slowly. The pipette is withdrawn as soon as the solution in it reaches the mark on the tip. If an attempt is made to stop the flow of the solution as it nears the mark by placing the finger over the end, in the usual way, some of the solution will frequently be drawn up from the tip by the contraction of the air in the pipette. Simple tilting of the pipette and the flask gives sufficient control to stop the flow when it reaches the mark. As soon as the pipette is empty it is removed and the flask sealed with a rubber stopper.

Where only one pipette is used to transfer the cuprous chloride solution to the iodine solution, three pipettfuls may be withdrawn without any error being introduced by the change in temperature of the solution, provided the pipette is again filled as soon as it is empty. In cool weather when a change may occur more quickly it may be necessary to use two pipettes so that the second 50 cc. quantity can be measured without waiting till the first pipette is empty or one pipette may be used and the volu-
metric flask placed in a large beaker containing water at about the temperature of the acid solution. The volumetric flask should be sealed with a rubber stopper to prevent the access of oxygen when the solution is not being taken from it.

The iodine remaining after the addition of the cuprous chloride solution is titrated with $\frac{N}{10}$ sodium thiosulphate,\(^7\) 2 cc. of starch solution being added when the end-point is nearly reached. The solution at the end of the reaction is a very light green, but it is so light and different from the iodo-starch blue that it does not interfere with an accurate determination of the end-point.

The difference between the number of cc. of $\frac{N}{10}$ thiosulphate solution oxidized by 25 cc. of iodine solution, as determined by the standardization, and the number of cc. of $\frac{N}{10}$ thiosulphate acted upon by the iodine remaining after the addition of the cuprous chloride solution, is multiplied by 14.315 to obtain the mg. of Cu$_2$O in the whole solution. The constant 14.315 is equal to four times the number of mg. of Cu$_2$O that would be oxidized by 1 cc. of $\frac{N}{10}$ iodine solution.

Amounts of dextrose from 4 mg. up to 150 mg. may be determined with these $\frac{N}{10}$ solutions, but of course by either changing the strength of the reagents or taking a smaller quantity of the cuprous chloride solution this method can be made to cover the determination of reducing sugar up to the limit of the Fehling method.

Data.

In order to test the method a sample of pure dextrose was obtained from the Bureau of Standards\(^8\) and a 0.5 per cent solution prepared. No copper salt was added to the sugar solution for these tests because they were made to determine the average accuracy of the method. These experiments, selected at random, will illustrate this accuracy.

\(^7\) Standardized against $\frac{N}{10}$ K$_2$Cr$_2$O$_7$ solution.

\(^8\) Bureau of Standards, Dextrose Sample No. 41; B. S. Test No. 16741-30.
Reducing Sugars

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<th>Temperature of water</th>
<th>N\textsubscript{25} thiosulphate solution used to titrate excess iodine</th>
<th>Dextrose present</th>
<th>Dextrose found</th>
<th>Error</th>
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<td>cc.</td>
<td>cc.</td>
<td>mg.</td>
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DISCUSSION.

The method described is one that may be used for the determination of widely varying quantities of reducing sugar, but if uniformly smaller quantities were to be determined the accuracy of the method may be increased by taking more of the cuprous chloride solution and using weaker titrating solutions. The method as outlined, however, is as accurate as the gravimetric one of Munson and Walker. All the figures in their table for less than 20 mg. of dextrose were obtained by interpolation, so there is no standard with which to compare the results obtained in the determination of the smaller quantities of dextrose. In working with this quantity of dextrose Munson's average result was 1 per cent lower and that of Walker 1 per cent higher than the amount of reducing sugar added.

As the results obtained in the determination of larger quantities of dextrose were as close to the theoretical values as those obtained with the gravimetric method it is evident that this volumetric method when properly manipulated will yield results fully as accurate as those of our standard gravimetric method.
If a blank Fehling’s solution is put through this procedure it is found that from 0.1 to 0.2 cc. of iodine has been reduced. It is believed, however, that when cuprous chloride is entering the iodine solution this quantity of iodine, which may be changed by the heat of the solution entering, is not without action on the cuprous chloride; because by taking the 25 cc. of iodine solution in distilled water as the quantity which is acted upon by the cuprous chloride the correct amount of cuprous oxide is obtained. If the iodine is lost, this way of calculating the result is only equivalent to adding a constant; so no error is introduced.

In these experiments no precipitation of cuprous oxide was obtained by boiling the blank Fehling’s solution. If there should be a precipitate of 0.3 or 0.4 mg. of cuprous oxide it could not be determined with these reagents, as one-fourth of it or 0.1 mg. would be equal to 0.03 cc. of the $\frac{7}{10}$ solution and this value would of course be covered by experimental error.

This method may be used for the quantitative determination of copper by reducing the metal in an alkaline solution with dextrose and then following the procedure.

**SUMMARY.**

A new volumetric method is described for the determination of cuprous oxide in Fehling’s solution. The cuprous oxide without filtration from the solution is converted into cuprous chloride and then pipetted into a known quantity of a dilute iodine solution. The iodine which is not reduced is then titrated with sodium thiosulphate solution.

The data indicate that the method will give results as accurate as those obtained with the gravimetric method.

Dextrose, maltose, lactose, invert sugar, and no doubt other reducing sugars may be estimated by this procedure.

This method yields results in one-quarter of the time required to obtain them by the gravimetric method.

The method may also be used for the quantitative determination of copper.
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