NOTES ON SUGAR DETERMINATION*

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In the present article two improved copper reagents for the quantitative determination of sugars are described, one for the colorimetric, the other for the iodometric method. These reagents were elaborated and used in the author's laboratory, and during the past 3 years were appreciated as useful in a number of other laboratories.

Colorimetric Technique

In 1944 Nelson (1) described a colorimetric method which represented a distinct step forward. He devised a new arsenophosphate solution as the chromogenic agent, and for copper reagent selected one of our older carbonate-tartrate solutions which was devised for the iodometric technique. In the following year, in a new alkaline copper reagent, we replaced the carbonate-bicarbonate buffer by phosphate as the alkali (2) and suggested that this reagent could be used for the colorimetric technique with Nelson's reagent. Subsequent observations convinced us, however, that this reagent is not suitable for accurate colorimetric work, because it interferes with the stability of the colors developed with chromogenic reagents, whereas, when carbonate is the alkali, Nelson's reagent yields very stable colors.

The copper-carbonate-tartrate reagent that Nelson selected (as well as the phosphate reagent we recommended) has, however, definite disadvantages when used for colorimetry. Since the reagent was devised for the iodometric technique, its copper content is far above the concentration needed in colorimetry and this excess copper requires a commensurate amount of tartrate to be kept in solution. Since tartrate measurably reduces copper, especially when the reagent is heated, in colorimetry it is the main cause of deviations from Beer's law. We have therefore decreased the copper content of the reagent to the amount actually usable in colorimetry, and reduced the concentration of tartrate to the minimum required. The self-reduction of such a solution, we found, is virtually negligible. 1 liter of this reagent contains 4 gm. of CuSO₄·5H₂O, 24 gm. of anhydrous Na₂CO₃, 16 gm. of NaHCO₃, 12 gm. of Rochelle salt, and 18 gm. of anhydrous Na₂CO₃.

Preparation of Reagent—The carbonate and Rochelle salt are dissolved

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in about 250 cc. of water, then the dissolved copper sulfate (e.g., 40 cc. of a 10 per cent solution) is introduced with stirring, and this is followed by the addition of the bicarbonate. The sodium sulfate is dissolved in about 500 cc. of hot water and boiled to expel air. After cooling, the two solutions are united and diluted to volume in a 1000 cc. graduated cylinder.

During the first few days, or a week, a slight amount of cuprous oxide settles, together with impurities of the ingredients of the solution; these are removed by filtration. Subsequently no more self-reduction takes place at ordinary room temperature, except, perhaps, under prolonged exposure to direct sunlight. In this laboratory no self-reduction was observed in reagents older than a week.

We have been advised by one laboratory that traces of cuprous oxide continued to be formed in their reagent, prepared as just described. The trouble may be caused by impurities and can be obviated by keeping the copper sulfate in a separate solution and uniting it with the other constituents directly before the reagent is to be used, as Folin and, later, Nelson have done. When this arrangement is chosen, the tartrate, carbonate, bicarbonate, and 144 gm. of sodium sulfate are dissolved in water and diluted to 800 cc. (Solution I), and the copper sulfate, with 36 gm. of sodium sulfate, is kept separately in 200 cc. of solution (Solution II). The reagent is readied for use by uniting 4 volumes of Solution I with 1 volume of Solution II.

The analytical procedure with this reagent is much the same as with other copper reagents used in colorimetry. A measured volume of the solution to be analyzed is mixed with an identical volume of the copper reagent and heated in a boiling water bath. For glucose and fructose a 10 minute heating period is adequate. After cooling, the chromogenic reagent, Benedict’s or Nelson’s, is added and care is taken to dissolve the cuprous oxide completely. The blue solution is then diluted to a volume selected in accordance with the quantities of sugar involved.

Rather than give stereotyped recipes, we wish to point out the elasticity and adaptability of the technique to a great variety of experimental conditions. For instance, one can use from 1 to 5 cc. of sugar solution per analysis with equal volumes of the copper reagent. The amount of the chromogenic reagent to be added need not exceed 2 cc. The final volume of the colored solution can be adapted to the prevalent color densities. In microanalysis, for example, when the amount of sugar that is being determined is not much above 10 µ, the final volume can be kept to 6 cc., which is the combined volume of 2 cc. portions each of the sugar solution, the copper reagent, and the chromogenic reagent (with proper precautions to prevent perceptible evaporation during heating for 10 minutes). At the other extreme, a dilution to 25 cc. is in order when the portion of solu-
tion used in a determination contains up to 0.6 mg. of glucose (or any other sugar of comparable reducing power). Color density being the limiting factor, about 0.6 mg. of glucose is the maximum and about 0.01 mg. the minimum amount of glucose that can be determined with this technique.

It is in place, perhaps, to outline the determination of blood sugar under the conditions most frequently encountered. The blood is deproteinized by the zinc-alkali or copper sulfate-sodium tungstate method, as described in a previous paper (3). 2 cc. of the filtrate, 1:10 dilution, are mixed with 2 cc. of the copper reagent in a test-tube of 16 or 18 mm. inside diameter, which is marked for a 25 cc. volume. The test-tube is covered with a glass bulb (or marble) and heated in a boiling water bath for 10 minutes. After cooling, 2 cc. of the chromogenic reagent are added, the cuprous oxide is brought into solution by mild agitation, and the fluid is diluted to the 25 cc. mark and mixed.

For color production Benedict’s phosphotungstate or Nelson’s arsenotungstate reagent may be used. The yellow color of Nelson’s reagent eliminates it from use in visual colorimetry, while Benedict’s colorless reagent is well suited for it. From 12 to 15 minutes are required for the development of maximum color density, which then persists for the next 40 minutes. The readings must, therefore, be completed within this interval. The color densities are in excellent accord with Beer’s law. Nelson’s reagent, on the other hand, possesses the virtue of developing maximum color density almost instantaneously, which remains unchanged for many hours.

Iodometric Technique

When maximum accuracy is desired in sugar analysis, we prefer the iodometric technique. Our copper-phosphate-tartrate reagent (2) seems to have served for this procedure satisfactorily in many laboratories, as well as in our own. After several years of experience we observed, however, that the sugar equivalents are subject to change. Such changes were not observable during the 1st year, but they came to our attention as we eventually prepared the reagent from varying batches of chemicals and as we checked on some of our solutions that were several months old. Since we were unable to find a full explanation of the trouble, we have reverted to the use of carbonate instead of phosphate as the alkali.

We recommend for accurate iodometric sugar analysis a reagent we have been using for the last 3 years. In order to make it suitable for determination of sugars that are more slowly oxidized than glucose, we eliminated bicarbonate and introduced NaOH in a quantity that is taken up in the copper-tartrate-alkali complex (somewhat as in our older “high alkalinity” reagent). Owing to the addition of sodium sulfate, the reagent, regardless
of its high alkalinity, allows the determination of as little as 0.015 mg. of glucose, with an upper limit of 3.0 mg. The reagent is perfectly stable; a 3-year-old sample is still crystal-clear, and its reduction equivalents are identical with those of freshly prepared batches.

Composition of Reagent—8 gm. of CuSO₄·5H₂O, 30 gm. of anhydrous Na₂CO₃, 30 gm. of Rochelle salt, 8 gm. of KI, 180 gm. of anhydrous Na₂SO₄, 40 cc. of N NaOH, and 10 to 25 cc. of N KIO₃.

Preparation of Reagent—The Rochelle salt and Na₂CO₃ are dissolved in about 200 cc. of hot water, the NaOH is added, then the copper sulfate dissolves in water (e.g., 80 cc. of a 10 per cent solution) is introduced with stirring, and the solution is boiled to expel air. The Na₂SO₄ is dissolved in about 500 cc. of hot water and the solution is boiled to expel air; then it is united with the solution containing the other ingredients. Finally the KI, dissolved in a small volume of water, and the KIO₃ solution are added. After cooling to room temperature, the solution is diluted to 1000 cc. in a graduated cylinder. The amount of the KIO₃ can be adapted to the amounts of the sugars to be determined. For microanalysis, for instance, involving the determination of from 0.02 to 0.5 mg. of glucose, 5 cc. of KIO₃ per liter will suffice, enabling one to use a fine 1 cc. burette for titration. For a range between 0.5 and 1.5 mg. of glucose, 12 cc. of KIO₃ are adequate. We prefer to omit the KIO₃ from the reagent and dilute it to 900 cc. instead of 1 liter; then we take an aliquot of the batch and adapt the amount of KIO₃ to the glucose concentration to be expected in a particular experiment, and make the final dilution to volume. Titration of unduly large excesses of iodine introduces slight opportunities for inaccuracy as well as waste of time.

The analytical procedure has been described previously. In our standard

<table>
<thead>
<tr>
<th>Glucose in 5 cc. solution</th>
<th>Reduction equivalents expressed in titration values of 0.005 N thiosulfate</th>
<th>Blood sugar equivalents when determined in 5 cc. of 1:10 filtrate mg. per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.015</td>
<td>0.10</td>
<td>3</td>
</tr>
<tr>
<td>0.080</td>
<td>0.5</td>
<td>16</td>
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</tr>
<tr>
<td>2.50</td>
<td>17.74</td>
<td>500</td>
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</table>
technique 5 cc. of sugar solution are mixed with 5 cc. of the copper reagent in a 25 × 200 mm. test-tube, covered with a glass bulb, and heated in a boiling water bath for 15 minutes. For further details the reader is referred to previous articles (2–4). This is not the only usable procedure with this reagent. When maximum accuracy is not imperative, smaller volumes of sugar solution may be used with equal amounts of the copper reagent. In our clinical laboratory, for example, we use 2 cc. of 1:10 blood filtrate with 2 cc. of the reagent in test-tubes of 18 mm. diameter. The smaller volume allows shortening the time for heating to 10 minutes and accelerates considerably the titration. (A reagent, 2 cc. of which give about a 10 cc. blank titration, is suitable for blood sugar concentrations up to nearly 600 mg. per cent.)

This reagent affords considerable accuracy and reproducibility in sugar analysis. In competent hands differences between duplicate determinations rarely exceed 2 γ of glucose, representing an error of no more than 0.5 mg. per cent in blood sugar values when determined in 5 cc. of 1:10 filtrates.

We recommend that for investigative work every worker determine the reduction equivalents of this reagent for himself with reliable standard glucose solutions. For convenience a few data are presented (Table I) which can be used for the preparation of a detailed table by interpolation with the aid of a graph.

SUMMARY

Two improved copper reagents for the quantitative determination of sugars are described, one for use in the colorimetric, the other for the iodometric technique.

BIBLIOGRAPHY

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