Carbohydrate Research, 28 (1973) 100–102

Note

Colorimetric determination of known disaccharides and oligosaccharides in mixtures containing monosaccharides

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(Received June 8th, 1972; accepted in revised form, October 25th, 1972)

To obtain the maximum amount of information on sugars in mixtures, separation of the individual sugars and determination of the amount of each one present must be made. Separation techniques are laborious and often unnecessary for a particular food or feed analysis.

The method presented here is based on the colorimetric analysis of reducing sugars in mixtures by use of the phenol–sulfuric acid reaction[1]. First, the total sugar content is determined on a portion of the sugar solution. Next, a part of the original sugar solution is treated with sodium borohydride to eliminate the reducing function of any reducing sugars present, and the phenol–sulfuric acid determination is applied again. The mono- and di- or oligosaccharide concentration is calculated by difference.

Earlier, Jørgensen and Jørgensen[2] applied the anthrone–sulfuric acid color reaction in like manner to the determination of the total sugar content. Then, the reducing aldohexoses were oxidized with hypobromite to give aldobionic acids, and the anthrone reaction performed again. The disaccharide content was calculated by difference.

The method proposed here is a two-fold improvement over the hypobromite oxidation method: there is no danger of destruction of sugars by overoxidation, and the reducing function of both aldose and ketose reducing sugars is eliminated rapidly and quantitatively.

RESULTS AND DISCUSSION

The equation for reduction of reducing sugars with sodium borohydride shows that 1 mole of sodium borohydride is required for 4 moles of reducing sugar[3], or 2.1 μg of sodium borohydride for 40 μg of D-glucose per test. Under the reaction conditions used here the system is not efficient and a great excess of sodium borohydride is required for a complete reduction in 30 min. Test solutions for reduction with sodium borohydride should be neutralized if acidic, otherwise the excess of acid will decompose the sodium borohydride added. Tests were made to determine the quantity of sodium borohydride required to convert the reducing function of sugars

into alcohols which do not give a color in the phenol–sulfuric acid test. One mg of sodium borohydride failed to reduce completely, within 30 min, the aldehyde group of more than 20 μg of D-glucose per ml of the test solution. However, 5 mg of sodium borohydride reduced more than 500 and less than 1000 μg of D-glucose under these conditions. Five mg of sodium borohydride per test is recommended for the analytical tests because there is often a ten-fold excess of monosaccharides as compared with oligosaccharides. The tests showed that the reduction of the aldehyde group of 40 μg of D-glucose, as measured by the phenol–sulfuric acid test, was complete within 30 min. The reducing monosaccharides (D-glucose, D-fructose, D-galactose, and L-arabinose) and the reducing disaccharides tested (melibiose, cellobiose, and maltose) were quantitatively reduced under these conditions.

Boric acid was shown by Lin and Pomeranz[4] to depress the color intensity obtained in the phenol–sulfuric acid sugar reaction. The absorbance of solutions containing hexoses was slightly reduced and that of pentoses greatly reduced by addition of boric acid, and the reduction was quantitatively related to the boric acid concentration. The effect of boric acid under the conditions of the experiments reported here is in agreement with those reported by Lin and Pomeranz[4]. Although the absorbancy values are linear with respect to the sugar concentration under these conditions (5 mg of sodium borohydride decomposed by acid to boric acid in each test), the depressive effect of boric acid reduced the absorbance to 75% of that observed in the absence of boric acid. This effect makes necessary the use of standards containing boric acid for quantitative tests.

The borohydride reduction and the quantitative colorimetric, enzymic, and iodimetric methods of analysis of sugars have proved useful in our analytical laboratory. It has been applied to analyze systems containing D-glucose and maltose, and D-glucose and sucrose, and other known mixtures of mono- and oligosaccharides.

EXPERIMENTAL

Apparatus and reagents. — A Bausch and Lomb Spectronic 20 spectrophotometer or an equivalent instrument was used with 17 × 150 mm closely matched tubes. The phenol solution was prepared by dissolving reagent-grade phenol (5 g) in water (100 ml) and was dispensed from an all-glass syringe pipette (Labindustries, 1802 2nd Street, Berkeley, California 94710)*. The sulfuric acid was reagent grade 96%, dispensed from an all glass-syringe pipette. The boric acid solution was prepared by dissolving sodium borohydride (1 g) in water (20 ml), and then adding sulfuric acid dropwise until the solution was acidified and the borohydride completely converted into boric acid. The Vortex Jr. Mixer was obtained from Scientific Industries, Inc., Springfield, Massachusetts*.

Standard curve of D-*glucose with boric acid.* — Standard curves were prepared

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for sugars to be analyzed. For example, D-glucose was used for maltose, D-galactose for melibiose, and sucrose for sucrose. Standards of 10, 20, and 40 μg of D-glucose per ml were prepared. In sequence, the standard solution (1 ml) was transferred into a 17 × 50 mm colorimeter tube, and 5% boric acid solution (100 μl) and phenol (5%, 1 ml) were added and mixed thoroughly. Conc. sulfuric acid (5 ml) was added rapidly and mixed immediately with the Vortex mixer. The solution was cooled for 30 min and the absorbancy was read at 490 nm and compared to that of a blank containing water, boric acid solution, phenol solution, and sulfuric acid.

Determination of maltose in the presence of D-*glucose.* — A neutral test-solution (1 ml) containing maltose (about 100 μg/ml) and D-glucose (500 μg/ml) was transferred into two colorimetric tubes (17 × 150 mm). Sodium borohydride (5%, 100 μl) was added, the solution mixed, and the reduction of the aldehyde groups of D-glucose and maltose was allowed to proceed to completion within 30 min. Phenol (5%, 1ml) was added and mixed, and sulfuric acid (1 ml) was added rapidly. After the solution had been kept at room temperature for 30 min, the absorbancy was read at 490 nm and compared with the curve of D-glucose standard with boric acid. The value of D-glucose found × 2 × 0.95 = maltose content.

Mixtures of D-*glucose and maltose.* — Samples (1 ml) of the neutral test-solution containing up to 60 μg of sugars (D-glucose and maltose combined) were transferred to four colorimetric tubes (17 × 150 mm). To each of two tubes was added boric acid (5%, 100 μl) and to the other two tubes the sodium borohydride solution (5%, 100 μl), followed by mixing. After 30 min the development of the color was allowed to proceed by addition of phenol and sulfuric acid, as just described. The D-glucose curve with boric acid was used to find the total sugar content, as D-glucose, before and after reduction. The value of D-glucose found after reduction with sodium borohydride determines the amount of 4-*O*-(α-D-glucopyranosyl)-D-glucitol resulting from the reduction of maltose. The difference of the D-glucose values before and after reduction × 2 × 0.95 = maltose content. The total sugar value less 2× (the D-glucose value after reduction) = D-glucose content.

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