THE DIFFERENTIATION OF MONOSACCHARIDES FROM DISACCHARIDES AND POLYSACCHARIDES AND IDENTIFICATION OF FRUCTOSE

By M. Z. Barakat and M. E. Abd El-Wahab

From the Biochemistry Department, Faculty of Medicine, Abbassia, Ibrahim Pasha El-Kebir University

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All carbohydrates give a positive Molisch test. To their free or potentially free aldehyde or ketone groups many sugars owe, in part, the property of readily reducing (in alkaline solution) the ions of certain metals such as copper, bismuth, mercury and silver. Upon this property of reduction the most widely used tests for sugars are based. Barfoed's test is a copper reduction test, but differs from Fehling's and other reduction tests in that the reduction is brought about in an acid solution.

A new qualitative test to distinguish monosaccharides from disaccharides and polysaccharides is described. It depends on the fact that monosaccharides, e.g., arabinose, glucose, fructose and galactose, reduce aqueous ammonium molybdate (8 per cent.), when heated in a boiling water-bath for 3 minutes only, to produce a blue colour, "molybdenum blue," which is probably a mixture of the lower oxides of molybdenum. Under the same conditions, disaccharides and polysaccharides do not reduce ammonium molybdate. The reaction is carried out in a neutral medium. The presence of acetic acid or dilute mineral acids hastens the reaction and stabilises the colour which appears within 1 minute, but the disaccharides may produce a faint blue colour, in the presence of acids, due to partial hydrolysis. The presence of alkali, even aqueous ammonia, prevents the development of the colour, even after heating in the boiling water-bath for 15 minutes.

This colour test is not specific for monosaccharides, since it is also given by glycerol, uric acid, citric, oxalic and tartaric acids and sodium hydrosulphite. It is very sensitive and can be used to detect small quantities (up to 20 μg.) of the monosaccharide. The intensity of the colour is proportional to the concentration of the monosaccharide.

Procedure: 0·1 g. of each of the following: arabinose, rhamnose, glucose, fructose, galactose, xylose, lactose, maltose, sucrose and starch were introduced respectively into 25-ml. pyrex test tubes and dissolved in 5 ml. of distilled water. To each tube 5 ml. of 8 per cent. aqueous ammonium molybdate solution was added and the contents well mixed. The test tubes were heated in a boiling water-bath for 3 minutes only. A definite blue colour appeared in the test tubes containing the monosaccharides, while the contents of the tubes containing the disaccharides and starch showed no colour or, in the case of maltose only, a very faint green colour, after heating for 5 minutes.

Identification of Fructose: Pinoff's test[1], which is said to be characteristic of fructose, is only valid in the absence of mineral acids. In this

test 0·1 g. of the sugar is dissolved in 10 ml. of water, then 10 ml. of a 4 per cent. solution of ammonium molybdate (freshly prepared) and 0·2 ml. of glacial acetic acid are added and the test tube is heated in a water-bath at 95° to 98°C. for 3 minutes; in the presence of fructose a deep blue colouration is produced.

Pinoff's test was carried out on the following sugars: arabinose, glucose, fructose, galactose, lactose, maltose and sucrose. After heating for 3 minutes in a water-bath at 95° to 98°C. a blue colour appeared in the tube containing fructose, a faint blue colour in the tube containing arabinose and galactose, and a very pale colour in the tube containing glucose. No colour appeared in the tubes containing lactose, maltose and sucrose.

After heating for 10 minutes at the same temperature, the blue colour in the tubes containing the monosaccharides was very definite and conspicuous, but was still lighter than that in the tube containing fructose. A faint green colour appeared in the tubes containing the disaccharides. After 20 minutes, the colour in the tube containing fructose became deep greenish-blue, while the colour in the tubes containing arabinose, galactose and glucose became deep blue and in the tubes containing the disaccharides faint blue. To reach a definite conclusion about the sugar under investigation by Pinoff's test, two parallel experiments with fructose and another monosaccharide should be carried out under the same conditions.

Modified tests which easily distinguish fructose from the other monosaccharides, disaccharides and polysaccharides were carried out under the following conditions:

(A) 0·5 g. of each of the following: arabinose, fructose, glucose, galactose, lactose, maltose, sucrose and starch was dissolved in 4 ml. of distilled water in 25-ml. pyrex test-tubes and 0·2 ml. of a freshly-prepared saturated solution of recrystallised ammonium molybdate added and the contents well mixed. The tubes were heated in a boiling water-bath for 5 minutes, and the colours obtained were as follows: arabinose, blue; fructose, yellow→orange; glucose, faint green; galactose, intense blue; lactose, maltose, sucrose and starch, no colour. When heating was continued for 10 minutes, the contents of the tube containing fructose became orange, while that containing glucose was faint green, and the other tubes were practically unchanged. After 20 minutes' heating, the tube containing fructose became deep orange, this colour remaining stable for several days. If this test is carried out on the monosaccharides by heating directly, the same colours are obtained within 3 minutes.

(B) 1 g. of the sugar was dissolved in 35 ml. of distilled water in a wide-mouthed pyrex test tube (capacity 50 ml.), then 2 ml. of a saturated ammonium molybdate solution (freshly prepared) was added. The contents were mixed and the tubes put in a boiling water-bath for 5 minutes; the following observations were recorded: arabinose, intense blue; rhamnose, faint green; xylose, yellowish-green; fructose, olive green; glucose, no colour or very faint yellowish-green; galactose, blue; lactose,

no colour; sucrose, no colour. The olive green colour of fructose is very characteristic.

Test for Sucrose in Milk[2,3] : Sucrose and lactose do not give a colour with ammonium molybdate in a neutral medium, but in dilute acid medium at 80°C., the hydrolysis of sucrose to glucose and fructose proceeds more quickly than the hydrolysis of lactose and the resulting monosaccharides, give rise to the blue colour. On the other hand, in a boiling water-bath, the hydrolysis of lactose into glucose and galactose is complete and the intense blue colour is also obtained. In this test, the sample of milk containing 0·4 per cent. of sucrose becomes an intense blue after heating at 80°C. for 5 minutes, while the genuine sample acquires only a faint blue colour. If the temperature is kept at 80°C., the colour of the genuine sample is intensified with time (after 15 minutes), which may give rise to the idea that the sample is adulterated with sucrose. If the temperature is raised to 98° to 100°C., the intensity of the colour of the genuine sample after 5 minutes becomes so strong that one cannot decide whether the sample contains sucrose or not. Therefore, in carrying out this test, the temperature should not be raised above 80°C., the heating should not exceed 5 minutes, and at the same time a genuine sample of milk should be tested under exactly parallel conditions in order to obtain a definite conclusion about the presence or absence of sucrose.

Summary

(1) Ammonium molybdate solution (8 per cent.) can be used as a reagent in a neutral medium to differentiate monosaccharides from disaccharides and polysaccharides.

(2) Modified tests to identify fructose and to distinguish it from other monosaccharides are described

(3) The conditions necessary to detect sucrose in milk by the ammonium molybdate reagent are described.

References

1. Pinoff, *Ber. dtsch. chem. Ges.*, 1905, **38**, 3317.
2. Cotton, *Chem. Centralbl.*, 1898, **1**, 130.
3. De Koningh, *Chem. Centralbl.*, 1899, **2**, 230.