A new method for the detection of sugars, specific for fucose, by thin layer chromatography

Work involving the isolation and identification of sugars from natural sources can be simplified by the use of thin layer chromatography.

Many reactions have been proposed, in the last few years, for the idendification of carbohydrates on chromatograms[1-6], but most of them are not suitable for very small amounts of these compounds.

The experimental procedure described here allows quantities as small as 0.1 μg of sugar to be detected on thin layer chromatograms by the use of a slight modification of the reaction of TAUBER[7]. Aldoses and ketoses, as well as some sugar derivatives, give a blue or blue-gray color with the reagent. Of all the carbohydrates tested, only fucose and galacturonic acid give a lilac color, making this method specific for these two compounds under the conditions described.

Experimental

Samples. All sugars and derivatives used were purchased from the Nutritional Biochemical Corporation, Cleveland, Ohio. The sugar solutions were made by dissolving 10 mg of each in 10 ml of pyridine, with the exception of lactose and cellobiose which were dissolved in water. These stock solutions were used for successive dilutions. The mixtures of the sugars were prepared in the same way.

Solvents. Merck or Riedel, specially prepared for chromatography.

Layer. Kieselgel G, Merck, A.G., Darmstadt.

Spreader and chromatography tanks. Desaga G.m.b.H., Heidelberg.

Reagent 1. 2.5 g of aminoguanidine sulfate monohydrate, p.a. are dissolved in 100 ml of water. The reagent is stable at room temperature.

Reagent 2. One ml of a 1 % solution of $K_2Cr_2O_7$ in water is mixed with 100 ml of concentrated H_2SO_4. This system is stable for approximately one month, at room temperature.

Chromatographic procedure. Aliquots (1–3 μl) of the different solutions of sugars and their mixtures were spotted on 20 × 20 cm glass plates coated with a thin layer (0.25 mm) of Kieselgel G, Merck, impregnated with 0.1 *N* H_3BO_3[8]. The spots were dried at room temperature, and the origin was 2.0 cm above the bottom edge of the plate.

Saturation of the tank atmosphere with the solvent was always allowed for 24 h before starting a run.

Chromatograms were developed, by the ascending technique for 50–60 min in the solvent system benzol–methanol–acetic acid (20:20:60, v/v) according to STAHL AND KALTENBACH[9], at room temperature. A running distance of 15 cm was generally used, then the plates were dried for half an hour at 60–70° and afterwards they were left at room temperature in order to eliminate the last traces of the solvent. The presence of acetic acid in the chromatogram resulting from the incomplete removal of the solvent did not, however, interfere with the color reaction.

The plates were sprayed with reagent 1, and immediately after, with reagent 2, and left for 10 min at 110°.

The sugars were revealed as intense blue or gray-blue spots, with exception of fucose (desoxy-galactose) and galacturonic acid, which gave a lilac color.

Decreasing amounts of the carbohydrates were tested in order to find the detection limit which is 0.1 μg. The optimum amount is between 0.3 and 0.5 μg. Nevertheless, quantities as big as 50 μg can be employed. In this case, the blue and blue-gray spots present a brown center and the lilac ones have an orange center.

TABLE I

COLOR REACTIONS ON CHROMATOGRAMS OF SOME SUGARS AND SUGAR DERIVATIVES WITH AMINOGUANIDINE SULFATE–SULFURIC ACID–$K_2Cr_2O_7$

Sugar	*Color*	*Sugar*	*Color*
Aldopentoses		*Disaccharides*	
L(+)Arabinose	Blue	Cellobiose	Blue
D(—)Lyxose	Blue	Lactose	Blue
D(—)Ribose	Blue	Maltose	Blue
D(+)Xylose	Blue	Melibiose	Blue
		Sucrose	Blue
Ketopentose		Trehalose	Blue
L(—)Xylulose	Blue	Turanose	Blue
Aldohexoses		*Trisaccharides*	
D(+)Galactose	Blue	Gentianose	Blue-gray
D(+)Glucose	Blue	Melezitose	Blue-gray
D(+)Mannose	Blue-gray		
Ketohexoses		*Uronic acids*	
D(—)Fructose	Blue	α-D-Galacturonic acid	Lilac
L(—)Sorbose	Blue	α-D-Glucuronic acid	Blue
Methylpentose		*Sugar derivatives*	
L(—)Fucose	Lilac	α-Methyl-D-mannoside	Blue
		Diacetone-fructose	Blue-gray

Results and discussion

The technique described can be used to identify microamounts of reducing and non-reducing sugars. (See Table I).

It has certain advantages over other published thin layer chromatographic methods of sugar detection. The sensitivity of the present reaction is greater than that of aniline phthalate (for reducing sugars only)[10], and of 1,3-dihydroxynaphthalene[8], with a limit of detection just below 4 μg.

Furthermore, in spite of being a little less sensitive than the anisaldehyde reagent of STAHL AND KALTENBACH[9], it has the advantage over the latter of being specific for sugars and a few sugar derivatives and of not being influenced by the boric acid used to impregnate the Kieselgel G.

The original reaction of TAUBER[7], executed without heating the system, gives a blue color for aldoses, red for ketoses and yellow for pentoses. Aldoses do not give a color in small amounts.

In our method, due to the formation of complexes between the sugars and boric acid used to impregnate the silica gel, the color given by reaction with aminoguanidine sulfate is blue or blue-gray with the exception of fucose and galacturonic acid. Also the sensitivity is much improved.

The method, therefore, allows the identification of fucose in a mixture of sugars, even when galacturonic acid is present, since they have different R_F values in many developing solvents.

It is interesting to note that the phosphate esters of hexoses, the lactones and the sugar alcohols do not give the reaction and this increases the specificity of the test.

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Department of Physical Chemistry, Escola de Engenharia, Universidade do Rio Grando do Sul, Porto Alegre (Brasil)

PAULINO M. MARTINS
YEDA P. DICK

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