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Note

Use of malonamide as a general spray reagent for the fluorimetric detection of reducing sugars on filter papers and thin-layer plates

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There are a number of reagents for fluorimetric determination of carbohydrates. However, most of them fluoresce only under strongly acidic conditions, so they are not suitable for paper chromatographic detection of carbohydrates. Recently we noticed that some aliphatic amines, especially ethylenediamine, were intensely fluorescent under weakly alkaline conditions, and devised a simple procedure for the detection of reducing sugars with this reagent[1]. This procedure allows the detection of as little as 0.5 nmole of reducing sugars, being more sensitive than ordinary colorimetric detection[2–5]. In continuation of the study of fluorimetric analysis of carbohydrates, we found that the malonamide is also usable as a fluorogenic reagent for reducing sugars.

The fluorescence produced by malonamide with reducing sugars shows both excitation and emission maxima at 328–382 and 383–425 nm, respectively. Fluorescence at the longer wavelength is several times as intense as that at the shorter wavelength, and is visible in the greenish-blue region.

Table I gives the lower limits of detection for individual carbohydrates. In this detection, carbohydrate samples were spotted in small circles (diameter, 5 mm) on a sheet of filter paper or a thin-layer plate, which was then sprayed with a 1% solution of malonamide in 1 *M* carbonate buffer (pH 9.2), heated for 5 min (filter paper) or 20 min (thin-layer plate) in an oven at 120°, and irradiated by a mercury lamp which emitted the 365 nm light most abundantly. It is indicated that all the aldoses, ketoses, amino sugars, uronic acids, and reducing disaccharides could be detected at levels as low as 0.25 nmole. The lower limit of detection for 2-deoxy-D-glucose was slightly higher (0.5 nmole). Aldonic acids, alditols, glycosides, non-reducing oligosaccharides, and polysaccharides were far more insensitive. When the spots of carbohydrate samples on a sheet of filter paper were detected after chromatographic development with an appropriate solvent system, such as *n*-butanol–acetic acid–water (4:1:5) (upper layer) or *n*-butanol–pyridine–water (6:4:3), the lower limit of detection rose to the nmole level due to diffusion of samples. Thin-layer chromatographic development did not cause blurring of the spots.

The fluorescence reaction of malonamide is highly selective; ordinary alcohols,

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TABLE I

LIMITS OF DETECTION OF CARBOHYDRATES

Carbohydrate	*Limit of detection (nmole)*	*Carbohydrate*	*Limit of detection (nmole)*
DL-Glyceraldehyde	0.25	Glycolic acid	1000
L-Arabinose	0.25	D-Gluconic acid	1000
D-Lyxose	0.25	Erythritol	10
D-Ribose	0.25	D-Glucitol	10
D-Xylose	0.25	Methyl α-D-glucoside	100
D-Galactose	0.25	Adenosine	1000
D-Glucose	0.25	Trehalose	200
D-Mannose	0.25	Sucrose	200
L-Fucose	0.25	Raffinose	200
L-Rhamnose	0.25	Maltose	0.25
D-Fructose	0.25	Cellobiose	0.25
L-Sorbose	0.25	Lactose	0.25
D-Galactosamine · HCl	0.25	Melibiose	0.25
D-Glucosamine · HCl	0.25	Starch	20
N-Acetyl-D-glucosamine	0.25	Glycogen	1000
D-Galacturonic acid	0.25	Dextran	1000
D-Glucuronic acid	0.25	Pullulan	20
2-Deoxy-D-glucose	0.5		

phenols, aldehydes, ketones, carboxylic acids, including amino acids, esters, amides, nitro compounds, sulfonic acids, thiols, and sulfoxides, did not show fluorescence at these wavelengths.

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