after development and visualisation while 1.0–5 μg quantities of the mixture were readily visualised as four distinct pink-purple spots.

This technique is currently being used to locate DNB and DNPH derivatives of compounds present in honey aroma after separation by TLC. It could have wider application as a general technique for reductions on thin layer chromatograms.

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Separation of cello-oligosaccharides by thin-layer chromatography*

While studying the initial products of hydrolysis of cellulose by rumen micro-organisms of the sheep it was found that separation of the cello-oligosaccharides by paper chromatography was time consuming and resulted in poor resolution. To achieve better separation of the oligosaccharides it was decided to make use of thin-layer chromatography (TLC).

The application of TLC for the separation and identification of malto-oligosaccharides has been reported by many workers[1–3]. On the other hand, little information is available on the resolution of cello-oligosaccharides by this method. BECKER *et al.*[4] reported on the separation of cellulose degradation products eluted from a charcoal–celite column by TLC on Kieselguhr G using *n*-butanol–ethanol–water (50:30:20, v/v). A suitable technique for the separation of cello-oligosaccharides by TLC is described in this communication.

Experimental

The chromatoplates (20 cm × 60 cm) were coated with Kieselgel G or Kieselguhr G (Merck & Co) to a thickness of 250 μ, according to the procedure described by STAHL[5]. Solutions of cello-oligosaccharides** (cellobiose, cellotriose, cellotetraose,

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cellopentaose, cellohexaose) were spotted on the plates in concentrations of 10 to 15 μg. Ascending chromatography was accomplished in closed glass tanks containing sheets of filter paper saturated with the developing solvent. Two different solvent systems were used for developing the chromatograms, *viz.* (a) isopropanol–water–ethyl acetate (1:2:1, v/v), and (b) *n*-propanol–ethyl acetate–water (6:1:3, v/v). When the solvent front reached 50 cm from the origin, the chromatoplates were removed and air dried. The presence of the cello-oligosaccharides was detected by spraying the plates with aniline phosphate reagent[6] and heating at 115° for 20 min. Alternatively, plates were sprayed with a 0.5% solution of potassium permanganate in 1 *N* sodium hydroxide[7] and heated at 100° for 1–2 min.

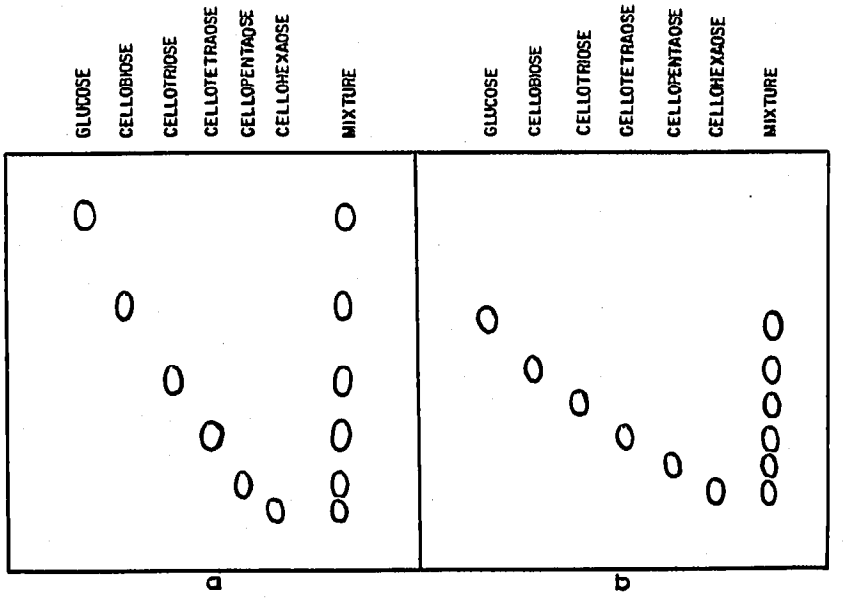

Fig. 1. Separation of cello-oligosaccharides by TLC using the following solvent systems: (a) isopropanol–water–ethyl acetate (1:2:1, v/v), and (b) *n*-propanol–ethyl acetate–water (6:1:3, v/v).

Results and discussion

Cello-oligosaccharides appeared as well-defined brown spots against a white background after treatment with aniline phosphate and as pale yellow spots on a purple background after treatment with alkaline permanganate. Fig. 1a and b shows the separation of individual and a mixture of oligosaccharides achieved using the two solvent systems. The R_F values of the oligosaccharides in the solvent systems studied are given in Table I. Under the conditions of the experiment solvent system (a) gave a better resolution of oligosaccharides than solvent system (b). To obtain repeatable results it was found necessary to have the atmosphere of the developing chamber saturated with the solvent system for a minimum of 30 min prior to development. This is easily achieved by placing a folded filter paper sheet soaked in the solvent so that it forms an inner lining to the wall of the chamber. It was also observed that the use of thick layers (300–350 μ) of Kieselgel G or Kieselguhr G rendered the detection of small quantities of oligosaccharides difficult. Comparatively Kieselgel G gave a better resolution of oligosaccharides than Kieselguhr G; the activation of the

TABLE I

R_F VALUES (× 100) OF GLUCOSE AND CELLO-OLIGOSACCHARIDES

Solvent systems: (a) isopropanol–water–ethyl acetate (1:2:1, v/v); (b) *n*-propanol–ethyl acetate–water (6:1:3, v/v).

	Solvent system	
	a	*b*
Glucose	45	46
Cellobiose	33	36
Cellotriose	23	30
Cellotetraose	15	23
Cellopentaose	8	18
Cellohexaose	5	13

prepared plates was not necessarily required. Spraying with the aniline phosphate reagent gave distinct spots whereas spraying with the alkaline permanganate reagent yielded spots which faded rapidly.

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