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Note

Separation of isomeric pentoses and hexoses by continuous-flow thin-layer chromatography

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Several thin-layer chromatographic (TLC) systems exist for the separation of the common pentoses and hexoses, using both micro-crystalline cellulose layers[1,2] and silica gel layers[3,4], but these often yield poor separation between isomeric pentoses and hexoses, especially when one of the sugars in a mixture is present in large amounts. The resolution of conventional TLC systems can, however, be increased by multiple developments[5] and by impregnation of silica gel layers with boric acid and phosphate[6–10]. In this paper a continuous-flow system is described to improve the separation of the common sugars using pre-coated silica gel TLC plates without impregnation.

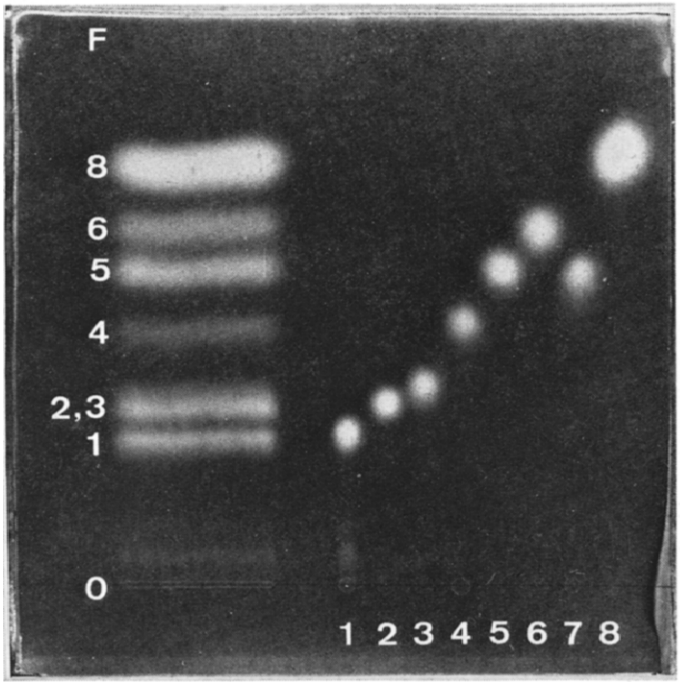
EXPERIMENTAL

Pre-coated, aluminium-backed silica gel G TLC plates, layer thickness 0.25 mm (Merck; No. 5553), were used unactivated with the solvent systems: (A) chloroform–methanol–water (65:35:4) and (B) acetone–water (15:1).

Continuous-flow TLC was performed in a sandwich chamber (Camag, Muttenz, Switzerland), modified to include a piece of absorbent paper (Whatman No. 1 filter paper) attached along the upper edge of the plates, allowing a continuous flow of solvent from the trough at the bottom of the plate, through the plate itself, and into the paper. Separations of the pentoses were performed using solvent system A for 9 h, while separations of the hexoses were performed using solvent system B for 7 h. Detection of the sugars was routinely performed using the sodium periodate–silver nitrate method[11], without subsequent sodium hydroxide treatment.

RESULTS AND DISCUSSION

A separation of standard sugars by continuous-flow TLC using solvent system A for 9 h is shown in Fig. 1. The system gave good separations of mixtures of rhamnose, fucose, xylose, arabinose, glucose, and galactose although it proved difficult to separate ribose and xylose, and glucose and mannose. However, glucose and mannose could be completely resolved by continuous-flow TLC in solvent system B for 7 h (Fig. 2). In both continuous-flow TLC systems a considerable improvement in



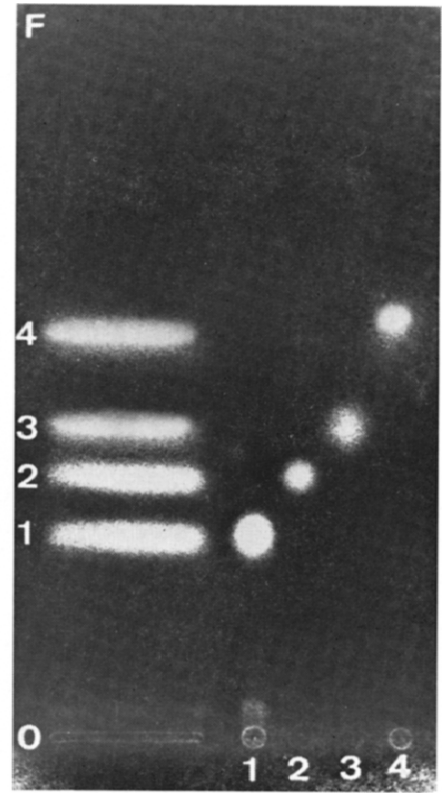


Fig. 1. Separations of the common pentose and hexose standards, both individually and as a mixture, by continuous-flow TLC using the solvent system chloroform–methanol–water (65:35:4) for 9 h. 0 = Origin, 1 = galactose, 2 = glucose, 3 = mannose, 4 = arabinose, 5 = xylose, 6 = fucose, 7 = ribose, 8 = rhamnose and F = top of the TLC plate (18 cm).

Fig. 2. Separation of galactose (1), glucose (2), mannose (3) and arabinose (4), both individually and as a mixture, by continuous-flow TLC. Development was for 7 h in the solvent system acetone–water (15:1).

resolution was observed when compared with normal TLC separations largely as a result of the increased spread of the sugars across the plate, enabling a reliable, easy and relatively rapid separation of the sugars commonly found in hydrolysates of glycosylated compounds.

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