CHROM. 6311

Orange Dextran as a void volume marker in thin-layer gel chromatography

Thin-layer gel chromatography (TLG) was developed soon after column gel chromatography[1], but only recently, following the introduction of improved techniques[2], has it become widely used. One of the present disadvantages of the method is that there is no satisfactory coloured material available for marking the rate of migration of high-molecular-weight solutes which are excluded from the gel beads: TLG experiments are normally continued until such substances approach the end of the gel-coated plate. Blue Dextran (mol. wt. $\geqslant 2 \times 10^6$), the material widely used for checking the packing of gel chromatography columns and determining their void volumes, is unsuitable for thin-layer work[3]. At low concentrations it is invisible on the gel layer, partly because of the poor colour contrast between the greyish gel and the pale blue solute. At concentrations at which they are easily visible solutions of Blue Dextran are too viscous and zone spreading occurs. The preparation of a satisfactory Dextran derivative is described in the present note.

Dextran Type 2000 (average molecular weight 2×10^6: Sigma Chemical Co.) was dyed with Procion Brilliant Orange 2RS (Dylon International Ltd., London) according to the procedure of DUDMAN AND BISHOP[4]. A 1:1 Dextran to dye ratio was used and the mixture allowed to stand overnight. Excess dye was removed by passing the mixture through a column of Sephadex G-25, using distilled water as the eluant. (Some dye became bound to the gel beads during this step and each column was used once only.) The salt-free dyed Dextran may easily be freeze-dried.

Both the free dye and the conjugated Dextran had an absorption maximum in the visible region of the spectrum at 494 nm, and at this wavelength the free dye had an $E^{1\%}_{1\,cm}$ value of 301.5. The same value was used to calculate the dye content of the Dextran conjugate: this was found to be 26.0%, in good agreement with the values obtained by DUDMAN AND BISHOP[4] with other Procion dyes used in similar conditions.

TLG was performed on 1-mm-thick layers of Sephadex G-200 Superfine (Pharmacia (G.B.) Ltd.), using 0.1 *M* NaCl as the eluant. Gel layers were equilibrated overnight before an experiment and used at an angle of approximately 10° to the horizontal. Samples of Orange Dextran with concentrations of 0.18% and 0.09% w/v were applied to the gel with microscope slide cover glasses[2], or micropipettes[5].

It was found that in all cases the coloured samples moved uniformly through the gel layer, without appreciable zone-broadening: in the conditions given the rate of migration was approximately 4 cm/h. When the sample concentration was 0.18% w/v the orange zone remained easily visible throughout the experiment with samples as small as 1 μl in volume. Even the 0.09% w/v solutions remained visible when the applied volume was 5 μl or more. A permanent record of the chromatogram could be obtained by taking a paper "print" of the gel[2] using Whatman No. 1 paper, and immediately drying it in warm air.

It was concluded that Orange Dextran solutions at concentrations of approximately 0.2% w/v are very suitable as void volume markers in TLG experiments. The simple dyeing procedure is applicable to many polysaccharides, and may be used in the determination of the molecular weights of Dextrans in the range 10,000–100,000, using the TLG method[6]. Orange Dextran solutions may also be used in column gel

chromatography, to determine void volumes and to check the packing of the gel bed: in this application, however, it does not seem to have any particular advantages over Blue Dextran.

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First received June 26th, 1972; revised manuscript received August 21st, 1972