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Automated molecular-sieve chromatography of polysaccharides

Molecular-sieve chromatography (MSC) is a useful technique for investigating the polymeric properties of acidic polysaccharides[1,2]. In earlier studies[3,4], elution patterns were obtained by collecting many small fractions which were then screened tediously by a colorimetric method[5].

A considerable range of agarose gels and porous glass or silica beads is now available for studies of high molecular weight polymers. This range will probably increase in the future. Manufacturer's data for such products are usually obtained from tests with characterised proteins, dextrans, or polystyrene fractions; their performance with other types of polymer (*e.g.* acidic polysaccharides) frequently differs[6] and must therefore be evaluated.

The different basic types of agarose preparations (gelled beads, and granular, physically disintegrated gels) vary fundamentally in chromatographic characteristics. Similar considerations apply to porous glass beads, which appear to be offered as two distinct types—"Bio-Glas" (Bio-Rad Laboratories, California) and "Haller Glass" (Corning CPG glass). The choice of a molecular-sieve with a suitable pore-size distribution has a significant influence on the degree of separation attainable[7]. Although HEITZ AND KERN predicted[8] that separation efficiency will eventually be determined by molecular coil dimensions rather than by experimental refinement, it seems reasonable to suppose that, for complex branched molecules with associated steric and charge effects, the most suitable molecular-sieve and the optimum chromatographic parameters for a particular analysis can best be found by experiment. In addition to variables such as the eluant composition and flow-rate, particle size of the molecular-sieve etc., the column shape[9] can be important—in addition to the column dimensions—if optimum results are to be obtained for polymer systems that have not previously been characterised rigorously. There are no standard methods for the preparation, purification, and analysis of polysaccharides. Successful procedures are frequently established only by series of successive refinements, and conditions devised for one polysaccharide system should not be taken automatically as optimum for others, even if they are similar in type or origin.

In all chromatographic techniques there have always been two time-consuming tasks: evaluating new or modified materials[10], and establishing optimum conditions for their use with different classes of compounds. At the present time, the extent of these committments in MSC clearly calls for an automated method of monitoring the effluent from the columns continuously, *e.g.* by differential refractometry[11]; spectroscopy[12]; radioactive labelling[13]; flame-ionisation, conductivity[14,15], and differential vapour-pressure[16] detectors; polarography[17]; and, most interesting of all, a return to true chromatography[18].

Selective dyes such as Toluidine Blue[19,20], Alcian Blue[21], and Mucicarmine[22] have long been used in differential staining reactions for electrophoresis and chromatography, and the recent resurgence of attention to mucopolysaccharides (glycosaminoglycans) has led[23] to renewed interest in dyed complexes.

DUDMAN AND BISHOP[18] observed that the reactivity of "Procion" dyes with polysaccharides was proportional to the number of primary hydroxyl groups present;

pure polygalacturonan was completely resistant to the dyes tested, and a polysaccharide containing *ca.* 30% uronic acid was dyed only to a slight extent. Tests on the behaviour of acidic gum polysaccharides were not reported. The uronic acid content of such materials frequently falls within the 5–25% range, but some botanical genera *(Khaya, Sterculia)* contain up to *ca.* 50%.

It was therefore of interest to evaluate the broad range of application of this colorimetric technique by studying the extent of the reactions of the dyes Procion Blue M3G and Procion Brilliant Red M2B (kindly provided by I.C.I. Ltd., Dyestuffs Division, Manchester) with a wide range of acidic gum polysaccharides from the *Acacia, Albizia, Araucaria, Azadirachta, Combretum, Khaya, Lannea* and *Sterculia* genera. Although the extent of reaction, as indicated by DUDMAN AND BISHOP[18], appears to be inversely proportional to the uronic acid content, those gums with the highest uronic contents available to us (*Brachychiton diversifolium*[24,25], 51%; *Khaya senegalensis*[26], 43%) nevertheless were dyed to an extent adequate for their colorimetric detection in the eluate from MSC columns containing the agarose gels "Bio-Gel A5" and "Sepharose 4B". For polysaccharides with low uronic acid content the amount of dyestuff used can be controlled, so that the molecular weight of the natural product is not significantly increased. Dextrans are also readily dyed; the characterised fractions available commercially can continue to be used as calibration standards for relative measurements[6] if more valid standards of closely similar chemical structure, characterised by fundamental methods, are not available.

Unfortunately, the use of Procion dyes is not of general applicability: a carrageenan did not react in the dyeing process.

The use of dyed polysaccharides (we prefer the use of Procion Brilliant Red M2B) leads to a simple method of monitoring the behaviour of the native polysaccharides during molecular-sieve chromatography, or for evaluating—with a selected, characterised polysaccharide—the performance of a molecular-sieve column. The dyed sample (1–3 mg) in 2 *M* sodium chloride (0.5 ml) is applied with care to the top of a silanised glass column (35 × 1.5 cm I.D.) containing an appropriate molecular sieve (porous glass or silica; agarose, dextran, or polyacrylamide gels). A suitable eluant is 1 *M* sodium chloride, at 0.5–1 ml/min; automated analyses then take about 2 h. The column effluent is passed directly via capillary tubing into the cell (modified to permit continuous flow) of a colorimeter (Unicam SP 1300). The photo-cell signal is fed via an impedance-matching device (Vibron 33B electrometer) into a recorder (Kent, 1 mV, chart speed 2 in./h). By increasing the recorder input resistor from 1 to 2.5 Ω the maximum signal from the photo-cell gives a recorder deflection of 10 in. This particular experimental combination of instruments is, of course, not unique. Micro-flow colorimeters (Phoenix MFC 800) give an optical path-length of 20 mm for a cell hold-up volume of only 0.035 mm, if increased sensitivity is essential.

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