Notes

CHROM. 4508

An automated gel filtration system for determining the molecular weight of polysaccharides

During the past few years, gel filtration has become an accepted method for determining the molecular size of polysaccharide molecules[1–4]. The technique usually involves measurement of elution volumes using a fraction collector fitted with either a syphon or a drop counter. Both of the latter devices must be calibrated to give accurate and reproducible deliveries. To determine the actual elution volume, each fraction has to be carefully analysed for carbohydrate content, a process which can be both laborious and time-consuming when a large number of samples are to be chromatographed. Furthermore, the error incurred in the above technique can be as high as ± 10 % (ref. 1). However, a rapid and accurate automated method for the lower polysaccharides has recently been described by DELLWEG and his co-workers[5]. Using a column of polyacrylamide gel coupled to an Auto-Analyser and a fraction collector, they showed that the elution volumes of a maltodextrin series could be accurately monitored and that a linear relationship existed between these elution volumes and the logarithms of the molecular weights of the maltodextrins up to a degree of polymerisation of 13. In this paper a fully automated system, which dispenses completely with a fraction collector, is described. The system can be used for measuring the number-average molecular weights of larger polysaccharides ranging from 4000 to *ca.* 100000.

Methods and materials

It has been reported[1] that the polyacrylamide gel, Bio-Gel P-300, is suitable for the filtration of polysaccharides whose number-average molecular weights ($\bar{M}_n$) range from 5000 to 150000. When used with a proportioning pump, however, this gel compressed considerably in the column and was therefore unsuitable for use with the Auto-Analyser system. An agarose gel (Bio-Gel A, 0.5 *M*, 200–400 mesh) which has a greater fractionation range than Bio-Gel P-300 (ref. 6) did not compress to the same extent and, despite being a carbohydrate itself, proved to be eminently suitable for the automated system.

A Pharmacia K9/30 column (0.9 × 30 cm), pretreated with dichlorodimethylsilane, was packed according to the manufacturer's instructions until the gel was level with the top of the column. The surface of the gel was then covered with a disc of nylon mesh. The column end-piece was modified for automated chromatography as shown in Fig. 1. A threaded glass T-piece, covered with a rubber septum, was screwed into the column end-block so that a 2-in. hypodermic needle could just reach the surface of the gel. Eluent was allowed to enter the column continuously through the side arm of the T-piece. Before use, the column was eluted for several days with

a 0.05 M solution of mercuric chloride to replace the sodium azide preservative on the agarose gel, since azide interferes strongly with colorimetric reagents such as orcinol. The mercuric chloride fulfilled the double role of preservative and eluting agent.

The void volume of the column was determined by eluting Blue Dextran 2000 (Pharmacia). The column was calibrated using the standard polysaccharides listed in the table.

The molecular weights were determined, by the manufacturers, using light scattering methods ($\bar{M}_w$) or by end group analysis ($\bar{M}_n$). Number-average molecular weights were not available for the Sigma dextrans. The dextran fraction (11640/1/111) was a gift from Dr. KIRSTI GRANATH of Pharmacia and the laminarin was a gift from Prof. D. J. MANNERS. The molecular weight of the laminarin was determined by end group analysis[7].

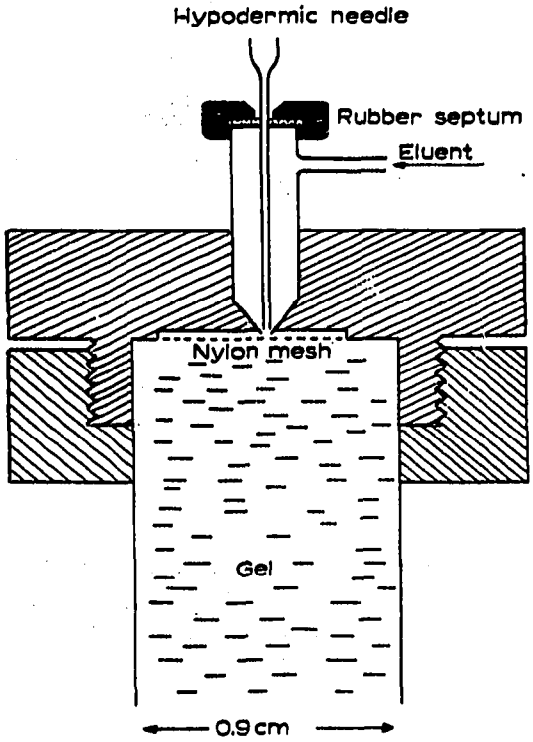

Fig. 1. Modified column end-piece and sample applicator.

Analytical procedure

An orcinol/sulphuric acid reagent[8] was used in a Technicon Auto-Analyser to detect polysaccharides eluted from the column. The flow diagram for the system is shown in Fig. 2. Unless otherwise indicated, Acidflex tubing was used throughout the system. The column eluent was mixed with 1 % aqueous orcinol before being introduced to, and mixed with, 72 % sulphuric acid. The complete reaction mixture was heated at 95° in an oil bath, then cooled, and the absorbance monitored at 420 nm in a 15 mm continuous flow cell.

By means of a 100 μl Hamilton syringe samples were applied to the top of the column when the recorder pen reached a pre-selected starting position on the

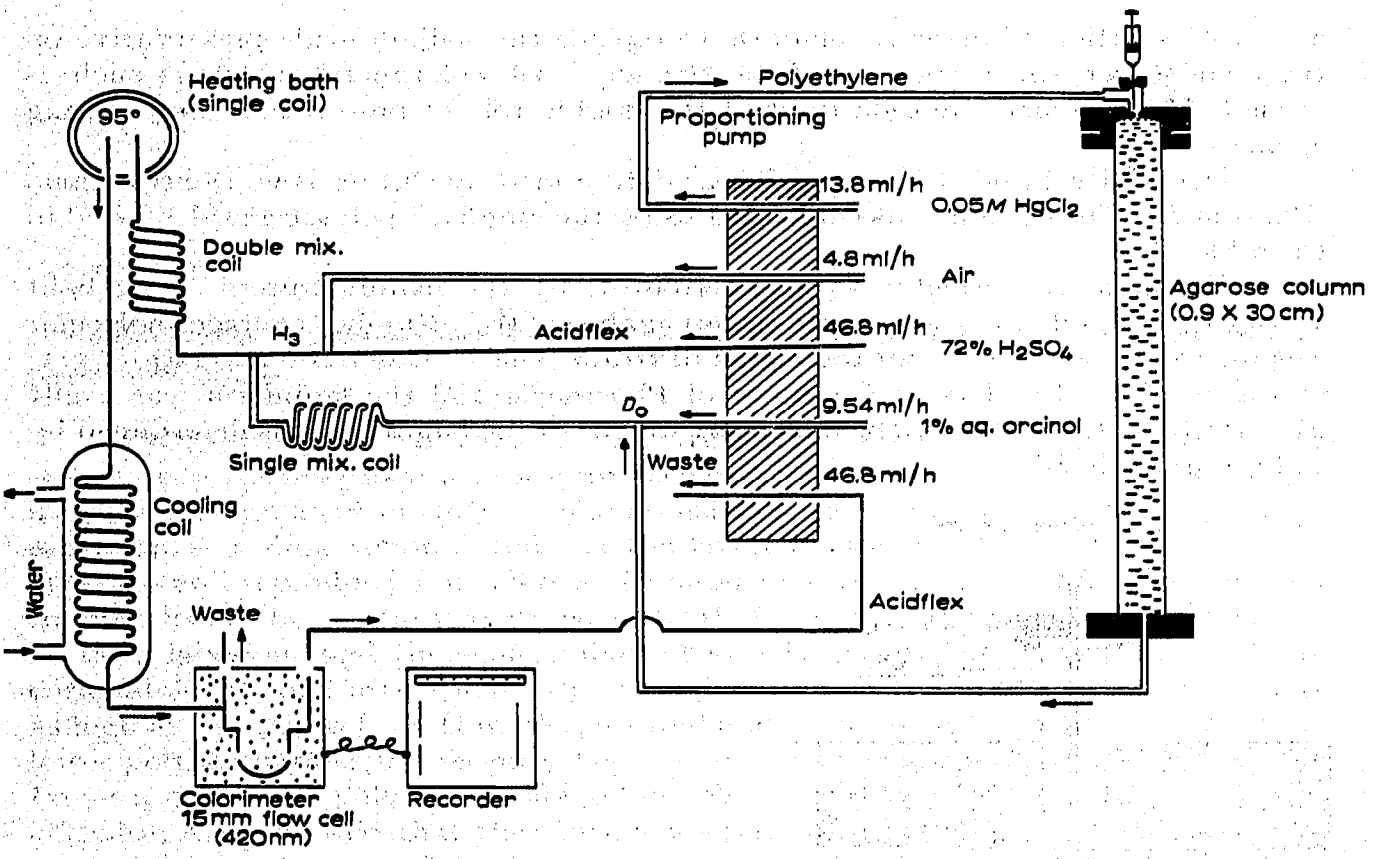

Fig. 2. Flow scheme for chromatography and analysis.

chart. The retention times on the column and in the Auto-Analyser were measured from this point to the centre of each peak (see Fig. 3).

Results and discussion

Since the retention time in the apparatus is proportional to the elution volume, a logarithmic plot of retention time against molecular weight ($\overline{M}_w$ and $\overline{M}_n$) was drawn (Table I and Fig. 3). Due to the almost perfect gaussian distributions obtained from

TABLE I

STANDARD POLYSACCHARIDES

Polysaccharide	*Manufacturer or origin*	*Number-average molecular weight* ($\overline{M}_n$)	*Weight-average molecular weight* ($\overline{M}_w$)	*Retention time (min)*
Dextran 2000	Pharmacia	—	> 2 000 000	85.4
Dextran 110	Pharmacia	80 000	113 000	100.2
Dextran	Sigma Chemicals	—	72 600	114.3
Dextran 40	Pharmacia	25 700	41 800	127.0
Dextran 20	Pharmacia	15 000	22 300	142.3
Dextran	Sigma Chemicals	—	20 800	146.9
Dextran 10	Pharmacia	5 700	11 200	163.2
Laminarin	See text	4 200	—	167.4
Dextran (11640/1/111)	Pharmacia	2 670	3 100	174.9

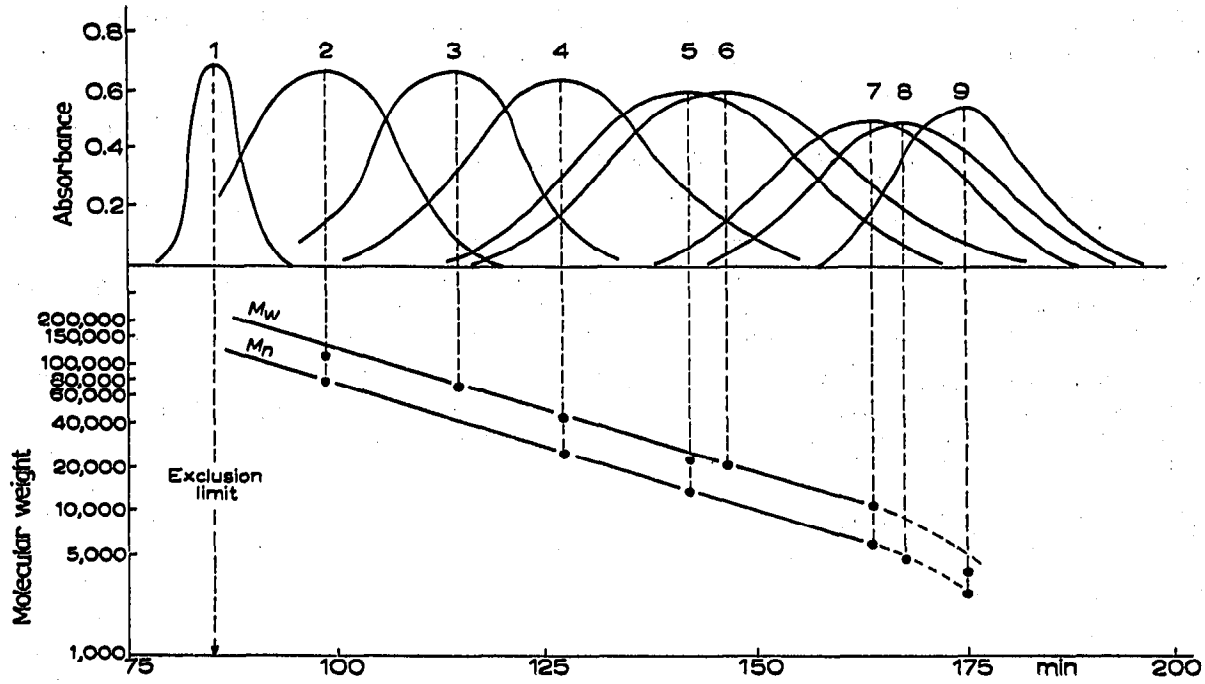

Fig. 3. Logarithmic plot of molecular weights ($\overline{M}_w$ and $\overline{M}_n$) *versus* retention time and molecular weight distributions of standard polysaccharides on Bio-Gel A (0.5 *M*). (1) Dextran 2000 (exclusion limit); (2) Dextran 110 ($\overline{M}_w$ = 113000, $\overline{M}_n$ = 80000); (3) Sigma dextran ($\overline{M}_w$ = 72600); (4) Dextran 40 ($\overline{M}_w$ = 41800, $\overline{M}_n$ = 25700); (5) Dextran 20 ($\overline{M}_w$ = 22300, $\overline{M}_n$ = 15000); (6) Sigma dextran ($\overline{M}_w$ = 20800); (7) Dextran 10 ($\overline{M}_w$ = 11200, $\overline{M}_n$ = 5700); (8) Laminarin ($\overline{M}_n$ = 4200); (9) Dextran 11640/1/111 ($\overline{M}_w$ = 3100, $\overline{M}_n$ = 2670).

all the standard polysaccharides, the mid-point of each peak could be determined with an accuracy of ± 1%. Repeated runs with each sample proved that retention times could be reproduced with a precision of ± 2%. Linear graphs were obtained for both weight-average and number-average molecular weights within the range $\overline{M}_n$ = 4000–80000 and $\overline{M}_w$ = 10000–100000.

Although requiring careful handling to avoid microbial infection, agarose gel gives high resolution of a wide range of molecular weights on short columns and at a relatively high flow rate. These latter attributes made this an excellent gel for use with the Auto-Analyser. For example, a complete determination could be carried out in less than 3 h without further attention to the apparatus after injection. The automated system offers several other advantages over the conventional technique. The sensitivity of the Auto-Analyser assay system is such that only very small amounts of material need be applied to the column; this in turn minimises band spreading and so increases the precision of each determination. Also, all the carbohydrate applied to the column is analysed and not just aliquots from individual fractions. The elution volume can therefore be established with accuracy and economy of material.

This method has been used successfully in this laboratory for measuring the molecular weight distributions of a number of β-glucan fractions and high-molecular-weight dextrins. In cases where these distributions were non-gaussian, average molecular weights could be calculated from planimeter readings taken directly from the recorder chart.

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