Journal of Chromatography, 171 (1979) 460–461

CHROM. 11,531

Note

Calibration of a Sephadex G-25 column with oligosaccharides of 2 to 50 units

JOHN H. McCLENDON
School of Life Sciences, University of Nebraska, Lincoln, Nebr. 68588 (U.S.A.)
(First received August 14th, 1978; revised manuscript received October 10th, 1978)

Calibration and interpolation of gel filtration columns are commonly done by plotting the elution volume against the log (molecular weight), which gives a linear function in the middle portion of the elution curve of any given system[1,2]. However, in the work in this laboratory, it was desired to extend the calibration to the void volume to estimate the degree of polymerization (DP) of the intermediate hydrolysis products of an *endo*-polygalacturonase acting on galacturonan (pectate). It was found that the plot of 1/DP *versus* elution volume gave a straight line in this region.

The calibration was made by taking a partial enzyme hydrolysate of galacturonan and reducing the terminal sugar moieties with ^{3}H-labelled $NaBH_4$. After destruction of the BH_4 with acid, a sample was chromatographed on a column of Sephadex G-25 (fine), about 2.4 m long, in 0.1 *N* acetic acid[3]. The sugars in the effluent fractions were determined by an anthrone method[3], and the radioactivity by liquid scintillation. Since each oligomer had DP—1 sugar units and 1 radioactive unit, the ratio (cpm) (anthrone color)$^{-1}$ (DP—1) was calculated for 16 fractions in which the peaks were well separated (dimer–pentamer). The mean value (standard deviation = 7%) of this ratio was then applied to each fraction of the effluent, calculating the DP from the measured values of radioactivity and color reaction. The plot of V_e *versus* log (DP) gave a straight line from a DP of 2 to 20. The plot of 1/DP *versus* V_e gave a straight line from V_0 to a DP of 10 (Fig. 1). The DP of the highest molecular weight material in this hydrolysate was about 40, while direct reducing group titration of the unhydrolyzed polymer gave a DP of about 50. By extrapolation, the void volumn of the column can be determined.

It was found in other experiments that the oligomers with reduced end groups co-chromatographed with normal oligomers as short as the tetramer, but that the trimer and especially the dimer and monomer were more retarded than the normal oligomers. The reduced dimer was eluted between the normal dimer and monomer. The radioactive impurity[4] chromatographed with the monomer, and was only eliminated by repeated evaporation of methanol–HCl.

Hjertén[5] has given a thorough thermodynamic treatment of gel filtration chromatography which predicts that the plot of $-\log(V_e - V_0)$ *versus* DP or *versus* $DP^{2/3}$ should give a straight line. These require that V_0 be known, which may be inconvenient. With the above data (and extrapolated V_0), a plot of $\log (V_e - V_0)$ *versus* DP gave a straight line only from a DP of 1 to 7. The more cumbersome plot *versus* $DP^{2/3}$ did appear to give a straight line if a slight adjustment to V_0 was made.

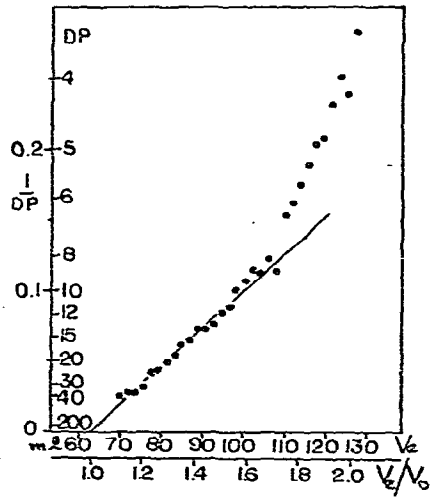

Fig. 1. Calibration of a gel filtration column near the void volume. The mean DP (degree of polymerization) of each eluted fraction was determined as detailed in the text, and its reciprocal plotted against the elution volume (V_e). A straight line was fitted to the curve by eye, and extrapolated to $1/DP = 0$ to give the void volume (V_0), and the ratio V_e/V_0 calculated as shown. The void volume thus derived is in approximate agreement with that given by a dextran blue marker in other experiments.

REFERENCES

1 H. Determan, *Gel Chromatography*, Springer, New York, 1968.
2 J. Coupek, M. Kubin and Z. Deyl, in Z. Deyl, K. Macek and J. Janak (Editors), *Liquid Column Chromatography*, Elsevier, Amsterdam, 1975, pp. 301–323.
3 J. H. McClendon, *Phytochemistry*, 14 (1975) 377.
4 G. N. Richards and W. J. Whelan, *Carbohyd. Res.*, 27 (1973) 185.
5 S. Hjertén, *J. Chromatogr.*, 50 (1970) 189.