Journal of Chromatography, 174 (1979) 443–446

CHROM. 11,811

Note

Comparison of open-column and high-performance gel permeation chromatography in the separation and molecular-weight estimation of polysaccharides

T. W. DREHER, D. B. HAWTHORNE and B. R. GRANT*

Russell Grimwade School of Biochemistry, University of Melbourne, Parkville, Victoria 3052 (Australia)

(Received February 19th, 1979)

Gel permeation chromatography (GPC) on polydextran (Sephadex) or polyacrylamide (Bio-Gel) gels has been widely used in the fractionation and molecular-weight estimation of polysaccharides[1,2]; for polysaccharides of very high molecular weights, agarose gels have been used[3,4]. In this paper, we compare the soft gel columns of Ultrogel (LKB, Bromma, Sweden) with the μBondagel columns (Waters Assoc., Milford, Mass., U.S.A.) used in high-performance liquid chromatography (HPLC) as a means of separating polysaccharides and estimating their molecular weights. Ultrogels are co-polymers of agarose with polyacrylamide and allow high flow-rates at low pressures.

The HPLC system consisted of a Waters Assoc. Model 6000A solvent pump, Model U6K injector and a Model R401 differential refractometer. Two stainless-steel columns 300 × 3.9 mm) were used; one had a specified fractionation range of 2000 to 2,000,000 daltons (μBondage E-linear), and the other had a narrower range of 3000 to 100,000 daltons (μBondagel E-300). Separations in the HPLC system were carried out at ambient temperature (25°), with either methanol–water (2:3, v/v) or 0.1 *M* sodium acetate–acetic acid buffer solution of pH 5.5 (containing 0.02% of sodium azide) as mobile phase; flow-rates were maintained at 1 ml min^{-1} by pressures of 1200 and 900 p.s.i., respectively.

The GPC on Ultrogel packings was carried out at 20° with columns (17 mm × 450 mm) of AcA-22 (fractionation range 60,000 to 1,000,000 daltons) and AcA-34 (fractionation range 20,000 to 400,000 daltons), with bed volumes of 95 and 98 ml, respectively. The fractionation ranges quoted for both Ultrogel and Bondagel are those obtained with globular proteins. The eluent was 0.01 *M* acetate buffer solution of pH 5.5 containing 0.02% of sodium azide. By using a head of 75 cm, constant flow-rates of 25 ml h^{-1} could be obtained with the AcA-22 column and 30 ml h^{-1} with the AcA-34 column. The polysaccharide content of the fractions was measured by the phenol–sulphuric acid method[5].

The T-series of dextrans, T-10 to T-500, and blue dextran (Pharmacia, Uppsala, Sweden), Dextrans D4133 and D5251 (Sigma, St. Louis, Mo., U.S.A.), Pullulan (Calbiochem, Los Angeles, Calif., U.S.A.) and Dextran B and D-glucose (BDH,

* To whom correspondence should be addressed.

Poole, Great Britain) were used to compare the systems. The weight-average molecular weights, $\bar{M}_w$, for the T-dextran samples used as calibration standards are listed in Table I. All samples used in HPLC were prepared as 2% (w/v) solutions in the mobile phase, and 4 to 5 μl of solution were injected in each run. Samples for separation on Ultrogel were applied as 0.3% (w/v) solutions, 1 ml to each column. All samples were passed through filters (pore size 0.4 μm) (Nuclepore, Pleasanton, Calif., U.S.A.) immediately before application to the column.

TABLE I

MOLECULAR WEIGHTS ($\bar{M}_w$) OF T-DEXTRAN SERIES USED IN CALIBRATION OF COLUMNS

Dextran	$\bar{M}_w$
T-10	10,400
T-20	21,600
T-40	44,400
T-70	68,500
T-150	154,000
T-500	450,000
Blue dextran	>2,000,000

By using the T-series dextrans as standards, the relationship between $\bar{M}_w$ and elution volume (V_e) shown in Table II was obtained from the various columns, within their specified fractionation ranges. By using these relationships, estimates of the molecular weights of other dextrans and pullulan were compared in different chromatographic systems; the results are shown in Table III. The reproducibility of the values was 2% for the Ultrogels and 1% for HPLC. The E-linear and AcA-22 columns fractionated over a similar range, whereas the AcA-34 column fractionated over a narrower range than the E-300. The fractionation range of both E-linear and E-300 columns may extend below 10,000, as a sample of a 1,3-β-glucan[6] ($\bar{M}_w$ 5000) gave an estimated molecular weight of 5100 on both E-linear and E-300 columns. The limit of resolution of both μBondagel and Ultrogel columns was similar. Compounds with $\Delta \ln \bar{M}_w$ values of 2.0 or greater were separated on the two μBondagel

TABLE II

RELATIONSHIP BETWEEN MOLECULAR WEIGHT ($\bar{M}_w$) AND ELUTION VOLUME (V_e) FOR μBONDAGEL AND ULTROGEL COLUMNS FRACTIONATING DEXTRAN STANDARDS

Column	*Solvent*	*Fractionation range*	*Linear regression*	*Regression coefficient*
μBondagel E-linear	Methanol–water (2:3)	10,000–2,000,000*	$\ln \bar{M}_w = 29.325 - 7.576 V_e$**	0.970
	0.1 *M* Acetate (pH 5.5)		$\ln \bar{M}_w = 28{,}076 - 7.011 V_e$	0.980
μBondagel E-300	Methanol–water (2:3)	10,000– 500,000	$\ln \bar{M}_w = 20.665 - 4.017 V_e$	0.992
	0.1 *M* Acetate (pH 5.5)		$\ln \bar{M}_w = 20.102 - 3.790 V_e$	0.979
Ultrogel AcA-22	0.01 *M* Acetate (pH 5.5)	20,000–2,000,000*	$\ln \bar{M}_w = 18.517 - 0.100 V_e$	0.990
Ultrogel AcA-34	0.01 *M* Acetate (pH 5.5)	20,000– 150,000	$\ln \bar{M}_w = 14.322 - 0.048 V_e$	0.970

* Blue dextran was fractionated slightly on these columns.

** V_e is the elution volume (in ml).

TABLE III

MOLECULAR WEIGHTS OF SEVERAL DEXTRANS AND PULLULAN ESTIMATED BY CHROMATOGRAPHY ON μBONDAGEL AND ULTROGEL COLUMNS

Sample	*Column*	*Solvent*	*Estimated molecular weight*
Dextran B	μBondagel E-linear	Methanol–water (2:3)	126,600
		0.1 *M* Acetate	125,500
	Ultrogel AcA-34	0.1 *M* Acetate	133,000
Dextran D5251	μBondagel E-linear	0.1 *M* Acetate	430,400
	Ultrogel AcA-22	0.01 *M* Acetate	442,000
Dextran D4133	μBondagel E-linear	0.1 *M* Acetate	50,040
	Ultrogel AcA-34	0.01 *M* Acetate	49,000
Pullulan	μBondagel E-linear	0.1 *M* Acetate	1,060,000
	Ultrogel AcA-22	0.01 *M* Acetate	1,623,000

columns, but those which differed in molecular weight by less than this simply gave flat-topped single peaks (Fig. 1) or broad single peaks, intermediate in position between the peaks of individual dextrans. With the Ultrogel columns, the $\Delta \ln \bar{M}_w$ value had to exceed 2.5 before separation could be achieved. It was of interest that, although the μBondagel E-linear column separated over a wider range of molecular weights than the μBondagel E-300 column, both columns showed similar resolution characteristics.

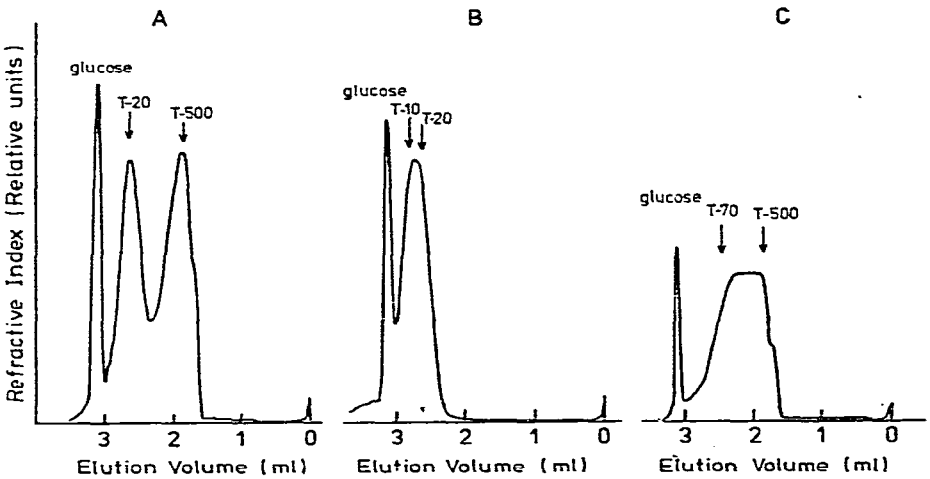

Fig. 1. Elution profiles of dextran mixtures on a μBondagel E-300 column, with methanol–water (2:3) as mobile phase. A, Mixture of D-glucose with Dextrans T-20 and T-500; B, mixture of glucose with Dextrans T-10 and T-20 (the single peak elutes between the positions of the two polymers); C, mixture of glucose, with Dextrans T-70 and T-500 showing a single, flat-topped peak extending over the individual elution positions.

The blue dextran normally used to locate the void volumes in GPC[7] fractionated on the μBondagel E-linear and the AcA-22 columns (this was also noted by Wu *et al.*[8], who used controlled-pore glass columns in an HPLC system. This leaves some uncertainty in the determination of the void volumes of these columns. When water is used as mobile phase, the blue dextran gives a sharper peak than

with acetate buffer solution; this is presumably due to hydrophobic absorptive interactions (which have previously been observed with μBondagel columns when separating proteins[9]). However, if charged polysaccharides such as dextran sulphates ($\bar{M}_w$ 500,000) were chromatographed with either water or buffer solution as mobile phase in either system, the apparent molecular weights obtained were abnormally large compared with those calculated from ultracentrifuge data.

The results obtained show that separations and estimations of the molecular weights of dextrans on the μBondagel and Ultrogel columns give similar values. The HPLC system has the advantage of speed (with complete separation in 5 min or less) and small sample size. Some 10 μg of carbohydrate could readily be detected with the refractive index detector. The Ultrogel open columns, although much slower (maximum elution rate 10 ml cm^{-2} h^{-1} for optimum separation[4]) are useful for preparative work with up to 20 mg of carbohydrate loaded in each run. We have used these systems to separate marine-algal polysaccharides with a wide range of molecular weights and linkage types.

REFERENCES

1 S. C. Charmo, *Advan. Carbohyd. Chem. Biochem.*, 25 (1970) 13.
2 A. Wasteson, *Biochem. Biophys. Acta*, 177 (1969) 152.
3 R. H. Pearce and B. J. Grimmer, *J. Chromatogr.*, 150 (1978) 548.
4 L. Hagel, *J. Chromatogr.*, 160 (1978) 59.
5 M. Dubois, K. A. Gibs, J. K. Hamilton, D. A. Rebers and F. Smith, *Anal. Chem.*, 28 (1956) 350.
6 D. B. Hawthorne, W. H. Sawyer and B. R. Grant, *Carbohydr. Res.*, in press.
7 L. Fischer, in T. S. Work and E. Work (Editors), *Laboratory Techniques in Biochemistry and Molecular Biology, Vol. I, Part II, An Introduction to Gel Chromatography*, North-Holland, Amsterdam, London, 1969, p. 166.
8 A. C. M. Wu, W. A. Bough, E. C. Conrad and K. E. Alden, Jr., *J. Chromatogr.*, 128 (1976) 87.
9 R. V. Vivilecchia, B. C. Lightbody, N. Z. Thimot and H. M. Quinn, *J. Chromatogr. Sci.*, 15 (1977) 424.