

## The Use of "Biogel-P" in the Gel Filtration of Polysaccharides

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LATHE and RUTHVEN<sup>1</sup> suggested that columns of swollen starch might provide a simple chromatographic method for determining the molecular size of proteins and polysaccharides. Gel filtration on cross-linked dextrans ("Sephadex") has, however, been much more successful, particularly for proteins,<sup>2-5</sup> and molecular weights of up to 225,000 can now be determined.<sup>6</sup> Cross-linked polyacrylamide gels have been used for gel-filtration studies on proteins.<sup>7</sup> These are now available commercially ("Bio-Gel P") and they offer the opportunity of studying the gel-filtration behaviour of polysaccharides on noncarbohydrate material.

Accordingly, we have examined different grades

of "Biogel-P" for their possible application to the estimation of the molecular size of polysaccharides. For "Bio-Gel P 300" the empirical relationship between  $\log \bar{M}_n$  and elution volume<sup>2,8</sup> is linear for values of  $\bar{M}_n$  between 5000 and 125,000; although the useful working range extends slightly beyond these values (cf. ref. 9) the exclusion limit of "Biogel-P 300" for polysaccharides appears to fall considerably below the value of 300,000 quoted commercially and found, presumably, for proteins. We have used columns measuring  $2.5 \times 50$  cm. and  $5.0 \times 50$  cm. at a loading of 2-10 mg. of polysaccharide. Calibration can be effected with dextran fractions having known values of  $\bar{M}_n$  and,

<sup>1</sup> G. H. Lathe and C. R. J. Ruthven, *Biochem. J.*, 1956, **62**, 665.

<sup>2</sup> P. Andrews and S. J. Folley, *Biochem. J.*, 1963, **87**, 3p.

<sup>3</sup> J. R. Whitaker, *Analyt. Chem.*, 1963, **35**, 1950.

<sup>4</sup> T. Wieland, P. Duesberg, and H. Determann, *Biochem. Z.*, 1963, **337**, 303.

<sup>5</sup> W. T. Roubal and A. L. Tappel, *Analyt. Biochem.*, 1964, **9**, 211.

<sup>6</sup> A. A. Leach and P. C. O'Shea, *J. Chromatog.*, 1965, **17**, 245.

<sup>7</sup> S. Hjerten and R. Mosbach, *Analyt. Biochem.*, 1962, **3**, 109.

<sup>8</sup> K. A. Granath and P. Flodin, *Makromol. Chem.*, 1961, **48**, 160.

<sup>9</sup> P. Andrews, *Biochem. J.*, 1964, **91**, 222.

when molar sodium chloride is used as eluant, we have found such a calibration to be valid for acidic polysaccharides. Thus two gum fractions (obtained<sup>10</sup> from *Acacia senegal* gum), for which osmotic-pressure measurement had indicated  $\bar{M}_n$  105,000 and 37,000, respectively, gave  $\bar{M}_n = 99,000 \pm 10,000$  and  $\bar{M}_n = 35,000 \pm 3,000$  by gel filtration. A sample of the degraded (auto-hydrolysis) gum, having  $\bar{M}_n = 4400$  (periodate end-group analysis, as formaldehyde) gave  $\bar{M}_n =$

$4800 \pm 500$  by gel filtration. The elution pattern of a de-ionised sample of the whole gum indicated that the molecular-weight distribution extended over a very wide range.

More experiments with "Bio-Gel P" materials are necessary to assess the importance of their application in fractionation and degradative studies and to establish their validity, applicability, and useful working range for molecular-weight estimations of polysaccharides.

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<sup>10</sup> D. M. W. Anderson and J. F. Stoddart, forthcoming publication.