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The effect of sample concentration on gel chromatography of polysaccharides on polyacrylamide gels

Sample concentration is known to affect separation efficiency in gel chromatography only if high solution viscosity, a zone broadening factor, accompanies increase in concentration[1]. Elution volume, V_e, has been generally considered to be concentration-independent. However, WINZOR AND NICHOL[2] noted a slight increase in V_e with increasing sample concentration (1–12 mg/ml) for certain proteins (*e.g.* bovine serum albumin, molecular weight 67000) on Sephadex G-100, ascribing this to concentration dependence of diffusion rates. These workers observed no such effect with a high molecular weight dextran ($\bar{M}_w$ 500000), but more recently others[3] have reported an increase in V_e with increasing concentration (5–20 mg/ml) of this dextran on Sephadex G-200. This was attributed to increased void volume, resulting from gel shrinkage due in turn to rising osmotic pressure of the non-penetrating solute in the mobile phase.

The inverse effect, decrease of V_e with increasing sample concentration, is shown by α-chymotrypsin on Sephadex G-100; this is due[4] to an increasing tendency to dimerisation at higher concentrations.

Since current determinations of polysaccharide molecular weight distributions by gel chromatography on polyacrylamide gels[5,6] depend upon the measurement of elution volumes, which must have reproducible, and meaningful, values, investigation of concentration effects in such systems was considered imperative.

Experimental

With 1 *M* NaCl as eluent[7], the elution volumes of D-glucose and of dextrans* $\bar{M}_w$ 500000 and 10000 on columns of the polyacrylamide gels Bio-Gel P-300 and P-10* *, and of a dextran* $\bar{M}_w$ 70000 on Bio-Gel P-300 only, were determined at various sample concentrations (2–20 mg/ml), the sample volume being 1 ml in all cases. 1-ml fractions were collected and assayed for carbohydrate[8].

The flow rate, maintained by hydrostatic pressure, was 16 ml/h/cm² in the case of the 60 × 1.2 cm Bio-Gel P-10 column, while a similar column of Bio-Gel P-300 gave 3 ml/h/cm² initially, decreasing to less than 1 ml/h/cm². When this occurred this column was replaced by one of dimensions 90 × 1.5 cm, in which flow was maintained at 3 ml/h/cm² by a peristaltic pump.

Chromatography was conducted at a constant temperature of 28 ± 0.5°.

Results and discussion

The elution volumes obtained at different sample concentrations are shown in Table I. For the dextrans $\bar{M}_w$ 10000 and 70000 values of the gel chromatographic distribution coefficient, K_d, are also tabulated. This is defined

$$K_d = \frac{V_e - V_0}{V_t}$$

where

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TABLE I

EFFECT OF SAMPLE CONCENTRATION ON V_e AND K_d

Solute	*Gel*	*Column dimensions (cm)*	*Sample concentration (mg/ml)*	V_e *(ml)*	K_d
D-Glucose	Bio-Gel P-300	60 × 1.2	1.9	70.1	
			19.6	70.1	
	Bio-Gel P-10	60 × 1.2	1.6	62.7	
			16.5	64.1	
Dextran $\bar{M}_w$ 500000	Bio-Gel P-300	60 × 1.2	2.1	18.9	
			18.6	18.9	
	Bio-Gel P-10	60 × 1.2	1.8	20.7	
			18.1	20.7	
Dextran $\bar{M}_w$ 10000	Bio-Gel P-300	90 × 1.5	3.8	100.0	0.64*
			8.5	100.4	0.64*
			19.9	100.6	0.64*
	Bio-Gel P-10	60 × 1.2	1.6	30.5	0.23
			10.3	31.9	0.26
			18.7	33.7	0.30
Dextran $\bar{M}_w$ 70000	Bio-Gel P-300	60 × 1.2	2.0	35.2	0.32
			10.1	35.5	0.32
			18.5	35.6	0.32

* Values of V_0 and V_t (28 and 113 ml, respectively) obtained in a previous calibration of this column were used in calculating K_d in this case.

V_0 = void volume of column, given by elution volume of dextran $\bar{M}_w$ 500000 (completely excluded),

V_t = inner volume of column, given[9] by difference between elution volume of D-glucose (fully penetrating) and V_0.

In the case of Bio-Gel P-10 V_e for D-glucose was found to be concentration-dependent (see Table I); the value at the appropriate concentration, estimated by interpolation, was used in calculating K_d.

With both gels the elution volume of the dextran $\bar{M}_w$ 500000 is independent of sample concentration over the range covered. At these solute concentrations, therefore, osmotic shrinkage has no appreciable effect in the case of polyacrylamide gels.

The elution volume of D-glucose on Bio-Gel P-300 is also concentration-independent over this range. The observed slight increases in V_e for the partially excluded dextrans $\bar{M}_w$ 10000 and 70000 with increasing concentration are within the degree of uncertainty (0.5 ml) of the elution volume measurements in most cases and therefore cannot be regarded as significant. The V_e and K_d values of polysaccharides on Bio-Gel P-300 can thus be considered to be independent of sample concentration. This is further exemplified by the striking reproducibility of polysaccharide elution patterns obtained on this gel, irrespective of varying sample concentration (Fig. 1).

In contrast, marked concentration dependence of V_e and K_d for the dextran $\bar{M}_w$ 10000 is observed with Bio-Gel P-10. The observed 10% increase in V_e would correspond to a decrease of the order of 20% in an estimated molecular weight value in this region. Sample concentration is thus clearly an important factor in such determinations on this gel. Molecular weight values obtained will be meaningful only if the concentrations of test substance and calibrating solutes are the same.

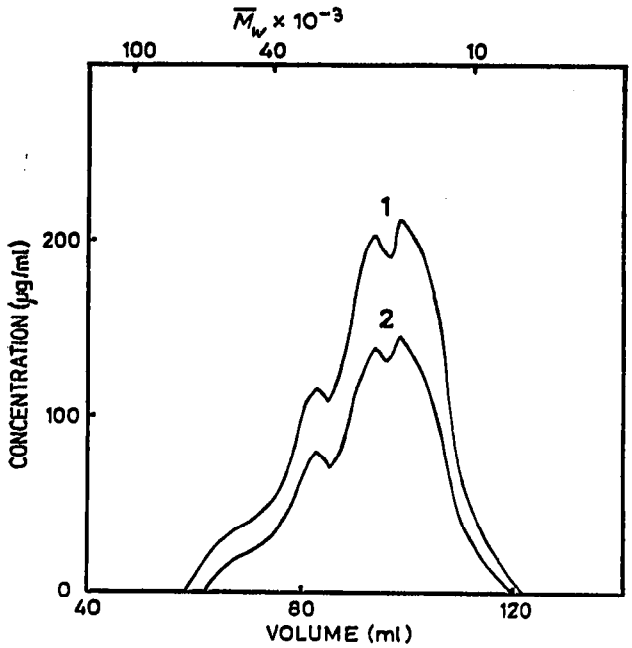

Fig. 1. Elution pattern on Bio-Gel P-300 of gum polysaccharide from *Acacia elata*. Column, 90 × 1.5 cm; eluent, 1 *M* NaCl; flow rate, 3 ml/h/cm². Sample concentrations: (1) 5 mg/ml, (2) 3 mg/ml. Molecular weight scale from calibration with dextrans of known $\overline{M}_w$.

According to ACKERS[10] adsorption and other solute–matrix interactions are probably enhanced in gels of low porosity, such as Bio-Gel P-10, resulting in increased diffusional restriction. With increasing concentration greater crowding of molecules within the gel pores may be expected to restrict diffusion further, delaying elution. The absence of appreciable concentration effects in the case of Bio-Gel P-300 may be ascribed to the relatively low degree of diffusional restriction in this highly porous gel.

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