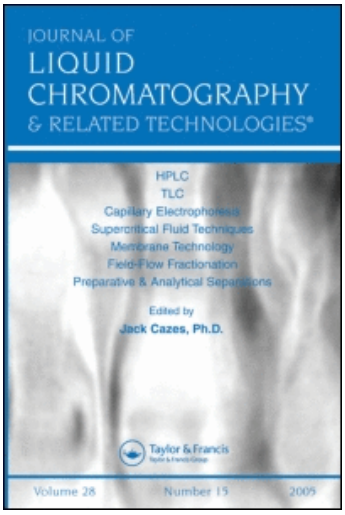

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Cooper, Anthony R. and Matzinger, David P.(1978) 'Characterization and Properties of Macromolecules. Part X. High Speed Aqueous Gel Permeation Chromatography Using Hydrogel Column Packing', Journal of Liquid Chromatography & Related Technologies, 1: 6, 745 — 759
To link to this Article: DOI: 10.1080/01483917808060031
URL: http://dx.doi.org/10.1080/01483917808060031

JOURNAL OF LIQUID CHROMATOGRAPHY, 1(6), 745-759 (1978)

CHARACTERIZATION AND PROPERTIES OF MACROMOLECULES. PART X. HIGH SPEED AQUEOUS GEL PERMEATION CHROMATOGRAPHY USING HYDROGEL COLUMN PACKING

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ABSTRACT

Hydrogel, a commercially available column packing, has been evaluated for high speed aqueous gel permeation chromatography. The commercial material designated as < 44μ particle size was classified into narrow particle size ranges using a sonic sifter. The pore volume was found to be independent of particle size. Efficiency was not found to be dependent upon particle size when these classified materials were dry packed. The application of Hydrogel to determine molecular weight distribution of a variety of water-soluble polymers was examined.

INTRODUCTION

Aqueous gel permeation chromatography (GPC) has been widely practiced for almost 20 years using organic hydrophilic gels *(1)*. These materials are capable of excellent resolution, but have a limitation of only being used at low operating pressures with concomitant long analysis times. Thus considerable effort has been expended on the design and development of rigid hydrophilic GPC column packings for faster analyses. These have been reviewed by us recently *(2,3)*, and show that progress has been made towards faster, high resolution analyses. We report here on our

investigations with Hydrogel, a polyethylene glycol dimethacrylate material, commercially available from Waters Associates *(4)*. A sonic sifter *(5)* has been employed as a method of sieving Hydrogel particles. The chromatographic properties of the sieved materials have been evaluated using aqueous and aqueous salt solvents.

EXPERIMENTAL

Chromatography

The gel permeation chromatograph employed a Miltron-Roy 5000 psi pump, a high pressure injection valve (Chromatronix HPSV-20) and a Waters Associates differential refractometer (Model No. R403) or a Varian Techtron spectrophotometer (Model No. 635) equipped with ISCO high pressure flow cells (19 μℓ internal volume).

The chromatographic conditions were as follows:

Column length	61 cm (2 ft)
i.d.	0.386 cm (0.152 in.)
o.d.	0.635 cm (0.25 in.)
Injection volume	0.2 ml
Injection concentration	1.0 mg/ml
Flow rate	1.0 ml/min

Columns were packed with dry packing material by vibrating the column for about 15 hours. The columns were then purged for 24 hours with solvent before use.

Particle Classification

Hydrogel was purchased with particle size < 44μ. These materials were then classified into narrower particle size distributions employing a sonic sifter with electroformed screens having hole diameters of 40, 30, 20 and 10μ. For efficient classification, we found that a maximum load of 2 g of material should be sieved. Additionally, only one sieve should be used, rather than several in cascade. The material on the sieve is agitated sonically to prevent agglomeration.

RESULTS AND DISCUSSION

Particle Classification

Hydrogel is available in three molecular weight separating ranges, which are coded II, IV and VI. Each of these materials, which were purchased as < 44μ size, was classified by sieving, and this allowed a mass distribution to be obtained in the following particle size ranges: 40-30μ, 30-20μ and 20-10μ. Typical results for Hydrogel IV are shown in Figure 1. Some retention of smaller particles on the coarser sieves is evident. The particles are mainly spherical, but a few irregularly-shaped particles are present. In general, the sieving was satisfactory for the purposes of investigating the chromatographic behavior of these materials as a function of particle size. Histograms of the weight percent of particles in each range as a function of the arithmetic mean particle size of that range are shown in Figure 2 for Hydrogel II, IV and VI. The particle size distributions of these materials are very similar.

Gel Permeation Chromatography

One column was packed with each type: II, IV and VI, of non-sieved Hydrogel. These were coupled in series, and calibrated with dextran *(6)* having the following molecular weights: 2×10^6, 5×10^5, 40,000 and 10,000. Glucose was used as an indicator of the total liquid volume. This calibration curve is shown in Figure 3. The column inlet pressure was 2500 psig at a flow rate of 1 ml/min for this three-column set. Several solvents were used, *viz.*, water, 0.1 and 0.01M sodium chloride and 0.01M phosphate buffer pH 7. The calibration curves for all these solvents are very similar, and show that solvent ionic strength has no significant effect on these packing materials. The separating range is from about 1×10^6 to ~500. A polyacrylamide sample having a molecular weight 5×10^6 confirmed that the 2-million molecular weight dextran was eluting at the void volume. Analysis time is about 15 minutes for this column set.

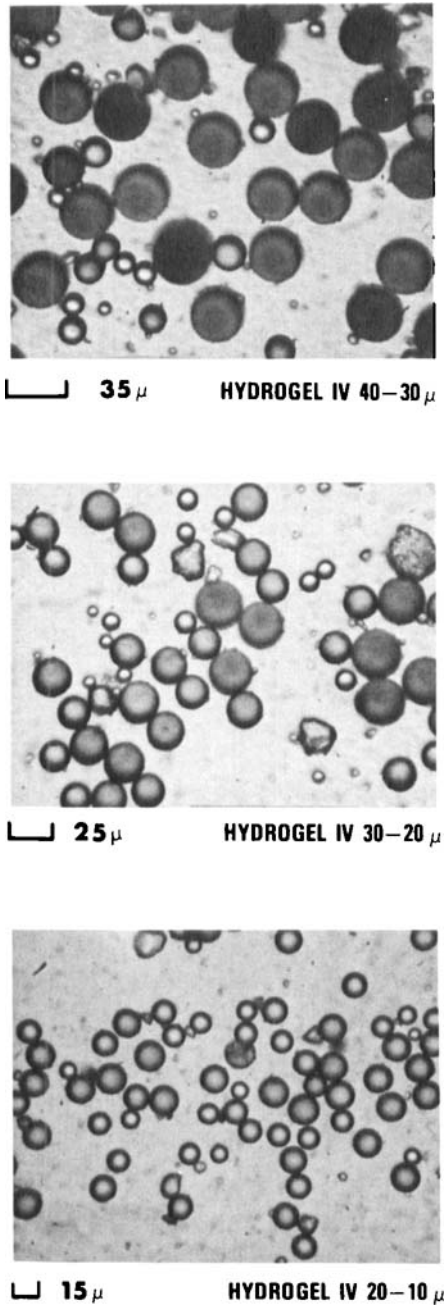

Figure 1 Photomicrographs of Classified Hydrogel IV Particles

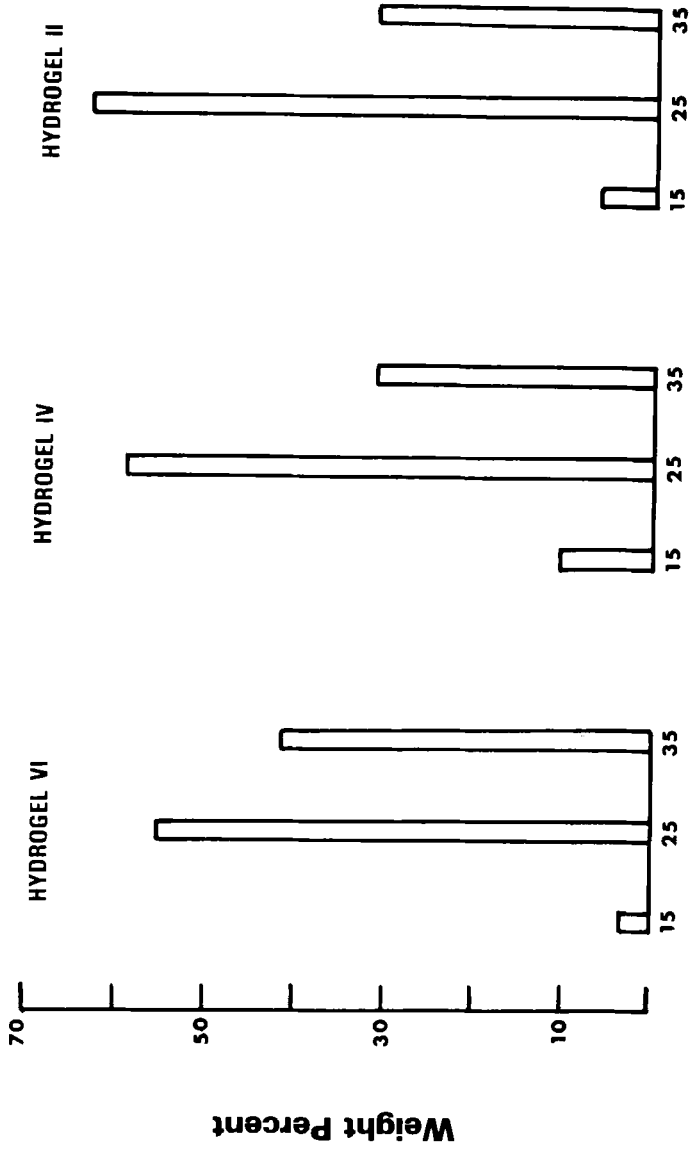

Figure 2. Particle Size Distributions of Hydrogel Samples. Ordinate: Weight Percent; Abscissa: Particle Diameter μ.

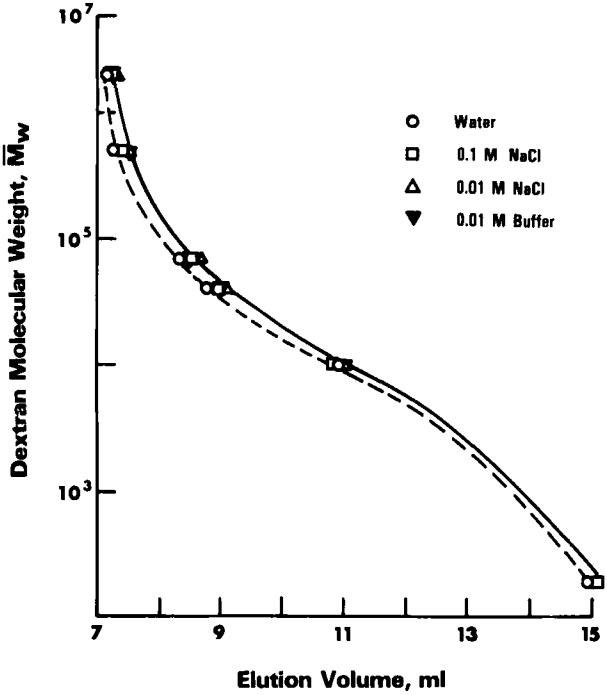

Figure 3 Calibration Curves for Hydrogel II, IV and VI Column Set Using Dextrans with Various Solvents

Ordinate	Dextran Molecular Weight, $\bar{M}_W$
Abscissa	Elution Volume, ml
Key	○ Water
	□ 0.1M NaCl
	△ 0.01M NaCl
	▼ 0.01M Phosphate Buffer

In order to investigate the effect of particle size on chromatographic properties, Hydrogel IV and Hydrogel VI columns were packed with classified particles in the 40-30μ and 30-20μ particle size ranges, and their performance was compared with the unmeshed material, < 44μ. There was insufficient material in the 20-10μ particle size range to pack columns. The calibration curves

for Hydrogel IV and Hydrogel VI as a function of particle size and particle size distribution using water solvent are shown in Figures 4 and 5. The calibration curve for Hydrogel IV, Figure 5, shows a fairly narrow separating range, but, because of the lack of availability of polymer molecular weight standards, it is difficult to

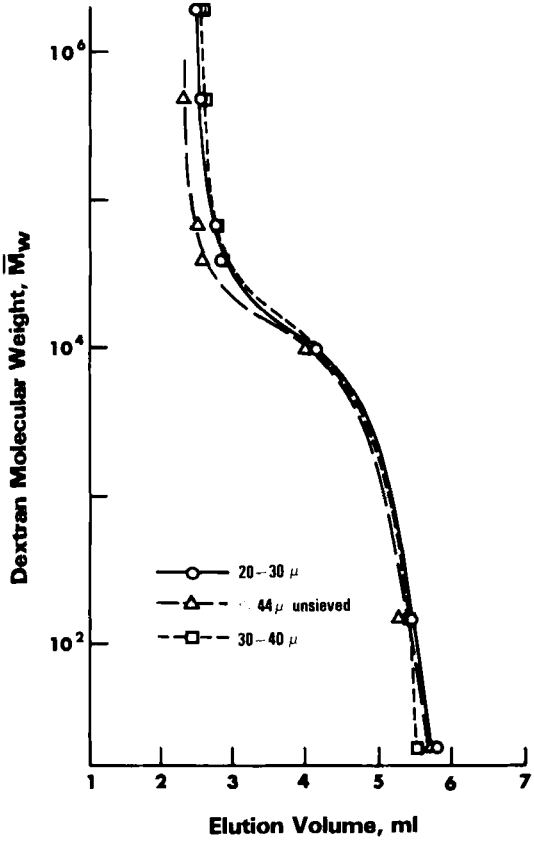

Figure 4 Dextran Calibration Curves for Hydrogel IV Columns with Distilled Water Solvent

Ordinate	Dextran Molecular Weight, $\bar{M}_W$
Abscissa	Elution Volume, ml
Key	O—20-30μ
	△—<44μ unsieved
	□---30-40μ

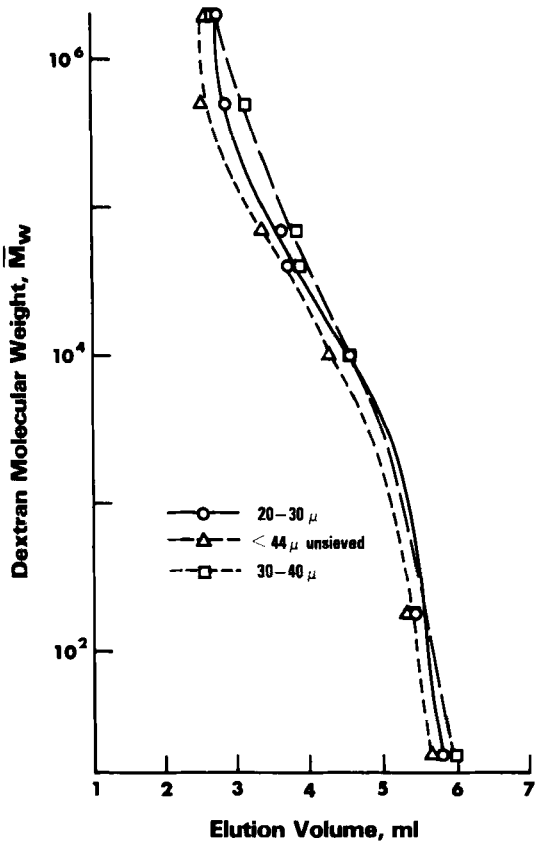

Figure 5 Dextran Calibration Curves for Hydrogel VI Columns with Distilled Water Solvent

Ordinate	Dextran Molecular Weight, $\overline{M}_W$
Abscissa	Elution Volume, ml
Key	○——20-30μ
	△— —<44μ unsieved
	□----30-40μ

assign. The upper molecular weight separating limit is 40,000, and the pore volume, as reflected by the calibration curves, is independent of particle size. Hydrogel VI has an extremely wide separating range, and the pore volume, as measured by the difference between the void volume and total liquid volume, is again independent of particle size.

Typical chromatograms are shown in Figure 6 for the 10,000 molecular weight dextran and Hydrogel IV columns. This polymer elutes in the middle of the separating range, and is therefore resolved. The column packed with unmeshed material is slightly more efficient, as evidenced by the narrower chromatogram. For the classified particles, the chromatogram broadens somewhat as the particle size is reduced. The chromatograms for the 40,000 molecular weight dextran and the Hydrogel VI columns are shown in Figure 7. The chromatograms are quite similar; however, in this case, there is a trend towards higher efficiency with decreasing particle size and particle size distribution. The dry packing technique for particles in this size range is reproducible, but does not produce higher-efficiency columns at reduced particle sizes which other packing methods, *e.g.*, slurry packing or balanced density, have been shown to do. For our columns, the pressure drop across the column was lower for the classified particles than the unmeshed packing material.

Efficiency of chromatographic systems may be expressed in many ways. To characterize these columns, we have used the number of theoretical plates per foot, n, the Resolution, R, and the Resolution Index, RI, defined by

$$n = \frac{1}{L}\left(\frac{V_e}{W_b/4}\right)^2$$

$$R = 2\,\frac{(V_{e1}-V_{e2})}{(W_{b1}+W_{b2})}$$

$$RI = \left[\frac{M_{W1}}{M_{W2}}\right]^{1/R}$$

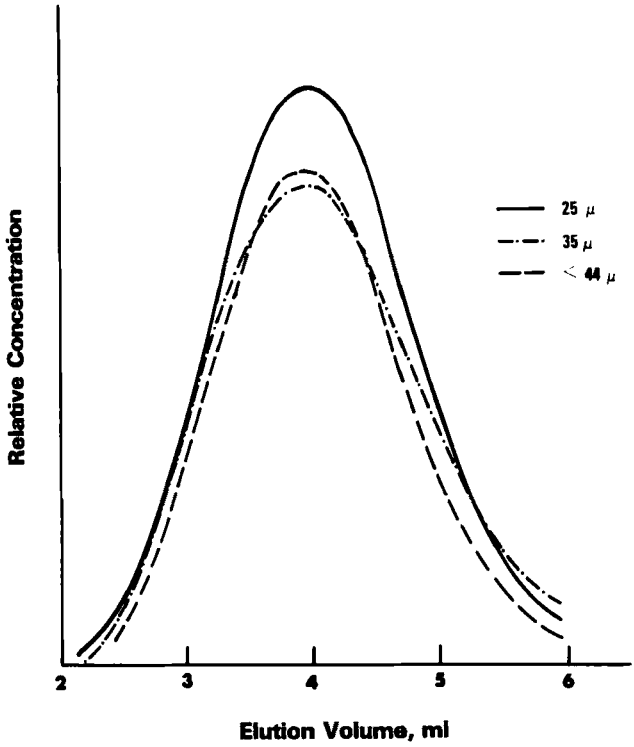

Figure 6 Chromatograms of a 10,000 Molecular Weight Dextran Using Hydrogel IV Columns

Ordinate	Relative Concentration
Abscissa	Elution Volume, ml
Key	——— 25μ
	— — 35μ
	----- <44μ

where L is the column length in feet,
V_e is the elution volume,
W_b is the peak base width,
M_W is the solute molecular weight.

The number of theoretical plates for small molecules has been used to compare the quality of column packing. Resolution determines

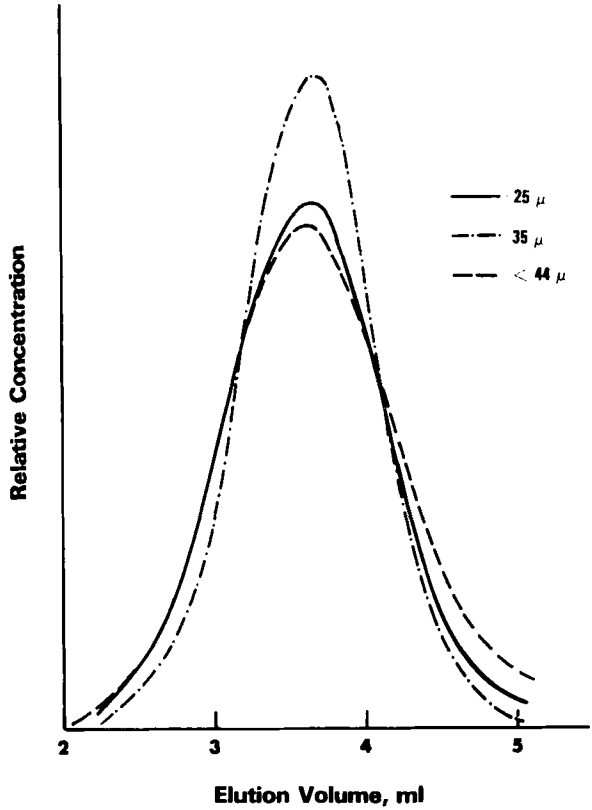

Figure 7 Chromatograms of a 40,000 Molecular Weight Dextran Using Hydrogel VI Columns

Ordinate	Relative Construction
Abscissa	Elution Volume, ml
Key	——— 25μ
	— — 35μ
	----- <44μ

that two polymers are baseline separated if R > 1. Resolution Index has been proposed to give a more meaningful efficiency parameter when the molecular weight axis is a logarithmic function of elution volume.

These values are shown in Table 1. The plate count for the two small molecules used, glucose and D_2O, are sometimes different, reflecting the fact that D_2O has a larger elution volume. For polymeric solutes at high molecular weight, the number of theoretical plates is insensitive to particle size or its distribution. As the polymer molecular weight is reduced, the number of theoretical plates increases in the order 40-30μ < 30-20μ < unmeshed packing. Resolution and Resolution Index for the polymer pair Dextran T40-T10 and Dextran T10-glucose show that the unmeshed packing material gave the best separation.

Table 1

EFFICIENCY OF GPC SEPARATIONS USING HYDROGEL COLUMN PACKINGS

Code	Dextran Molecular Weight	Number of Theoretical Plates/Ft., n					
		Hydrogel VI			Hydrogel IV		
		40-30μ	30-20μ	Unmeshed <44μ	40-30μ	30-20μ	Unmeshed <44μ
T70	70,000	23	24	26	44	37	42
T40	40,000	22	26	36	28	23	55
T10	10,000	37	44	63	11	14	20
Glucose		91	57	187	70	60	77
D_2O		147	118	269	126	76	129
		Resolution, R					
Solute Pair							
T40-T10		0.30	0.43	0.35	0.48	0.55	0.77
T10-glucose		0.50	0.44	0.80	0.50	0.51	0.66
		Resolution Index, RI					
T40-T10		0.010	0.041	0.030	0.056	0.081	0.167
T10-glucose		0.0003	0.0001	0.0067	0.0003	0.0004	0.0022

For the characterization of polyelectrolytes, it is necessary to add ionic solutes to the solvent to suppress polyelectrolyte expansion and prevent ion exclusion and inclusion *(7)*. Several solvent systems were investigated in order to chromatograph blue dextran and polymeric azo dyes *(8,9)*. These results are summarized in Table II. With water solvent, dextrans, blue dextran, polyacrylamide and glucose eluted normally. Low molecular weight polymeric azo dyes eluted at the column void volume and polyethylene oxides were absorbed. The use of 0.1N NaCl, 0.01N NaCl, and 0.01M phosphate buffer pH 7 solvents allowed dextrans to be chromatographed normally, but, in all cases, blue dextran and polymeric azo dyes were

Table 2

SUMMARY OF SOLVENT SYSTEMS USED WITH HYDROGEL COLUMNS

SOLVENT	SOLUTE	GPC ELUTION BEHAVIOR			
		NORMAL	AT VOID VOLUME	AFTER TOTAL LIQUID VOLUME	ADSORBED
WATER	DEXTRANS	X			
	BLUE DEXTRAN	X			
	POLYMERIC AZO DYES		X		
	POLYETHYLENE OXIDES				X
	POLYACRYLAMIDE	X			
	GLUCOSE	X			
	METHANOL			X (K_d = 1.43)	
	ETHANOL			X (K_d = 1.95)	
	ACETONE			X (K_d = 3.47)	
0.1N NaCl	DEXTRANS	X			
	BLUE DEXTRAN				X
	POLYMERIC AZO DYES				X
0.01N NaCl	DEXTRANS	X			
	BLUE DEXTRAN				X
	POLYMERIC AZO DYES				X
	POLYACRYLAMIDE	X			
	POLYVINYL PYRROLIDONE				X
0.01M PO_4, pH 7	DEXTRANS	X			
	BLUE DEXTRAN				X
	POLYMERIC AZO DYES				X
0.01M PO_4, pH 7+1% MeOH	DEXTRANS	X			
	BLUE DEXTRAN				X
	POLYMERIC AZO DYES				X
5% METHANOL IN WATER	DEXTRANS	X			
	BLUE DEXTRAN	X			
	POLYMERIC AZO DYES		X		
	GLUCOSE	X			

totally adsorbed. From the elution volumes of methanol, ethanol and acetone with water solvent, it is evident that these compounds have a strong interaction with the Hydrogel surface. Thus we tried to modify the adsorptive properties of Hydrogel by the addition of 1% methanol to the 0.01M phosphate buffer pH 7. Dextrans eluted normally in this solvent, but again the blue dextran and polymeric azo dyes were totally adsorbed. Employing a 5% methanol in water solvent allowed dextrans, blue dextran and glucose to be chromatographed, but low molecular weight polymeric azo dyes eluted at the void volume in the absence of an electrolyte.

The elution behavior of tartrazine and Sunset Yellow was found to be extremely unpredictable. Using water solvent, tartrazine eluted at the void volume of the column when the injection concentration was 1.0, 0.1 or 0.01 mg/ml. Sunset Yellow injected at a concentration of 1.0 mg/ml eluted with a peak maximum at the total liquid volume of the column, but was extremely broad. Using 0.01M phosphate buffer pH 7 solvent, tartrazine and Sunset Yellow injected at 1 mg/ml concentration eluted long after the total liquid volume. These elution volumes were not effected by the addition of 1% methanol to the solvent. Using 5% methanol in water solvent, both tartrazine and Sunset Yellow eluted at the void volume when the injection concentration was 1 mg/ml.

CONCLUSIONS

Hydrogel column packing material has been classified into narrow particle size distributions, and the chromatographic behavior of dextrans shows that the pore size and pore volume is essentially independent of particle size. These materials are able to withstand high pressures and allow high speed analyses. By optimizing the packing technique, these materials should be useful for high resolution aqueous GPC. Dextrans and polyacrylamide elute normally with water and aqueous salt solutions, with or without methanol added. Polyvinylpyrrolidone and polyethylene oxides do not elute with water. Methanol, ethanol and acetone interact strongly with this

packing material, and as such should be suitable cosolvents when adsorption is encountered. Hydrogel does not appear to be applicable to the characterization of some polyelectrolytes because the required supporting electrolyte causes adsorption.

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