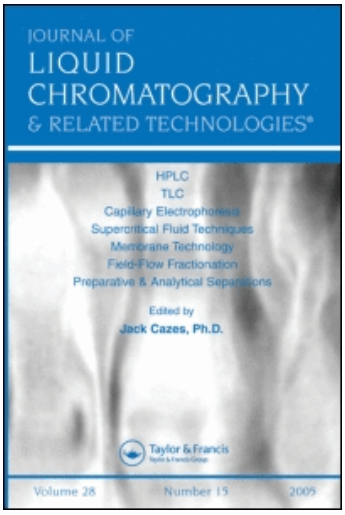

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Recent Advances in Aqueous Gel Permeation Chromatography

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RECENT ADVANCES IN AQUEOUS GEL PERMEATION CHROMATOGRAPHY*

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ABSTRACT

The recent literature on aqueous gel permeation chromatography is reviewed. The chromatography of charged and uncharged solutes has been considered. A major emphasis has been to document the newer types of column packings for use in aqueous systems and their typical applications.

INTRODUCTION

Sephadex *(1)*, a cross-linked dextran gel, became available in the early 1960's, and has been widely used for the characterization of biological materials. The uses *(2)* and applications *(3)* of Sephadex have been described. Several other packing materials formed from cross-linked polymers became available. Those based on polyacrylamide came into general use. These systems are capable of excellent resolution, as shown in Figures 1a, 1b. Here, glucose oligomers are separated, using a polyacrylamide gel, Bio-gel P2. Although the resolution is good, the analysis time of five to ten hours is undesirable. These

* Based on a transcript of a lecture delivered at the "Chromatography of Polymers Symposium," Chicago ACS Meeting, 8/77.

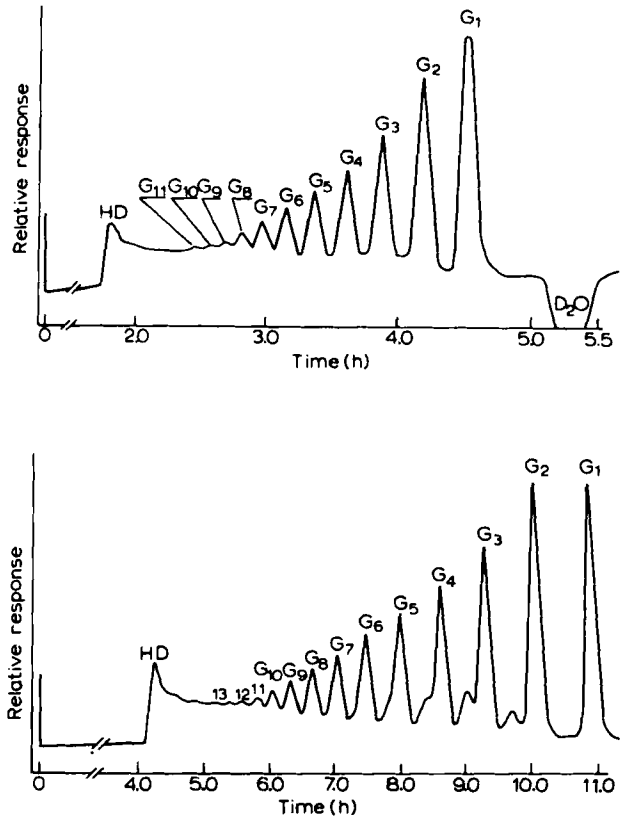

FIGURE 1a

Gel Chromatogram of Glucose Polymers on Bio-Gel P2 with a 1-m Column *(4)*

FIGURE 1b

Gel Chromatogram of Glucose Polymers on Bio-Gel P2 with Two 1-m Columns in Series *(4)*

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gels do not have good mechanical strength, and thus are unable to withstand the higher pressures necessary for faster analyses. Their volumes are also affected by changes in pH, ionic strength

and temperature. With the advent of new generations of instrumentation and a wider selection of column packings, the quest for faster analyses began.

This review summarizes the current theory of aqueous GPC, instrumentation, calibration and the newer column packing materials and their applications.

THEORY

Ideally, in GPC based on a steric exclusion mechanism, polymer solutes should elute between the column void volume (interstitial volume plus connecting tubing volume) and the total liquid volume (void volume plus the liquid volume contained in the pores). Exceptions to this situation are well known; for example, solute and packing materials interact, solutes elute later than the total liquid volume. On the other hand, solutes may associate, causing earlier elution than if they were molecularly dispersed. These exceptions can occur in aqueous and non-aqueous GPC systems. If ionic factors are present in aqueous GPC systems, they become important in determining elution volumes. Charges on the packing material, solute or solvent components all have to be considered.

Ion Exclusion

Small molecules which are capable of entering pores on a size basis may be excluded if similar charges are present on the column packing. When water is used as the eluent, the elution profile of a low molecular weight charged solute is highly dependent upon the amount injected *(5-7)*. Chromatograms demonstrating this effect are shown in Figure 2 *(7)*. The elution profiles are characterized by a skewed leading edge, which always starts at the column void volume, and a sharp tail. As the injected solute concentration is increased, the peak maximum is displaced to larger elution volumes. The peak elution vol-

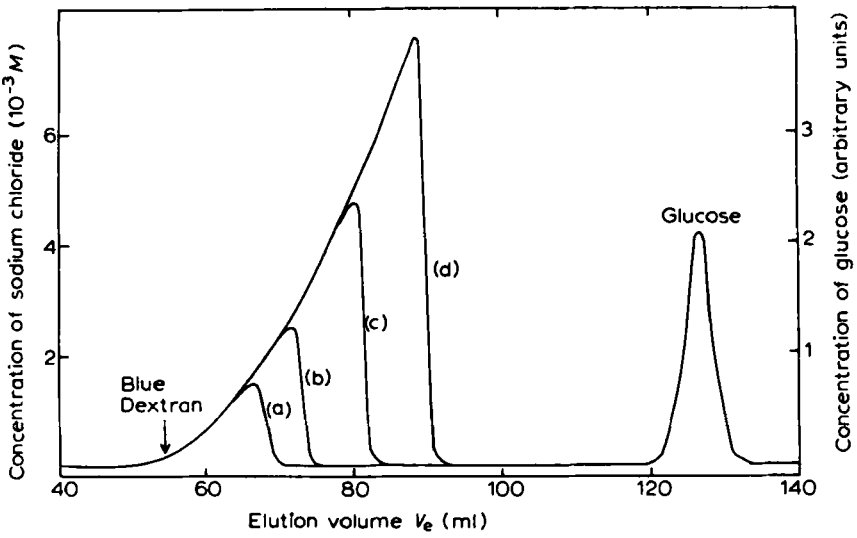

FIGURE 2

Elution Profile for NaCl on Enzacryl Gel K1 in Water at a Sample Concentration of (a) 0.01M, (b) 0.02M, (c) 0.05M, and (d) 0.1M *(7)*

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umes do not approach that of a small neutral solute, glucose for example.

Sephadex, which contains carboxylic acid groups at a concentration ~1% of a typical ion exchange resin, would demonstrate this type of behavior *(5)*. The packing material used to obtain the data of Figure 2 was considered to be essentially neutral, and additional exclusion mechanisms were postulated *(7)*.

Although the exact mechanism of this exclusion phenomenon is unknown, it is very easily eliminated by the addition of sufficient electrolyte to the solvent. Adding 0.01M electrolyte, having a small hydrodynamic volume and a common ion with that of ionic solute overcame this problem *(5)*.

This effect has been exploited to separate low molecular weight species, such as amino acids, on the basis of net charge.

The separation shown in Figure 3 *(8)* was achieved by using an anionically derivatized Sephadex packing with low ionic strength solvent, 0.01M.

When polyelectrolytes are chromatographed in water with charged packing materials, they also will exhibit ion-exclusion. This is shown in Figure 4 *(9)*. In the absence of added electrolyte, Curve 1, the chromatogram is distorted by a low elution volume plateau. The high elution volume peak was trapped and re-injected successively four times (Curves 2-5). As the sample size decreased, the low molecular weight peak completely disappeared (Curve 5). Again, this effect can be eliminated by adding sufficient electrolyte to screen the charge on the packing material.

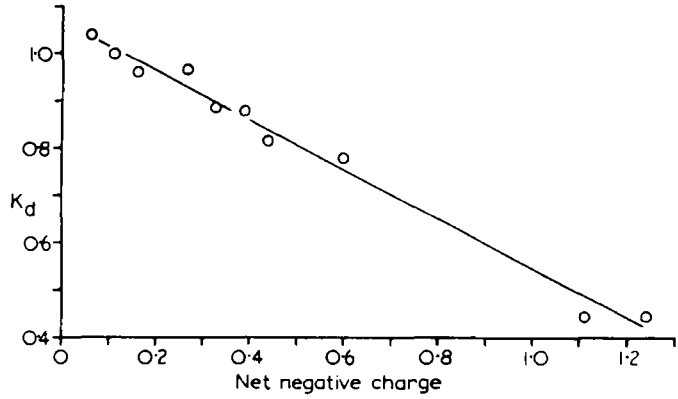

FIGURE 3

The Relationship between K_d and the Net Negative Charge on a Series of Amino Acids When Eluted from a CM-Sephadex C-50 Column at pH 9.0 in Borate Buffer. The amino acids from left to right are: β-alanine, α-alanine, leucine, tryptophan, methionine, serine, threonine, asparagine, aspartic acid and glutamic acid *(8)*

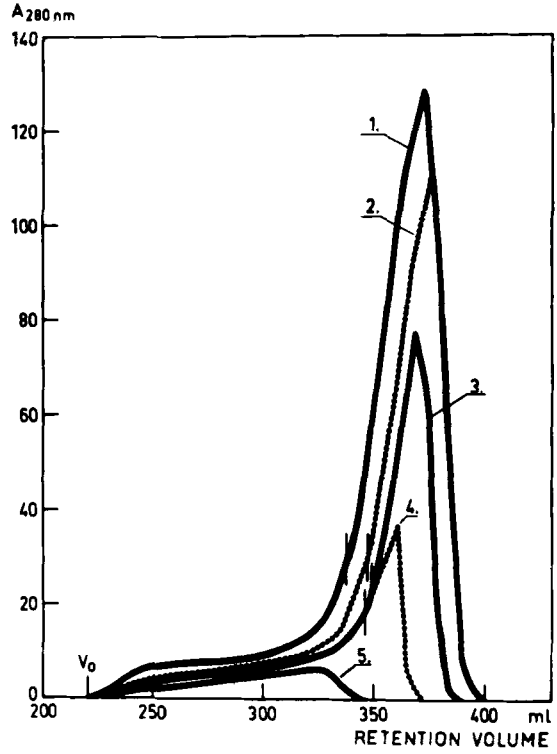

FIGURE 4

Influence of Sample Size on Ion Exclusion When a Low-MW Calcium Lignosulfonate Fraction is Eluted from Sephadex G-25. Flow Rate 16 ml/hr *(9)*

Ion Inclusion

An effect has been demonstrated *(9)* where a high molecular weight excluded polyelectrolyte causes a lower molecular weight polyelectrolyte to be retained longer than if it were eluted with water or a dilute solution of low molecular weight electrolyte. The mechanism is depicted in Figure 5 *(9)*. The excluded high molecular weight polyelectrolyte causes a Donnan equilib-

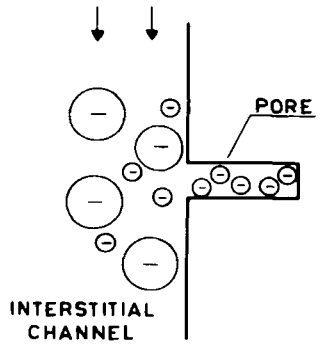

FIGURE 5

Inclusion of a Low-MW Polyelectrolyte Component into a Pore in a Gel (Schematic Drawing) *(9)*

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rium to be established, where the lower molecular weight polyelectrolyte has a higher concentration in the pore than in the interstitial volume solution. This delays the elution of the included species. This effect may also be eliminated by the presence of electrolyte added to the solvent, because the charges on the excluded polyelectrolyte are screened.

INSTRUMENTATION

Most modern instruments designed for high pressure liquid chromatography are also suitable for aqueous GPC. High pressure capability is often required with the advent of the newer microparticulate GPC packings. Stainless steel (316) columns, pumps, injection valves, fittings and connecting tubing have been in use in our laboratory for over three years, with no corrosion problems using dilute phosphate buffers. Occasionally, detectors may contain glass flow cells, which may dissolve in high pH buffers. Typically, syphons are used to measure elution volumes. These may produce erratic dump volumes with pure water as the

solvent. This may be overcome, using the device shown in Figure 6 *(10)*.

Detection is most often accomplished by differential refractometry or ultraviolet absorption. The specific refractive index increment $\frac{\partial n}{\partial c}$ is often favorable for organic polymers dissolved in water and buffers. The newly-developed laser light-scattering detector *(11)* should be particularly sensitive for aqueous GPC, since the response is proportional to $(\frac{\partial n}{\partial c})^2$.

CALIBRATION

Calibration is usually performed for each solute-solvent-column packing combination of interest by chromatographing narrow MWD fractions or a broad MWD sample of a polymer, or proteins. Commercial materials and sources are listed in Table 1.

The size of polyelectrolytes in solution may easily change by a factor of two by varying the ionic strength of the aqueous

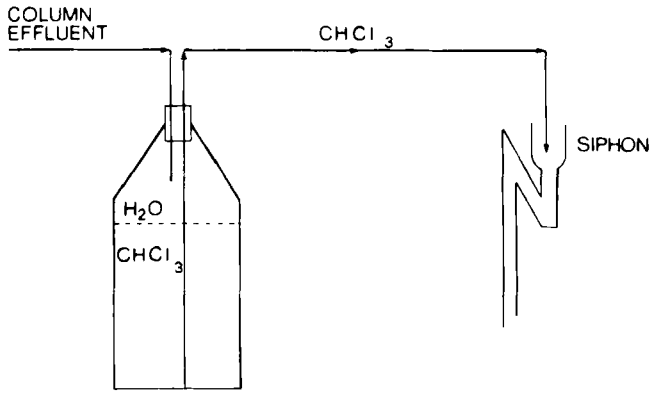

FIGURE 6

Modified Syphon Counter for Aqueous Gel Permeation Chromatography *(9)*.

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TABLE 1

Polymer Standards Suitable for Calibrating Aqueous GPC Columns

Polymer	$\bar{M}_w$	$\bar{M}_w/\bar{M}_n$	Supplier
Poly(styrene sulfonate)*	1,800	< 1.25	Pressure Chemical Co.
MW based on	4,600	< 1.10	(Pittsburgh, Pennsylvania)
Na salt	8,000	< 1.10	
	18,000	< 1.10	
	35,000	< 1.10	
	100,000	< 1.10	
	220,000	< 1.10	
	400,000	< 1.10	
	780,000	< 1.10	
	1,200,000	< 1.10	
Dextran	10,000	~1.6	Pharmacia Fine Chemicals
	40,000	~1.3	(Piscataway, New Jersey)
	70,000	~1.65	
	500,000	~2.9	
	2,000,000		
Poly(vinyl pyrrolidone)	10,000		Fluka A.G. (Switzerland)
	24,000		U.S. Tridom Chemicals,
	40,000		Hauppauge, New York
	360,000		
Poly(acryl-amide)	5.04×10^6	2.0	Professor A. E. Hamielec,
	3.24×10^6	2.0	Dept. Chemical Engineering
	5.83×10^6	2.43	McMaster University
			Hamilton, Ontario, Canada

*These may be synthesized by sulfonating the polystyrene standards also supplied by Pressure Chemical Co. See W. R. Carroll and M. Eisenberg, *J. Poly. Sci.*, 42, 4, 599 (1966), J. E. Sutherland Polymer Preprints 17, 434 (1976).

solvent. Thus, solvent ionic strength becomes very important when charged solutes or packing materials are used.

In order to simplify GPC calibration, the universal calibration approach has been pursued. In this method, the hydrodynamic volume, measured by the product of molecular weight (MW) and intrinsic viscosity $[\eta]$, is plotted against peak elution volume. If a steric exclusion mechanism is operative, all polymers,

whether linear or branched, etc., should fall on this universal calibration. It is extremely encouraging that the universal calibration has been verified for dextran and poly(styrene sulfonates), using aqueous salt solvent with porous glass packings *(12)*. The normal calibration curve, log MW *vs.* elution volume, is shown in Figure 7, using 0.2M Na_2SO_4. The curves are coincident at low MW, but diverge at higher MW. When the universal calibration plot is performed (Figure 8), at both 0.2M and 0.8M Na_2SO_4, all the data fall on a single curve. This demonstrates that all charge effects have been suppressed, and a molecular size separation has been achieved.

NEWER COLUMN PACKING MATERIALS AND THEIR APPLICATIONS

Porous Glasses or Silicas

These materials offer excellent mechanical and physical properties for use as GPC packings. Table 2 lists some pertinent properties for a variety of commercial materials *(13)*. A recent review article *(14)* also discusses suitable materials. Generally, for GPC, the desirable properties are availability in a range of pore sizes, a large pore volume, and small particle size. All porous silicas or glass should be useful as aqueous GPC packings unless a surface modification, such as chemical coupling or heat treatment, has been performed which renders them hydrophobic. The effects of heat treatment may be reversed by hydrolysis.

Neutral synthetic polymers, such as dextran *(15)* or polyacrylamide *(16)*, can be successfully chromatographed, using water as solvent. Anionic polyelectrolytes, poly(styrene sulfonates) *(12,17)* and poly(acrylates) have been analyzed by GPC, using simple electrolytes to prevent ion exclusion, ion inclusion, and polyelectrolyte expansion.

The selection of solvent ionic strength is most important in aqueous GPC with porous inorganic packings. We have been using a four-column set, packed with 2000 Å, 700 Å, 240 Å, and 75 Å

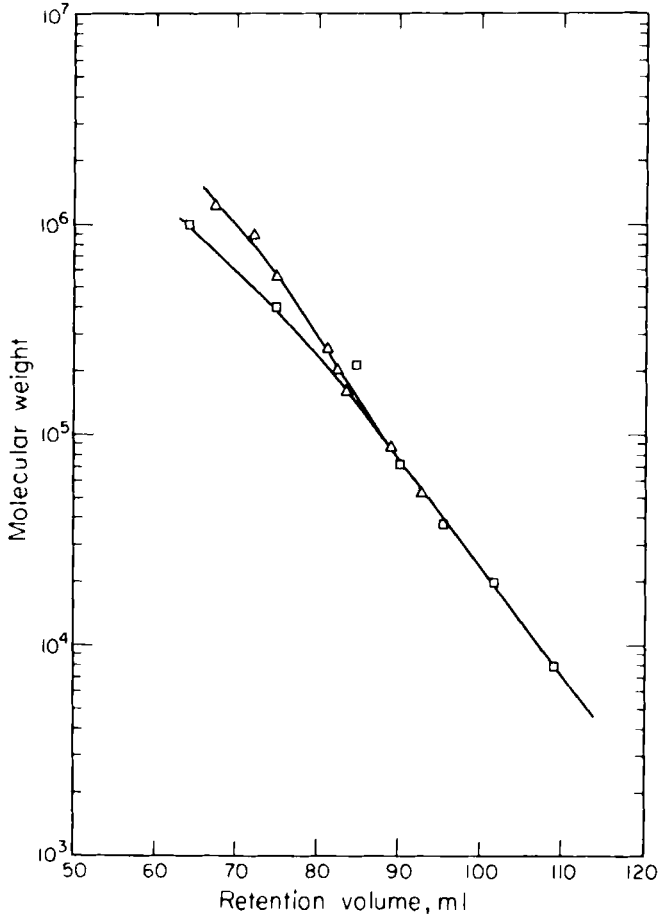

FIGURE 7

Semilogarithmic Calibration Curves of $\bar{M}_w$ *vs.* Elution Volume for Sodium (Polystyrenesulfonate) (□) and Dextran (△) Samples in 0.2M Sodium Sulfate *(12)*.

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Corning porous glass. At low ionic strength (0.05M phosphate buffer, pH 7), two distinct calibration curves are obtained for dextrans and poly(styrene sulfonates) (NaPSS); see Figure 9.

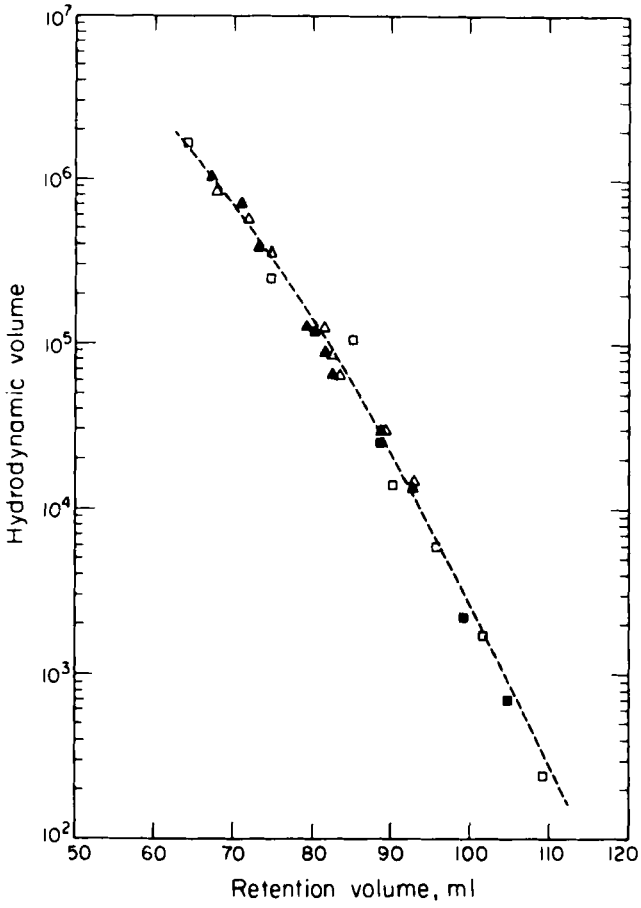

FIGURE 8

Hydrodynamic Volume Calibration Curves *vs.* Elution Volume for the Hydrophilic Polymers Studied at Two Salt Concentrations.
0.2M sodium sulfate, dashed line, □ - sodium polystyrenesulfonate, △ - dextran
0.8M sodium sulfate, ■ - sodium polystyrenesulfonate, ▲ - dextran *(12)*.

TABLE 2
PHYSICAL PROPERTIES OF TEN SILICA GELS (13)

Adsorbent	*Average particle size (μm)*	*Surface area (m^2/g)*	*Shape**	*Mean pore diameter*** *(Å)*	*Pore volume**** *(ml/g)*	*MW exclusion limit‡*	*Permeation range MW‡‡*	*Bulk density (dry) (g/ml)*	*Bulk density (liquid) (g/ml)*	*Mass of silica gel in column (g)*	*N (benzene)‡‡‡*	*Peak capacity*
Partisil 10	10	400+	I	40–50	0.661	$3.40 \cdot 10^4$	140– 34,000	0.465	0.220	3.33	10,000	10
LiChrosorb 10	10	200+	I	60	0.631	$1.90 \cdot 10^4$	200– 19,000	0.465	0.213	3.12	10,100	9
Silarex II	10	300	I	130	0.894	$3.00 \cdot 10^4$	230– 30,000	0.382	0.201	2.83	19,600	15
Spherosil 10	10	200+	S	–	1.040	$1.35 \cdot 10^5$	520–135,000	0.387	0.295	2.65	5,064	8
Biosil A	20–44	200+	I	<100	0.550	$1.10 \cdot 10^4$	190– 11,000	0.497	0.359	3.07	3,734	5
Biosil HA	<44	200+	I	<100	0.520	$1.10 \cdot 10^4$	220– 10,000	0.519	0.372	3.10	8,002	7
Sil-LC	44	–	I	–	0.566	$8.40 \cdot 10^3$	190– 8,400	0.564	0.448	3.27	1,840	4
Porasil C	37–75	50–100	S	300	0.966	$1.80 \cdot 10^5$	1,000–180,000	0.404	0.377	2.65	1,799	5
Porasil A	37–75	350–500	S	<100	1.107	$2.30 \cdot 10^4$	180– 23,000	0.424	0.395	2.44	1,406	4
CPG-10	37–75	177	I	75	0.560	$1.96 \cdot 10^4$	240– 19,600	0.486	0.411	2.91	2,326	3

* I = irregular, S = spherical.
** Given by the manufacturer.
*** Exptl. determined as total pore volume/g of silica gel.
‡ Determined from exptl. data as the MW corresponding to 5% of the total pore volume.
‡‡ Determined from exptl. data as the MW range corresponding to 5–95% of the total pore volume.
‡‡‡ Calculated from exptl. data assuming the same peak width of benzene for all solutes in the permeation range.

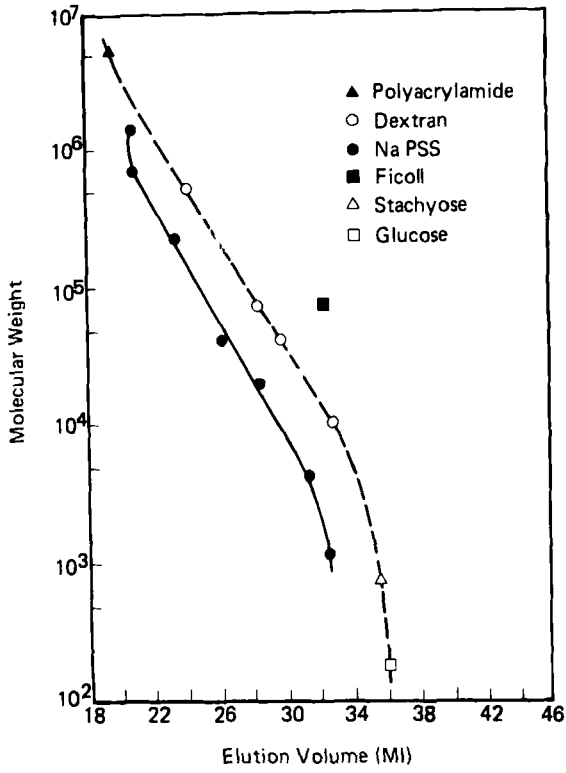

FIGURE 9

Calibration Curves for CPG-10 (2000, 700, 240, 75 Å) Column Set. Solvent: 0.05M phosphate pH 7 buffer. Sample concentration: 1 mg/ml. Flow rate: 1 ml/minute.

The flow rate was 1 ml/min, and the sample concentration was 1 mg/ml. The low molecular weight NaPSS elutes at a smaller elution volume than would be predicted on the basis of molecular size. This is attributed to ion exclusion of the charged solute by the charged surface. It is obvious that the universal calibration approach does not hold here. Spatorico has demonstrated *(12)* that the universal calibration is valid for these dextrans and NaPSS, using 0.2M Na_2SO_4 with these packing materials. We therefore chose to work with a phosphate buffer at the same

ionic strength, *viz.*, 0.27M, and repeated the calibration process. The results shown in Figure 10 show that there is a dramatic shift of the lower molecular weight NaPSS samples to higher elution volumes. The lower part of the dextran and NaPSS elution curves is now coincident. The general nature of the two curves is similar to that obtained by Spatorico (see Figure 7).

Although we have not measured intrinsic viscosities in this solvent, it is probable that the universal calibration approach is valid at 0.27M phosphate buffer, pH 7. At a given molecular

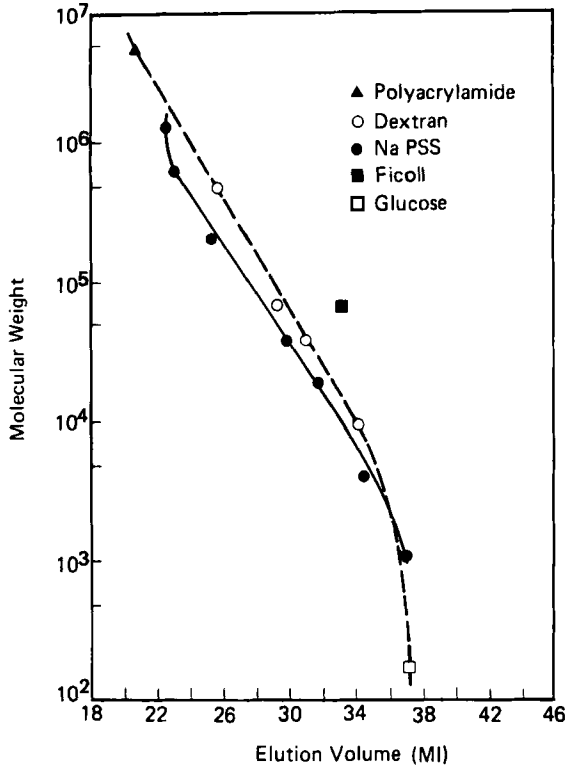

FIGURE 10

Calibration Curves for CPG-10 (2000, 700, 240, 75 Å) Column Set. Solvent: 0.27M phosphate pH 7 buffer. Sample concentration: 1 mg/ml. Flow rate: 1 ml/minute.

weight, dextrans elute later than NaPSS, particularly at higher molecular weights, because the former are branched.

We had performed a considerable amount of work at the lower phosphate buffer concentration 0.05M. We were interested to know if there was any dependence of elution volume on flow rate, sample concentration, or sample ionic strength when using a solvent where an ion exclusion effect was present. The calibration curves obtained at two flow rates (0.96 and 1.85 ml/min) are shown in Figure 11. No significant change in elution volume could be detected for either dextrans or NaPSS.

All previous calibrations were performed at 1 mg/ml, so concentrations above and below this, 2.5 and 0.25 mg/ml, were investigated. The results are shown in Figure 12. Generally in GPC, higher solute concentrations lead to larger elution volumes because polymer coil size decreases with increasing polymer concentration. This effect is seen in our system. However, the magnitude of the effect appears to be smaller for the charged solutes than for the neutral solutes. The effect of sample concentration becomes more important at higher molecular weight.

We are often involved with the analysis of polyelectrolytes containing significantly different amounts of simple inorganic salts. In these experiments, the polymer concentration was 1 mg/ml and the NaCl in the sample solution was varied from 0 to 6%. The calibration curves obtained are shown in Figure 13. For the polyelectrolyte, NaPSS, the elution volumes decrease with increasing sample ionic strength. The effect is relatively minor and does not eliminate ion exclusion. There is also an effect with dextrans; the magnitude of the effect seems to be larger than for NaPSS.

High adsorption of biological materials is a common problem with porous glass packings. An early solution to the problem was to coat the glass surface with polyethylene oxide (MW = 20,000). This treatment has allowed high recoveries of many materials which would otherwise have been irreversibly adsorbed on the bare glass

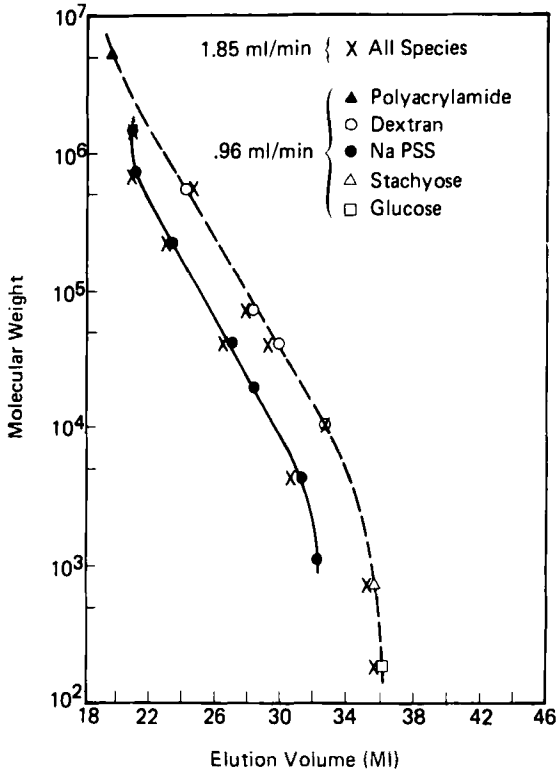

FIGURE 11

Effect of Flow Rate on Elution Volume for CPG-10 (2000, 700, 240, 75 Å) Column Set. Solvent: 0.05M phosphate pH 7 buffer. Sample concentration: 1 mg/ml.

surface. Porous glass having a pore diameter of 3000 Å and coated with polyethylene oxide has been used to fractionate casein micelles *(18)*. However, even with a poly(ethylene oxide) coating, porous glass demonstrates an ionic exclusion mechanism towards proteins up to a solvent ionic strength of 0.2 *(19)*. Unfortunately, the use of high ionic strengths caused the protein peaks to tail towards lower elution volumes.

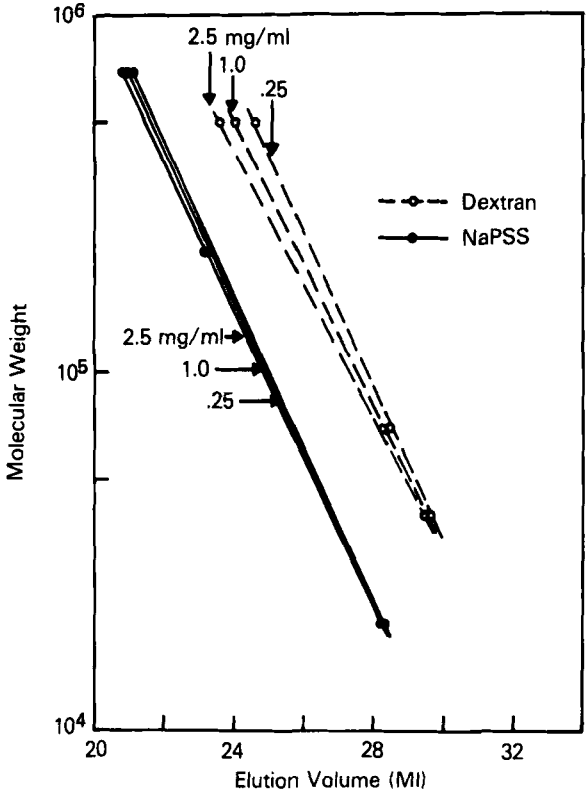

FIGURE 12

Effect of Polymer Concentration on Elution Volume for CPG-10 (2000, 700, 240, 75 Å) Column Set. Solvent: 0.05M phosphate pH 7 buffer. Flow rate: 1 ml/minute.

A different approach has been to modify the adsorptive properties of porous glass by covalently bonding a hydrophilic group such as glycerolpropylsilane onto the glass surface. This has been applied to small particle diameter glass to achieve high speed separations *(20)*. Figure 14 shows a protein separation on 100 Å glycophase G porous glass with a dilute buffer. The peaks appear to be symmetrical when chromatographed in the presence of dilute

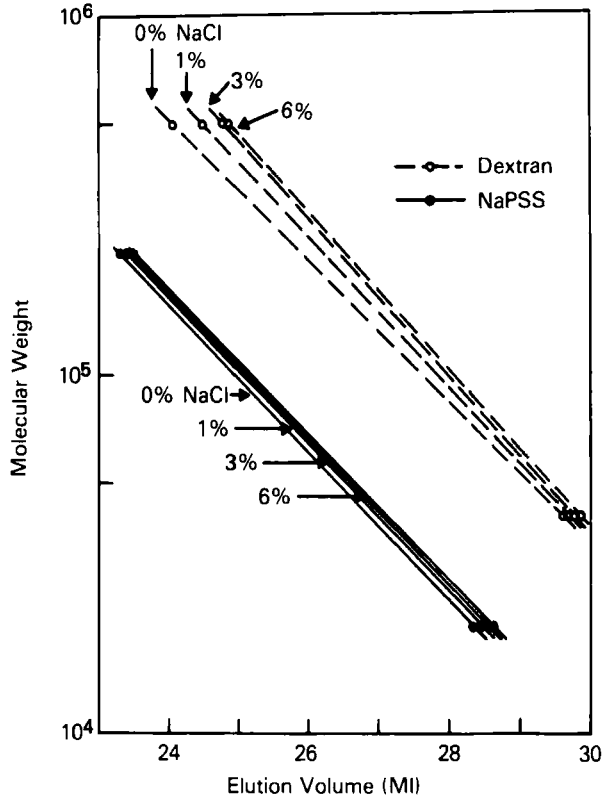

FIGURE 13

Effect of Sample Ionic Strength on Elution Volume for CPG-10 (2000, 700, 240, 75 Å) Column Set. Solvent: 0.05M phosphate pH 7 buffer. Flow rate: 1 ml/minute.

buffer. These packing materials have also been used to analyze human serum components, nucleic acids and dextrans *(21)*.

A comparative study of a covalently bonded glyceryl packing material and a polyethylene oxide-coated glass showed that the bonded material afforded much greater stability *(22)*. However, it was demonstrated that for polyvinyl alcohol, the covalently bonded

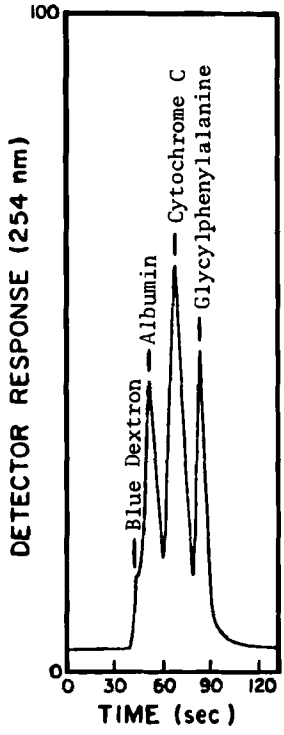

FIGURE 14

Steric Exclusion Chromatography of Proteins. Column, 300 x 4.1 mm I.D. stainless steel. Packing glycophase G/CPG (100 Å pore diameter, 5-10 μ particle size); temperature, 25°C. Solvent: 0.1M KH_2PO_4, pH 6; flow rate: 7 mm/sec; pressure 2700 psi *(20)*.

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material produced poorer resolution than the coated glass packing.

Evidence that polycations, which are notorious for adsorbing to porous glass, may be chromatographed from glyceryl bonded packing has been reported *(23)*. Chitosan which contains free amine groups may be eluted from this packing, using 2% acetic acid in water as solvent.

Other surface treatments have been applied to porous glasses or silicas, and have extended the usefulness of these materials. A proprietary surface treatment to produce deactivated Porasil *(24)* has been successfully applied to characterize polyvinyl alcohol with water as solvent. It has also been investigated for the characterization of maleic anhydride-divinyl ether copolymer *(25)*.

μBondagel E is a newer column packing which is silica-based and has an organosilane bonded surface. This material is useful for aqueous and non-aqueous applications *(26)*. Calibration curves for dextran in water as a function of pore size are shown in Figure 15 *(27)*.

Surface bonding of various functionalities on silica has been performed *(28)*. The structures are summarized in Table 3, together with the elution behavior of dextrans, polyethylene glycols and proteins in water. In this table, excluded means the polymer

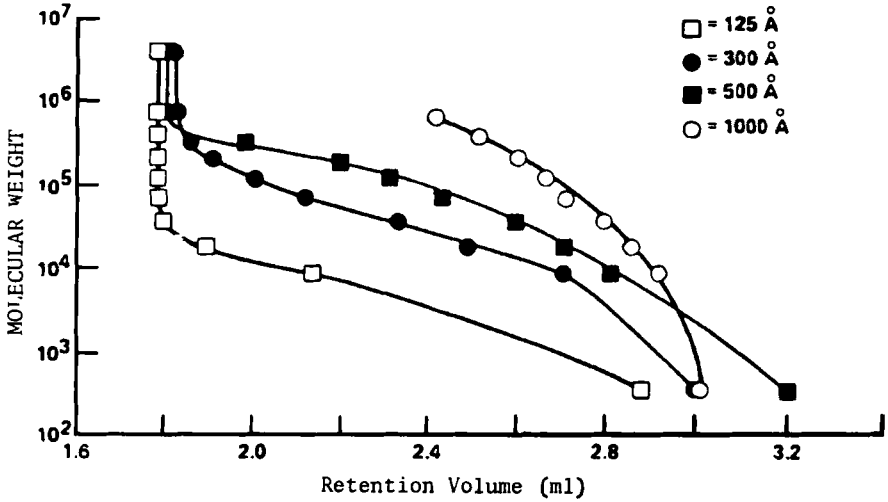

FIGURE 15

μ Bondagel Calibration by Dextran Standards *(27)*.

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Table 3

Exclusion Chromatography of Water-Soluble Polymers with Water as Eluent *(28)*

Stationary Phase	Structure	Elution Properties		
		Dextrans	Polyethylene Glycols	Proteins
Silica	$-OH$	Excluded	Strongly retarded; PEG 200, $k' \simeq 3$	—
RP-C_{18}	$-(CH_2)_{14}-CH_3$	Retarded	Strongly retarded; PEG 200, $k' > 6$	Strongly retarded
"Trifluoroamide"	$-NH-CO-CF_3$	Excluded	Retarded; PEG 200; $k' \simeq 0.2$	Strongly retarded
"Sulphonamide"	$-NH-SO_2-CH_3$	Excluded	Retarded; PEG 4000, $k' \simeq 0.5$	Retarded
"Glycol"	$-O-CH_2-CHOH-CH_2OH$	Excluded	Retarded; PEG 200, $k' \simeq 0.1$	Retarded and excluded
"Glycinamide"	$-NH-CO-CH_2-NH-CO-CH_3$	Excluded	Excluded	Weakly retarded
"Amide"	$-NH-CO-CH_3$	Excluded	Excluded	Excluded

elutes before the total liquid volume of the column. Dextrans are separated by size on all columns except the $-C_{18}$. Polyethylene glycols were eluted only satisfactorily on the "glycinamide" or amide columns. Proteins exhibited variable behavior, and should probably be chromatographed in a different solvent *(19-22)*.

Other approaches to preventing adsorption have been directed towards adding components to the solvent. Addition of such amino acids as glycine DL analine, or β analine to common phosphate, carbonate, borate, acetate or citrate buffers, at pH 8, has prevented adsorption of bovine serum albumin *(29)*.

A variation of this method has been proposed to separate proteins of similar molecular weight and charge *(30)*. The protein mixture is applied to the column and eluted with a stepwise or gradient elution with a series of buffers, *e.g.*, water, phosphate, tris hydrochloride and glycine buffers.

Other solvent additives for preventing protein adsorption which have been used are 7M urea and sodium dodecyl sulfate (SDS). Proteins having molecular weights between 17,000 and 385,000 could be chromatographed as SDS complexes using 500 Å pore diameter porous glass *(31)*. The solvent was 0.025M PO_4, 0.1% SDS, pH 7.0 (NaOH). It was found that by adding 6M urea to 0.5% SDS, 0.05M PO_4, pH 7 solvent and using a 123 Å pore diameter glass, the effective separating range was 3,500-12,000 *(32)*.

A master equation relating protein molecular weight to elution coefficient (k) has been reported *(33)*. This was obtained by chromatographing 13 protein SDS complexes with four different pore diameter porous glasses, *viz.*, 197 Å, 280 Å, 470 Å and 650 Å. These data are plotted in Figures 16a and 16b. The master equation is

$$(k-1.234)^2 + (y-1.234)^2 = 1.577,$$

where $y = (2.13M^{0.413}/p)^2$,

M is the molecular weight,

p is the pore diameter in Å,

k is the normalized peak position.

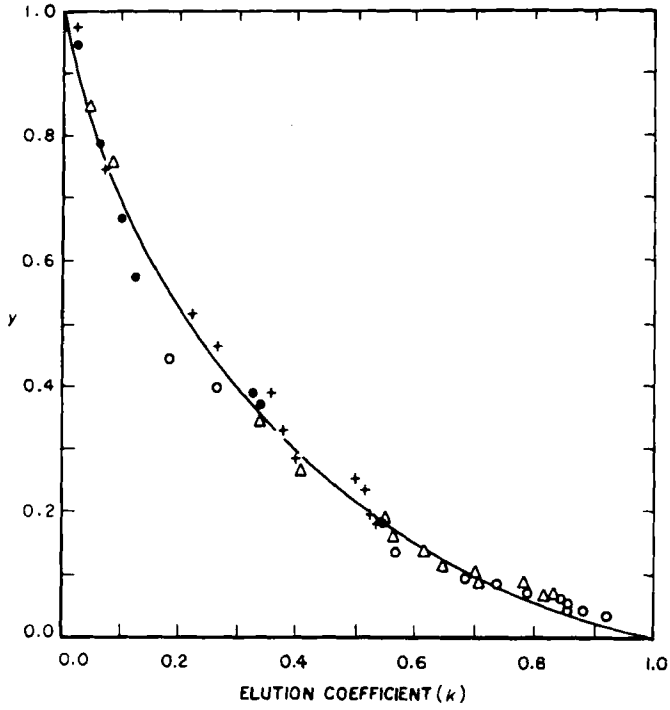

FIGURE 16a

Master Curve Relating Elution Coefficient to Protein Molecular Weight and Pore Diameter of Glass. Pore diameter (P): ●, 197 Å; +, 280 Å; △, 470 Å; ○, 650 Å *(33)*.

Porous glasses and silicas are chemically unstable at high pH. A special silicate material designed to be stable at high pH has been developed *(34)*. This packing material has been examined as a support for the chromatography of underivatized cellulose using Cadoxen solvent (cadmium ethylene-diamine hydroxide).

Soft Organic Gels

These materials are still finding many applications, and are versatile packings when proper consideration is given to solvent composition, ionic strength and pH. The elution of poly(L-histi-

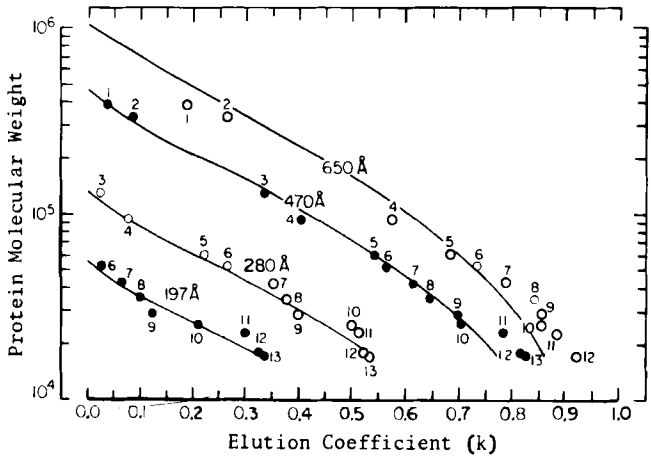

FIGURE 16b

Log Protein Molecular Weight *vs.* Elution Coefficient. Proteins are: (1) hemocyanin, (2) thyroglobulin, (3) β-galactosidase, (4) phosphororylase, (5) catalase, (6) glutamate dehydrogenase, (7) ovalbumin, (8) pepsin, (9) carbonic anhydrase, (10) α-chymotrypsinogen, (11) trypsin, (12) β-lactoglobulin, (13) nyoglobin *(33)*.

dine) from Sephadex G-75 is shown in Figure 17 *(35)* using 0.5M LiCl at pH 2.35. At higher pH values, 4.95, elution was delayed, and at pH 5.4, complete adsorption occurred.

Similarly, oligomers formed by hydrogen-transfer polymerization of acrylamide may be separated using Sephadex G10 with 0.1N HCl as solvent. Using water as solvent produced poorer resolution *(36)*.

A recent study on the application of GPC to characterize gelatin using Sepharose 4B has been reported *(37)*. Using 0.1M urea as solvent, a poor correlation was found with the universal calibration curve developed using dextran standards. When 0.1M NaCl was added to the solvent, the universal calibration approach was improved.

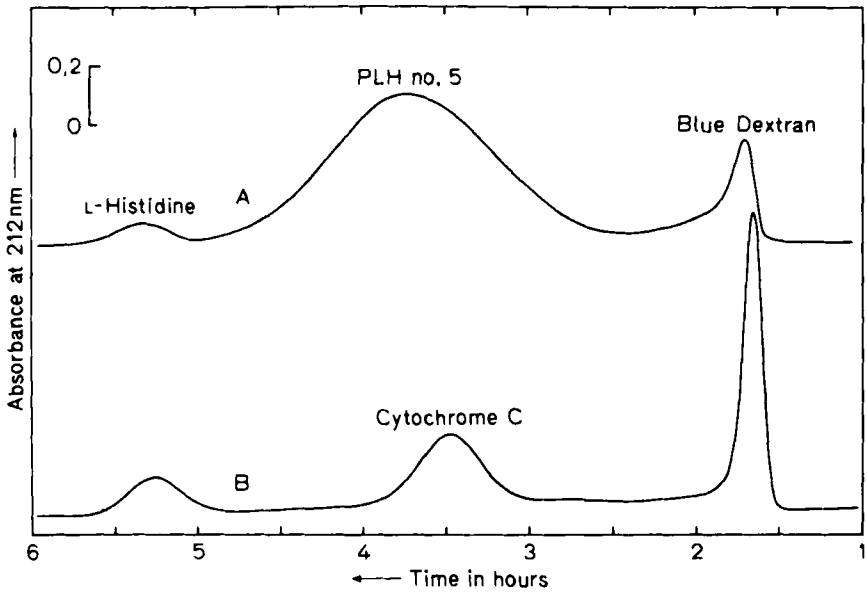

FIGURE 17

Typical Gel Filtration Elution Curves With Sephadex G-75: A, for fractionated poly(L-histidine) (0.2 mg), and B, for cytochrome C (0.4 mg); flow rate: 0.4 ml/min; eluant: pH 2.35 buffer; the effluent was monitored at 212 nm *(35)*.

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The application of Sephadex gels for the characterization of lignosulfonates has been thoroughly discussed *(38,39)*. Using Sephadex G50 and 10^{-1} to 10^{-4}M NaCl solvents, the data obtained conformed to the universal calibration approach for sodium lignin sulfonate fractions.

Rigid Organic Gels

1. *Hydrophobic Gels.* Styragel (styrene-divinylbenzene) gels have been employed to analyze dextrans by utilizing 0.1% sodium lauryl sulfate as the eluent *(40)*. The calibration curve for dextran is shown in Figure 18 for three styragel columns designated as 10^4,

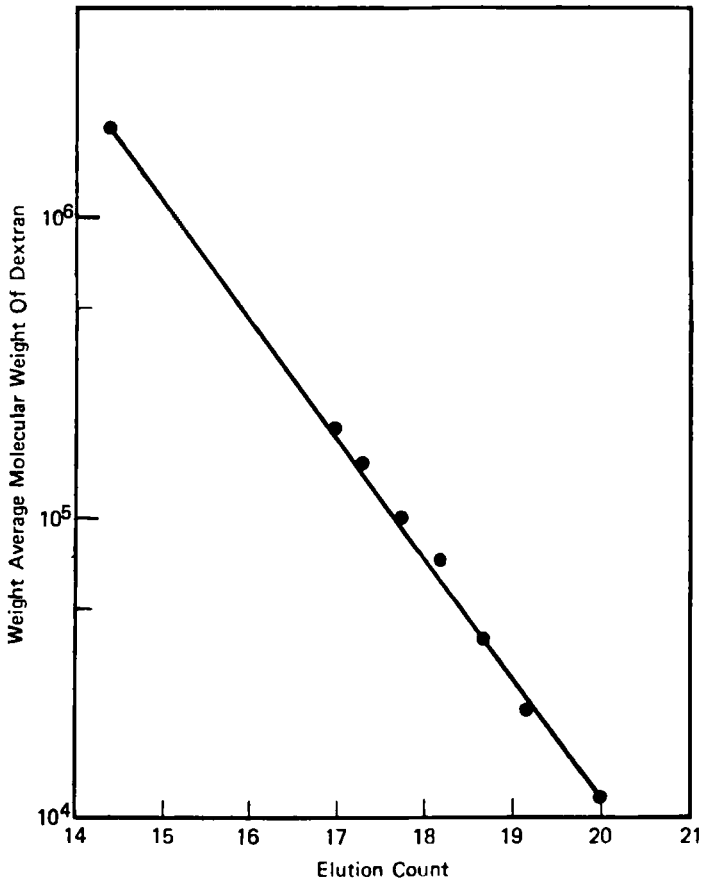

FIGURE 18

Calibration Curve of Dextran. Elution counts: 0.15 ml/count *(40)*. Reproduced by permission of Elsevier Publishing Co.

10^5 and 10^6 Å. This should be generally applicable to all hydrophobic gels.

2. *Hydrophilic Gels.* Various semi-rigid organic gel packings have recently become commercially available, which are specifically

designed for aqueous GPC. These are often obtainable in small particle sizes to allow high speed analyses.

TSK-Gel Type W (41). This material promises to be extremely useful for aqueous GPC. Efficiencies of over 4,000 plates/ft are reported. Dextrans, polyacrylamides and even polyethylene glycols elute normally using water as solvent.

Hydrogel (42). Hydrogel has been used to analyze dextrans and polyacrylates, and is stable up to 3000 psi.

Polyvinyl Alcohol Gels. Polyvinyl alcohol gels produced by hydrolysis of polyvinylacetate copolymer gels have been described *(43,44)*. Dextrans and polyethylene glycols elute normally in water. A typical chromatogram of polyethylene oxide oligomers is shown in Figure 19 *(43)*. Polyvinyl alcohol gels also appear to be useful for characterization of proteins using 0.05M pH 7.5 tris buffer *(44)*.

3. *Hydrophilic/Hydrophobic Gels.* Several column packings have recently been introduced, which are suitable for operation both in water and organic solvents.

Crosslinked Poly(Acryloyl Morpholines). The synthesis *(45)* and applications *(7,46)* of these materials has been described. They are suitable for characterization of dextrans, polyethylene oxides, oligosaccharides and proteins in aqueous solvent. These materials are also compatible with many organic solvents, including chloroform, dimethylformamide and pyridine.

Polyethylene Glycol Dimethacrylate. EM Gel PG M 2000 is commercially available as a packing for use in aqueous and organic solvents. It has been applied to the separa-

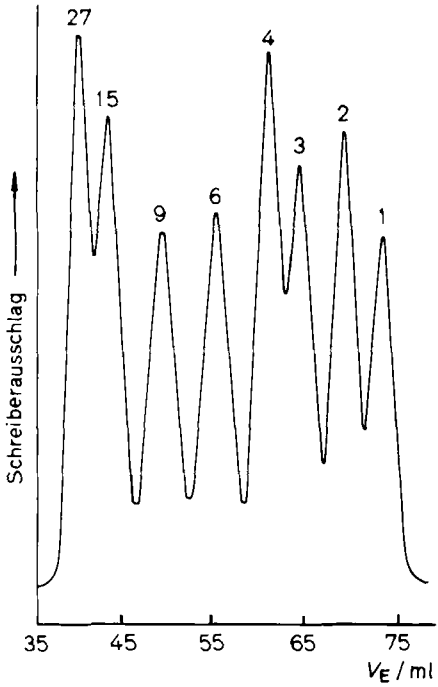

FIGURE 19

Chromatogram of a Mixture of Polyethylene Oxide Oligomers $HO(CH_2CH_2O)_nH$. The number on each peak is the value of n in the molecular formula. Solvent: water; column: 100 x 1.2 cm; flow rate: 15 ml/hr; injection volume: 0.5 ml; injection concentration: 20 mg/ml *(43)*.

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tion of polyethylene oxides in water, proteins in 1% acetic acid and glucose oxidase in 0.05M tris buffer pH 6.5 + 1.0M NaCl *(47)*.

Spheron/Uniflex Gels. The copolymerization of 2-hydroxyethyl methacrylate and ethylene dimethacrylate to form suitable gels for GPC has been reported *(48)*. Typical calibration curves for aqueous and nonaqueous

operations are shown in Figure 20. Quite high concentrations of acetic acid (1M) were employed in the aqueous solvent mixture. A later publication *(49)* reported the analysis of dextrans and proteins using 0.02% sodium triazide in distilled water as solvent.

A later study with this column packing *(50)* reported that proteins exhibited adsorption effects at low ionic strength. This effect was exploited to separate some natural biopolymers which are difficult to resolve.

Hydroxyethyl Cellulose Gels. Crosslinking of hydroxyethyl cellulose with epichlorohydrin produces porous gels which are swelled by water and organic solvents *(51)*. Chromatography of oligosaccharides and polyethylene

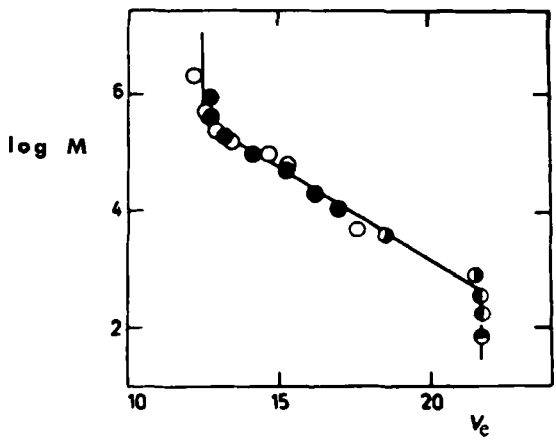

FIGURE 20

Universality of Uniflex 300. ○ Polystyrene in THF; ◑ polyethyleneglycols in THF; ○ dextrans in 1M solution of acetic acid in water; ◑ sugars in 1M solution of acetic acid in water; ◒ glycin in 1M solution of acetic acid in water; Ve: elution volume in counts *(48)*.

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oxides were reported. Sorption effects were considered to be smallest with this gel when compared with dextran and polyacrylamide gels.

CONCLUSIONS

Aqueous GPC has become a useful analytical and preparative technique for separating molecules of different size and charge. New column packings have extended the separating range and resolution from those previously available. The rigid packings can withstand higher flow rates and pressures, and thus enable faster analyses. Surface coatings and bonding on the packing surface have been used to improve separation and prevent adsorption of solutes. The separating range and resolution may be adjusted by the addition of ionic species to the solvent. Higher ionic strengths also usually have the beneficial effect of masking ion inclusion or exclusion effects, although these effects can be exploited to achieve a desired separation.

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