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Exclusion Chromatography Using Porous Glass. II. Application to Hydrophilic Polymers

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Synopsis

A chromatograph employing five columns packed with porous glass of pore size 1250 Å to 75 Å provided peak retention volumes (V_R) that were reproducible and essentially independent of sample size and flow rate when aqueous eluents were used. Calibration was carried out with a series of dextran fractions and polystyrene sulfonate samples, both of moderately narrow molecular weight distribution. The universal calibration method, based on hydrodynamic volume, was tested for four different polymer types. All four types produced a common curve within experimental error, which indicates that absolute molecular weight distributions may be derived from aqueous exclusion chromatography data for at least these polymer types. Additional study using a higher salt concentration produced hydrodynamic-volume plots that superposed with those above. The use of the same set of porous glass columns with polystyrene standards in three different organic solvents produced calibration curves that agreed well with the aqueous curves after corrections were made for differences in available pore volumes.

INTRODUCTION

The development of reproducible systems for determining the molecular weight distribution of water-soluble polymers has lagged behind the rapid progress made in characterization of hydrophobic polymers by gel permeation chromatography (GPC). This is partly because the introduction of stable, rigid porous media is only fairly recent, and partly because of the unavailability of synthetic polymer standards for calibration in aqueous media. An earlier work describes the preliminary stages of development of a system for determining the molecular weight distribution of water-soluble polymers by aqueous exclusion chromatography (AEC) on porous glass.[1] A more complete calibration of this chromatographic system is presented here, and tests with several polymers of different character are given. An investigation of a universal calibration procedure based on the hydrodynamic-volume concept[2] is reported. This study complements a previous work[3] that describes the use of these porous glasses for GPC separations.

EXPERIMENTAL

Apparatus

The five stainless steel columns of 0.17-in. I.D. and 5-ft length used in this study were packed with CPG-10 porous glass of nominal pore sizes of 1250,

670, 500, 190, and 75 Å. The controlled porosity glasses were obtained from the Corning Glass Co., Corning, N. Y., or the Electronucleonics Co., Fairfield, N. J., and were from the same lots described earlier.[3] These columns were connected in series and degassed; 0.20*M* sodium sulfate eluent was pumped through them at a constant rate, usually at 0.5 ± 0.1 ml/min. Samples of 1.0 ml were injected from a 2-ml sample loop, and the composition of the eluate from the column was monitored by a differential refractometer (Waters Model 401). Next, the eluate passed into an automatic balance that provided a mark at each 1-g increment on the elution curves, and these were traced on a strip-chart recorder.

Materials

The polystyrene sodium sulfonates (PSSNa) were prepared by sulfonation of narrow-distribution polystyrene according to the procedure of Carroll and Eisenberg,[4] which is claimed to produce complete monosulfonation without degradation or sulfone formation. (Careful adherence to the procedure is required. We have found incomplete reaction when commercial-grade fuming sulfuric acid was used, or when the polystyrene particles were not sufficiently subdivided.) The fractions of dextran were prepared by a column-elution technique[5] in which Pharmacia dextran samples were coated in a thin layer on a porous support. A continuous gradient of water–methanol compositions was used for progressive extraction of components of increasing molecular weight. The fractions of polyacrylic acid (PAA) were prepared by fractional precipitation upon addition of 1,2-dichloroethane to a dilute solution of the polymer in *n*-propanol. Fractions of the copolymer of acrylic acid and ethyl acrylate (AA–EA) (mole ratio 1:1) were prepared by a similar procedure with the use of isopropanol as the solvent and heptane as the precipitant. The polymers containing AA were neutralized with sodium hydroxide before the AEC and viscosity measurements were performed.

Dilute-Solution Characterization

Light-scattering measurements were performed at 23°C with the use of a SOFICA Model 42000 Photometer. Scattered intensities were measured at 11 angles ranging from 30° to 150°, with the use of unpolarized light of 436 nm. The light-scattering data from four or five concentrations (about 0.2 to 1.0 g/dl) of each sample were analyzed by the Zimm technique[6] to obtain weight-average molecular weight values. The solvents used in this study were aqueous 0.2*M* sodium sulfate; methanol containing 0.01*M* HCl; and 2-butanone containing 0.01*M* HCl for the dextran, PAA, and AA–EA polymers,[7] in that order. The specific refractive increment (dn/dc) for each system was measured with a differential refractometer with the use of a Brice-Phoenix split cell. These measurements were performed before and after clarification by filtration, so that concentration corrections could be applied when necessary.

The intrinsic viscosity measurements were performed in 0.20*M* and 0.80*M* sodium sulfate at 25.00 ± 0.05°C with the use of Cannon-Ubbelohde semimicro dilution viscometers. Solvent flow times were greater than 120 sec; under

these conditions kinetic energy corrections were found to be negligible. Solution flow times were measured at four concentrations, and the intrinsic viscosity was calculated by the Kraemer method.[8]

Number-average molecular weights were obtained with the use of a Melabs CSM-2 membrane osmometer at 23°C, with *p*-dioxane employed as the solvent. A Schleicher and Schuell 07 membrane was used after conditioning by a procedure very similar to that described by Billmeyer and Holleran.[9]

Polymer Characterization

The molecular weights and intrinsic viscosities of the polymers used in this study are given in Table I, and the intrinsic viscosity–molecular weight relationships in 0.2*M* sodium sulfate are given in Figure 1. The viscosity–molecular weight behavior of the dextran fractions is typical of a highly branched polymer.[10,11]

The modest *increase* in intrinsic viscosity in 0.8*M* sodium sulfate observed in some of these dextran fractions was unexpected. It will be shown later, however, that the increase in intrinsic viscosity is supported by a decrease in retention volume (corresponding to larger molecular size) in the chromato-

TABLE I
Results of Polymer Characterization

Sample		$[\eta]^{25°C}$, 0.2*M* Na_2SO_4	$[\eta]^{25°C}$, 0.8*M* Na_2SO_4	$\bar{M}_w(\times 10^{-3})$	$\bar{M}_n(\times 10^{-3})$[a]
Dextran	1	0.71	0.86	1230.	
	2	0.66	0.80	890.	
	3	0.64	0.68	560.	
	4	0.48	0.51	259.	
	5	0.43	0.46	204.	
	6	0.40	0.42	160.	
	7	0.33	0.34	88.0	
	8	0.27	0.25	53.0	
PSSNa	1	1.70	—	985.[b]	
	2	0.64	0.30	400.[b]	
	3	0.50	0.12	220.[b]	
	4	0.19	—	73.[b]	
	5	0.16	0.06	37.5[b]	
	6	0.09	0.06	20.0[b]	
	7	0.03	—	8.0[b]	
PAANa	1	1.91		490.	184.
	2	1.50		403.	143.
	3	1.07		171.	129.
	4	0.60		65.2	43.8
AA–EA	1	2.70	—	568.[c]	193.
	2	1.10	—	151.[c]	95.6
	3	0.44	—	62.5[c]	29.8
	4	0.27	—	29.6[c]	—

[a] Molecular weight determined by osmometry as the free acid, but converted to the appropriate value of the sodium salt.

[b] Calculated from the molecular weight of the starting material, with assumption of complete monosulfonation without degradation.

[c] Light-scattering measurements from ref. 7.

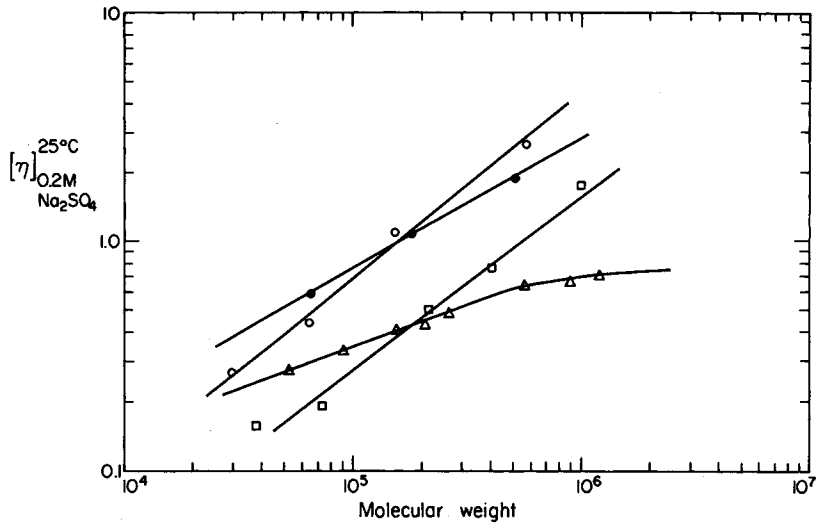

Fig. 1. Log intrinsic viscosity vs. log molecular weight for materials used in AEC studies: (⊡) = PSSNa; (▲) = dextran; (⊙) = AA–EA; (●) = PAANa.

graphic studies. We have not explored possible explanations for this increase in size of dextran molecules with increasing salt concentration.

The characterization of the PSSNa standards has been complicated by the presence of small, but variable, amounts of insoluble material in all preparations of molecular weight greater than about 70,000. Several efforts to modify the tedious preparation method of Carroll and Eisenberg[4] have not overcome this problem entirely. The amount of insoluble material is so small that it does not interfere with viscosity, AEC, or ultracentrifuge measurements. However, repeated attempts to determine the molecular weights of representative preparations by the light-scattering method have produced

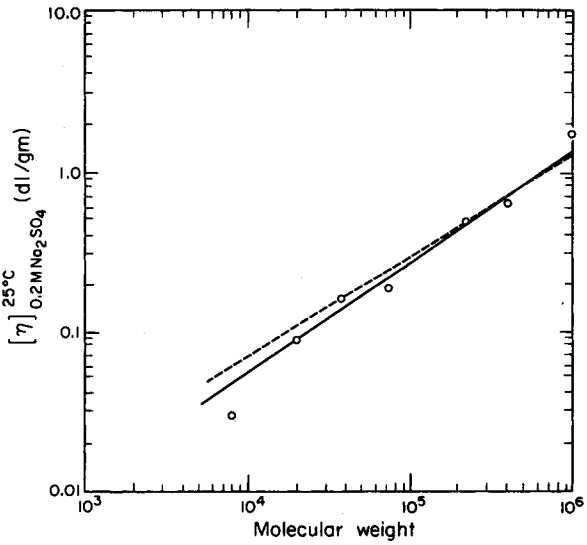

Fig. 2. Viscosity–molecular weight relationships for PSSNa: — = this work; - - - - = ref. 12.

unreasonably high molecular weight values. It appears that our usual method of clarification by filtration through sintered glass and/or Millipore filters has not removed a small amount of highly swollen insoluble polymer. The relatively small samples prepared (about 300 mg) have prevented the use of more extensive clarification methods that have been effective with similar problems in the past.

The molecular weights of the PSSNa samples listed in Table I have been calculated from the molecular weights of the initial narrow-distribution polystyrene samples with the assumption of complete monosulfonation without degradation. Support for these values is provided by several methods: First, determinations of absolute molecular weights by ultracentrifugal sedimentation have been made for two PSSNa samples of nominal molecular weight 4×10^5 and 2×10^4. The Archibald and meniscus depletion equilibrium techniques were used for each sample, and the molecular weights obtained by extrapolation to infinite dilution agreed within ±5% and were in good agreement with the assumed molecular weight values.

In addition, the viscosity–molecular weight relationship shown in Figure 2 for all the PSSNa standards at an ionic strength of 0.6 is in fairly close agreement with literature results[12] for PSSNa fractions at about the same ionic strength (0.5). Finally, the chromatographic results that follow further support the assumption that negligible degradation occurred during preparation of these standards.

RESULTS AND DISCUSSION

Operational Variables

The reproducibility of the peak retention volumes, V_R, was studied for the PSSNa and dextran samples. Three component blends of each of the above polymer types were injected on four consecutive days to obtain the data given in Table II. These samples were also examined individually and gave the same retention volumes as they did when examined as a blend. The data in Table II indicate that the retention volumes can be measured reliably to ±0.2 ml, which is equivalent to ±2% in peak molecular weight.

The dependence of V_R on sample size was evaluated by injecting the blends of PSSNa and of dextran samples at several concentrations and was found to be negligible, i.e., within our experimental error, over the concentration range of 0.02–0.1 g/dl. The variation of V_R with volumetric flow rate at constant

TABLE II
Reproducibility of Peak Retention Volumes

Molecular weight ($\times 10^{-3}$)	V_R, ml				
	Run 1	Run 2	Run 3	Run 4	Average
Dextran 1230.	67.6	68.1	67.6	67.6	67.7 ± 0.3
Dextran 161.	82.8	83.4	83.2	83.3	83.1 ± 0.2
Dextran 53.	92.3	93.0	92.8	92.8	92.7 ± 0.3
PSSNa 985.	64.0	64.6	64.1	64.0	64.2 ± 0.3
PSSNa 220.	84.6	84.9	85.2	85.0	84.9 ± 0.2
PSSNa 20.	101.7	101.9	101.9	101.8	101.8 ± 0.1

concentration, 0.10 g/dl, for the PSSNa and dextran blends was also studied. The maximum change observed in V_R was about 0.2 ml over the flow rate studied (0.2–1.0 ml/min). Furthermore, the data are very similar to results obtained when a similar chromatograph was operated in the GPC mode.[3] Thus, the concentration and flow-rate dependence of these charged and uncharged calibrants are not very different.

Calibration

The porous glass chromatograph was calibrated with PSSNa preparations of narrow molecular weight distribution and with dextran fractions; the semilogarithmic plot of weight-average molecular weight ($\bar{M}_w$) versus retention volume (V_R) is shown in Figure 3.

The two calibration curves are fairly similar at $M \leq 1.5 \times 10^5$; at higher molecular weight ($M \sim 1 \times 10^6$) the curves diverge sharply. This observation is not surprising, since dextrans are known to be highly branched, a characteristic leading to a more compact conformation and, therefore, to lower viscosities (and larger V_R) than those observed for a linear macromolecule of equivalent molecular weight.

The molecular weight and polydispersity, $\bar{M}_w/\bar{M}_n$, of the calibrants were determined by AEC with use of the respective calibration curves shown in Figure 3. The polydispersities of the PSSNa and dextran samples are fairly

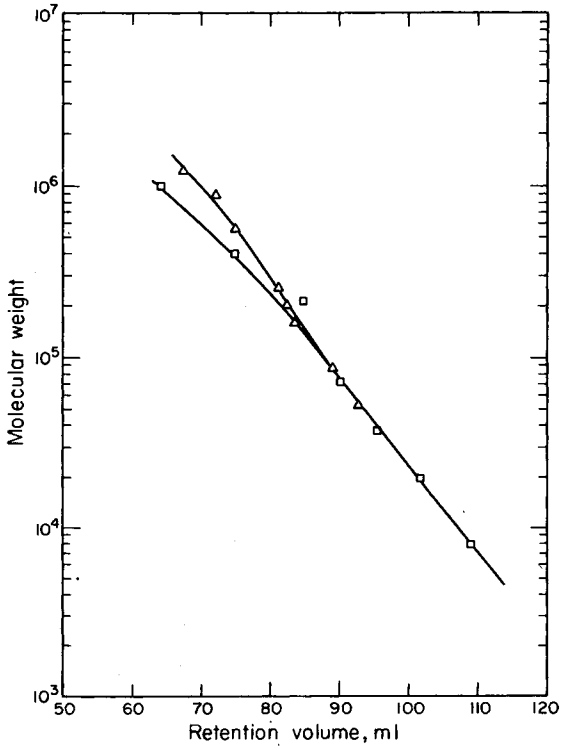

Fig. 3. Semilogarithmic calibration curves of $\bar{M}_w$ vs. V_R for PSSNa (□) and dextran (▲) samples in 0.2M sodium sulfate.

TABLE III
Polydispersity Estimates of AEC Calibrants

Dextran		PSSNa	
$\bar{M}_w$ (× 10^{-3})	$\bar{M}_w/\bar{M}_n$	$\bar{M}_w$ (× 10^{-3})	$\bar{M}_w/\bar{M}_n$
1230.	1.35	985.	1.29
890.	1.45	400.	1.12
560.	1.45	73.	1.07
259.	1.20	37.5	1.12
204.	1.20	20.0	1.07
160.	1.20	0.8	1.06
88.	1.15		
53.	1.15		

low; i.e., $\bar{M}_w/\bar{M}_n = 1.10 \pm 0.05$ and $= 1.30 \pm 0.15$, respectively, as is summarized in Table III. (Corrections for axial dispersion have not been applied in either case; such corrections would make the polydispersities substantially lower.) The very small values of $\bar{M}_w/\bar{M}_n$ found for the PSSNa samples support the assumption that no appreciable degradation occurred during the sulfonation process.

The fairly narrow distribution and markedly different structures of these primary calibrants make them suitable for testing the applicability of the hydrodynamic-volume approach to universal calibration of AEC. The PSSNa samples are linear and highly charged polyelectrolytes, whereas the dextrans are uncharged, but highly branched, polysaccharides.

The Hydrodynamic-Volume Concept in AEC

The hydrodynamic-volume concept developed by Benoit et al.[2] has gained wide acceptance in GPC as a universal calibration procedure. The extension of this approach to AEC has been complicated by the lack of chromatographically stable stationary phases and the unavailability of characterized flexible polymer molecules having narrow distributions of molecular weight and covering a wide range of molecular weights.

The product, $[\eta]\cdot M$, a quantity proportional to the hydrodynamic volume, J, for randomly coiled chains, was calculated for each sample. The common practice of relating log J to peak retention volumes is satisfactory for polymers having narrow molecular weight distributions, e.g., the dextran and PSSNa samples studied here. However, Dawkins[13] has shown that substantial errors can occur when one is analyzing polymers of broad molecular weight distribution. Since it appeared that the PAANa and AA–EA samples might not fit the log normal distribution function, the molecular weight of these materials was related to the peak position by a method similar to that of Ring and Holtrup,[14] as described below.

The weight-average molecular weights were related to the peak retention volumes for the series of PAANa and AA–EA samples. Estimates of $\bar{M}_w$ and $\bar{M}_n$ were then calculated from the AEC distribution curves; and the calibration plots were adjusted by an iterative method until the computed $\bar{M}_w$ and $\bar{M}_n$ values of each of the series of AA polymers agreed, within experimental error, with the measured values given in Table I. It was found that a single

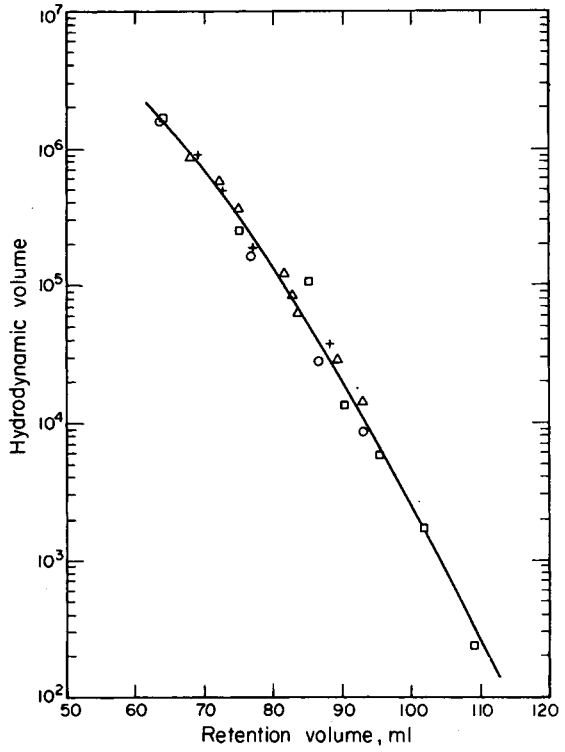

Fig. 4. Hydrodynamic-volume calibration curves showing log J vs. V_R for the hydrophilic polymers in 0.2M sodium sulfate: (⊡) = PSSNa; (▲) = dextran; (⊙) = AA–EA; (+) = PAANa.

minor adjustment of the slope of the M versus V_R calibration was required to obtain molecular weight values, $\bar{M}_w$ and $\bar{M}_n$, that agreed well with the data given in Table I.

A plot of log J versus V_R (corresponding to that value where $\bar{M}_w$ occurs) is given in Figure 4. The close approach to superposition for all four polymer types demonstrates the applicability of this approach for various water-soluble polymers.

Since the end-to-end distances of polyelectrolyte molecules are strongly affected by ionic strength,[15,16] changes in salt concentration should influence

TABLE IV
Retention Volume of AEC Calibrants at Different Salt Concentrations

Dextran			PSSNa		
$\bar{M}_w$ (× 10^{-3}), g/mole	V_R (0.2M), ml	V_R (0.8M), ml	$\bar{M}_w$ (× 10^{-3}), g/mole	V_R (0.2M), ml	V_R (0.8M), ml
1230.	67.7	66.3	985.	64.0	—
890.	72.1	71.0	400.	74.8	80.3
560.	75.0	73.2	220.	85.2	89.8
259.	81.4	79.6	73.	90.1	—
204.	82.7	81.7	37.5	95.3	99.1
160.	83.0	82.7	20.0	101.7	104.2
88.	89.1	88.9	0.8	109.0	—
53.	94.8	94.4			

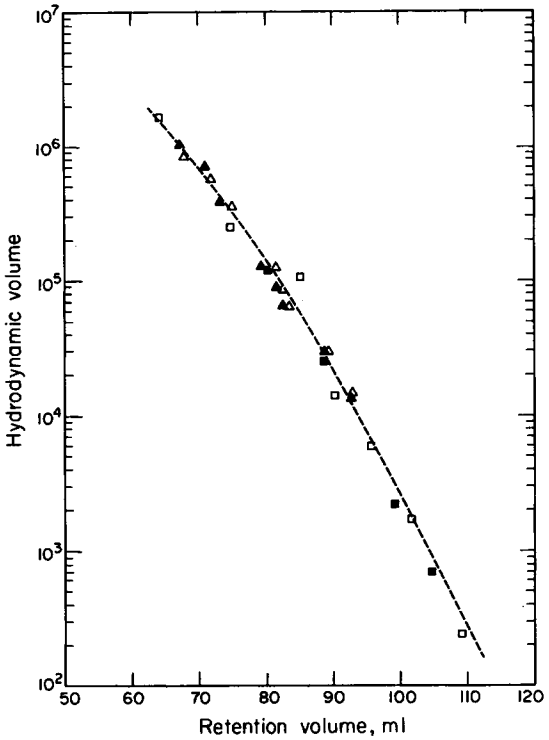

Fig. 5. Hydrodynamic-volume calibration curves vs. V_R for the hydrophilic polymers studied at two salt concentrations: Data from Fig. 4 in 0.2*M* sodium sulfate shown as dashed line; (□) = PSSNa; (△) = dextran. For studies at 0.8*M* sodium sulfate concentrations: (■) = PSSNa; (▲) = dextran.

V_R. To investigate this effect, the AEC studies using the PSSNa and dextran samples were also performed in 0.8*M* sodium sulfate.

Table IV lists values of V_R found at the two concentrations of supporting electrolyte for dextran and PSSNa samples. A graph of log hydrodynamic volume versus V_R at both salt concentrations is given in Figure 5. It can be seen that despite the decrease by a factor ~3 in the intrinsic viscosity for the PSSNa samples, occurring at the higher salt concentration, the accompanying shift in V_R causes all points to fall on the same line as that found at the lower salt concentration. The shifts of V_R and intrinsic viscosity were small for the dextrans, but again, a good fit to the same common line was found. These results show that the hydrodynamic-volume concept is applicable for these polymer types over the range of 0.2*M* to 0.8*M* sodium sulfate concentration.

Exclusion Chromatography with Organic Solvents

A comparison of the behavior of porous glass columns operated with aqueous and organic solvents was made, to determine whether the hydrodynamic-volume curve for each solvent type would superpose. This behavior might be expected if the porous structure, available for the separation process, was the same in all cases. The same column-set previously used with aqueous salt so-

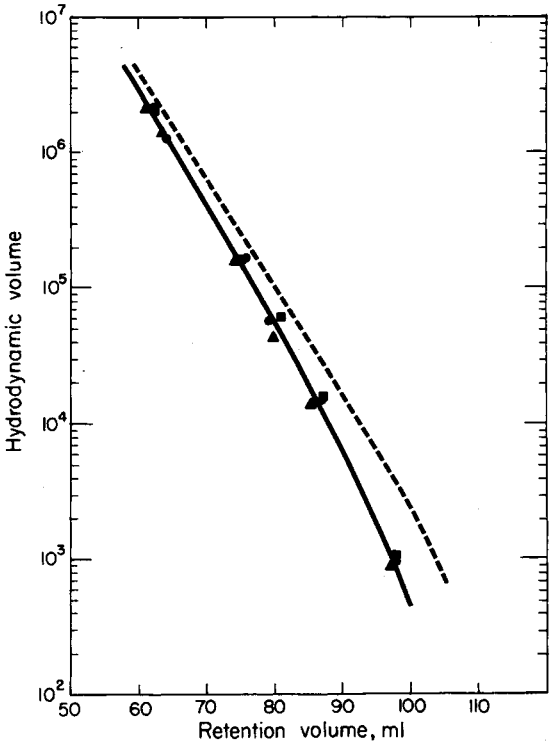

Fig. 6. Log J vs. V_R for porous glass chromatographic system operated in the GPC (—); and AEC (0.2M sodium sulfate) (- - - -) modes. (●) = THF; (▲) = $CHCl_3$; (■) = benzene.

lutions was purged with water and then tetrahydrofuran (THF) until constant refractive index was reached. (Eluent exchange to the other organic solvents did not require the water treatment.)

A series of narrow-distribution polystyrene samples (Pressure Chemical Co.) was chromatographed in THF, chloroform, and benzene at a flow rate of 0.5 ml/min. On the basis of the intrinsic viscosity measurements in each solvent, and of the molecular weights reported by the manufacturer, the respective hydrodynamic-volume calibration curves were constructed and are shown in Figure 6. Some systematic differences among the results for the three organic solvents may be observed. These have tentatively been attrib-

TABLE V
Column Parameters in Exclusion Chromatography

Solvent	V_0, ml[a]	V_T, ml
0.2M Na_2SO_4	56.2	117.4[b]
0.8M Na_2SO_4	—	117.7[b]
THF	56.1	112.8[c]
Benzene	55.6	113.0[d]
Chloroform	55.7	112.3[c]

[a] Determined with a polystyrene or PSSNa sample of high molecular weight.
[b] Determined with a solution of slightly higher sodium sulfate concentration.
[c] Determined with benzene.
[d] Determined with THF.

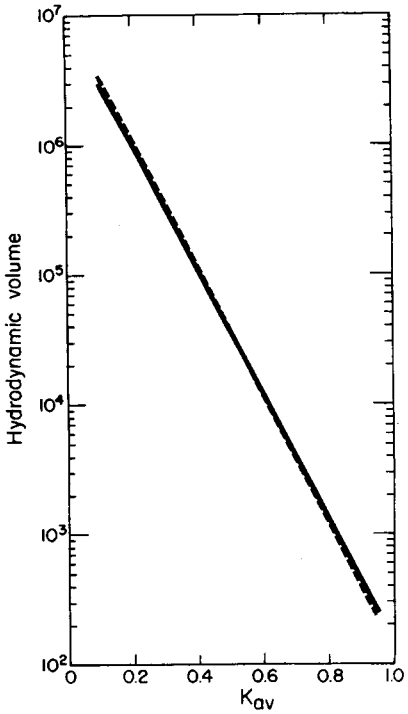

Fig. 7. Log J vs. the normalized retention volume parameter K_{av} for the AEC (- - - -) and GPC (—) studies performed with these porous glasses.

uted to differences in the rate of evaporation of the solvents, resulting in some errors in V_R. The line drawn in Figure 6 is an average for the three solvents. It is apparent that the data obtained with the organic solvents do not agree with similar data obtained with aqueous eluents.

It was found that the value of V_0, the void volume, remained essentially constant for all solvents studied, whereas the values of V_T, obtained with solutes of very low molecular weight—e.g., benzene and water—differed substantially, as shown in Table V, for the aqueous and organic solvent systems. These differences are attributed to changes in the effective pore volume available in the two solvent types. The nature of this change in available pore volume with various solvent media may be related to differences in surface tension and/or interfacial tension between the porous glass and the solvent media employed. These interactions, in the presence of a dilute polymer solution, make it difficult to determine what changes might be taking place in the pore-size distribution of these glasses. A study of the source of these differences has not been made.

The results observed in the two chromatographic modes can be compared by normalizing the data through the use of the parameter K_{av} where

$$K_{av} = \frac{V_R - V_0}{V_T - V_0}$$

in which V_R = retention volume of the solute, V_0 = interstitial volume, and V_T = total liquid volume in the columns. Figure 7 shows the hydrodynamic volume plots with the normalized K_{av} as abscissa for all the polymers studied

in aqueous and organic solvents. It is obvious that the data for all systems agree well, once this correction for differences in pore volume is made.

The behavior described above was confirmed by preparation of a new set of columns packed with a similar series of porous glasses. These were standardized in both aqueous and organic solvent systems, as previously described. Shifts in the calibration curves of Figures 3 and 5 were found, but the difference between V_T values in the aqueous and organic solvents was, within experimental error, the same as shown in Table V. This indicates that the difference in available pore volume was not a result of changes occurring with extended use of the first set of columns.

Whereas a number of uncharged and anionic polymers, in addition to those just discussed, have shown no sign of adsorption problems, all efforts to study polymers containing cationic groups have been unsuccessful. Various polymers containing quaternary ammonium groups and even gelatin, at a pH far above its isoelectric point, were found to adsorb strongly to the porous glass. Treatment of the glass with polyethylene oxide or quaternary surfactants to reduce adsorption effects has not proven entirely successful in our laboratory. Various alternatives to allow analysis of cationic polymers with porous glass[17,18] or other rigid packings may solve this problem soon.

CONCLUSIONS

The demonstration of the applicability of the hydrodynamic-volume concept to four markedly different hydrophilic polymers suggests that this approach may be generally suitable in aqueous systems as well as for polymers in organic solvents.

The stability of the porous glass packings and the near independence of the peak elution volume on flow rate and sample concentration, which have been shown here, suggest that this AEC technique should be suitable for general use in characterizing uncharged and anionic hydrophilic polymers. The use of the same columns with aqueous and organic solvents was found to produce nearly identical hydrodynamic-volume plots, after a correction for the difference in the available pore volume was applied.

Difficulties have been experienced with adsorption to the porous glass of polymers containing cationic groups; and treatment of the glasses with polyethylene oxides, or surfactants, to reduce adsorption effects has not proven successful in our laboratory.

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