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GEL PERMEATION CHROMATOGRAPHY OF CATIONIC POLYMERS ON PW GEL COLUMNS

PAUL L. DUBIN*,* and IRVIN J. LEVY
Clairol Research Laboratories, 2 Blachley Road, Stamford, CT 06922 (U.S.A.)
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SUMMARY

Universal calibration studies show that several cationic polyelectrolytes can be characterized by aqueous exclusion chromatography, without adsorption effects, using PW (Toyo Soda) hydrophilic gel columns. No other commercially available, high-performance packings are suitable for polycations, primarily because of charge-induced adsorption. In the case of the present columns, such interactions are minimized by 0.2 *M* NaCl in the mobile phase, which also provides an ionic strength sufficient to suppress Donnan equilibrium salt peaks.

INTRODUCTION

Until rather recently, aqueous exclusion chromatography was associated primarily with biopolymer separations, namely "gel filtration" of proteins on cross-linked polyacrylamide and dextran gels. Because these supports are not mechanically strong, aqueous exclusion chromatography did not at first share in the rapid progress in high-pressure liquid chromatography towards high resolution and short analysis time. With the advent of commercially available hydrophilic stationary phases for high-pressure exclusion chromatography, aqueous gel permeation chromatography (GPC) currently represents a field of expanding applications which may now fully benefit from the technology and theories developed for non-aqueous systems.

Substrates for aqueous GPC are of three general types: porous silica or glass, derivatized siliceous supports, and "semi-rigid" cross-linked polymer gels. The characteristics of these packings have been thoroughly reviewed[1], and the burgeoning literature on aqueous GPC contains numerous references to their applications to dextran, acidic polysaccharides, non-ionic synthetic polymers such as poly(ethylene oxide) and poly(vinyl alcohol), acrylic polyanions, and proteins. In contrast, only two references describe the exclusion chromatography of cationic polymers. The first report presents fragmentary data for a quaternized Styragel-type column with very limited resolution[2]. The second describes the chromatography of (uncharacterized) quaternized poly(4-vinylpyridine) and poly(N,N-diallyldimethylammonium

* Present address: Department of Chemistry, Purdue University, Indianapolis, IN 46205, U.S.A.

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chloride) on column packings made by coupling porous glass or silica to a quaternized aminopropylsilane[3]. Among the difficulties encountered in the latter work were (1) the necessity for strong acid (0.1 *N* HNO_3) in the mobile phase to ionize unquaternized bonded phase amine, and (2) low column efficiencies (*ca.* 500 plates ft.$^{-1}$) and correspondingly long run times (2–3 h). In any event, neither of these two quaternized substrates is commercially available. Since the principal applications of cationic polymers —as flocculants in water treatment, paper making and sewage processing— are ones in which the polymers' molecular weight distribution (MWD) plays a central role, the lack of readily available high efficiency aqueous GPC columns has major consequences for these technologies.

Siliceous packings may be presumed to always contain some level of acidic silanol groups capable of dissociation and consequent electrostatic binding to polycations. Thus, a non-ionic semi-rigid gel would appear less prone to adsorption. PW packings (Toyo Soda) are hydrophilic cross-linked polyether gels containing $-CH_2-CH(OH)CH_2O-$ repeat units[4] whose application to non-ionic linear polymers, dextrans, acidic polysaccharides and proteins have been well documented[4–7]. In this report we discuss the characterization of cationic synthetic polymers with PW columns[8].

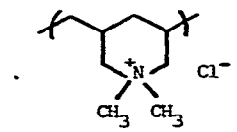
EXPERIMENTAL

Apparatus

The GPC system consisted of a Milton Roy Minipump, a Rheodyne Model 7010 injector equipped with a 200-μl loop, and a Waters Associates R401 differential refractometer. The columns employed, Toyo Soda G3000 PW and G5000 PW, both 30 cm long, were preceded by an in-line stainless steel fritted filter (Rheodyne). At a flow-rate of 30 ml h^{-1} the back pressure was 100–200 p.s.i. per column.

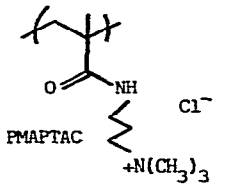
Materials

Narrow MWD poly(ethylene oxide) (PEO) samples with MWs $< 2 \cdot 10^4$, as determined by end group analysis, were supplied by Union Carbide or Dow Chem. High-molecular-weight broad distribution PEO samples characterized by light-scattering were from Aldrich. Dextrans were supplied by Pharmacia with intrinsic viscosities, and with MWs from light-scattering. Poly(dimethyldiallylammonium chloride) (PDMDAAC) was a commercial product "Merquat 100" or low MW homologs, all from Calgon. Their MWs were determined from intrinsic viscosities in 0.1 *M* NaCl at 30°C, using the relationship $[\eta] = 3.5 \cdot 10^{-4} \cdot \bar{M}_w^{0.62}$, which was developed on the basis of viscosity and light-scattering data supplied by the manufacturer along with similar data from the literature[9]. Narrow MWD fractions of polyethyleneimine (PEI) were kindly provided by Professor R. Stratton, Institute of Paper Chemistry, along with viscosity data and MWs from ultracentrifugation[10]. Poly(N-vinylacetamide) (PVAc) fractions were gifts from Dynapol, with MWs obtained by osmometry and GPC[11] and intrinsic viscosities calculated from the relationship[12] $[\eta]_{H_2O,30°C} = 1.6 \cdot 10^{-3} \cdot \bar{M}_n^{0.52}$. A sample of polyvinylamine (PVA) was from the same source, its MW determined from that of the PVAc precursor. Globular proteins were from Sigma. A high-molecular-weight cationic polymer for water treatment, poly(methacrylamidopropyltrimethylammonium chloride) (PMAPTAC) was a gift from Texaco. An ionene polymer of unknown MW was kindly provided by Professor

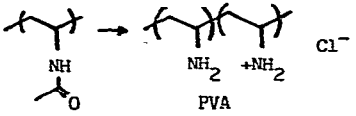


PDMDAAC

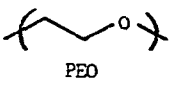
Poly(dimethyldiallylammonium chloride)



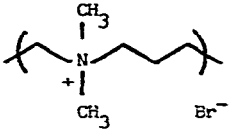
Poly(methacrylamidopropyltrimethylammonium chloride)



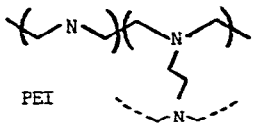
PVAc ⟶ poly(vinylamine)



Poly(ethyleneoxide)



4,4 Ionene



Polyethylenimine

Fig. 1. Structures of synthetic polymers used in this study.

P. Ander, Seton Hall University. Structures of the synthetic polymers mentioned above are shown in Fig. 1.

Methods

Polymers were dissolved in the mobile phase and clarified with 0.45-μm HAWP filters (Millipore). The concentration of polymer injected onto the column ranged from 0.1 to 1.0 wt. % corresponding roughly to RI detector attenuations of $\frac{1}{2}\times$ to $4\times$. The lowest concentrations were necessary for the higher-molecular-weight polymers in order to avoid signal noise due to viscosity-related pressure pulses in the refractometer cell as the sample eluted from the column.

PEI and PVA samples were typically applied to the columns as the free base, at pH = 10. Since the pH of the sample eluents were more than 9, it could be assumed that these polymers were largely un-ionized during chromatography.

Intrinsic viscosities of PDMDAAC were measured in 0.1 *M* NaCl at 30°C using a Schott AVS/N semi-automatic elution viscometer.

RESULTS AND DISCUSSION

Mobile phase supporting electrolyte

In the absence of simple salt, polyion molecular dimension —and hence retention volume— would be extremely sensitive to intra-molecular electrostatic repulsion. This effect would result in strong divergence of the elution behavior of non-ionic and ionic polymers and an excessive influence of salt impurities for the latter. Supporting electrolyte is thus a prerequisite for interpretable chromatograms. In the presence of 0.1 *M* citrate (selected for its buffering capacity and indifference to stainless steel) chromatograms of PDMDAAC were obscured by large and erratic negative peaks as shown in Fig. 2. Since these peaks were eliminated if the polymer solution was first brought to dialysis equilibrium with the mobile phase, they may be ascribed to Donnan equilibrium effects. When the polyion shares a common co-ion with the simple salt, the activity of the latter in the domain of the polymer is increased. The volume within the small pores, which may be regarded as analogous to an external dialysis solution from which polymer is excluded, then acquires a concentration of simple salt in excess of the bulk mobile phase. This salt is subsequently eluted as a so-called "ion-inclusion" peak[1]. In the present case, the Donnan equilibrium results in a depression in citrate ion activity and a consequent increase in citrate concentration in the immediate vicinity of the polymer. The concomitant depletion of citrate from the bordering volumes results in large negative peaks, since the refractive index increment of citrate is large. Such Donnan effects could be eliminated by choosing a salt of lower refractive index and valency, and maintaining a large salt:polymer ratio. Thus with 0.1–0.2 *M* NaCl in the mobile phase, and sample loads of less than 1 mg, no such interfering peaks were encountered.

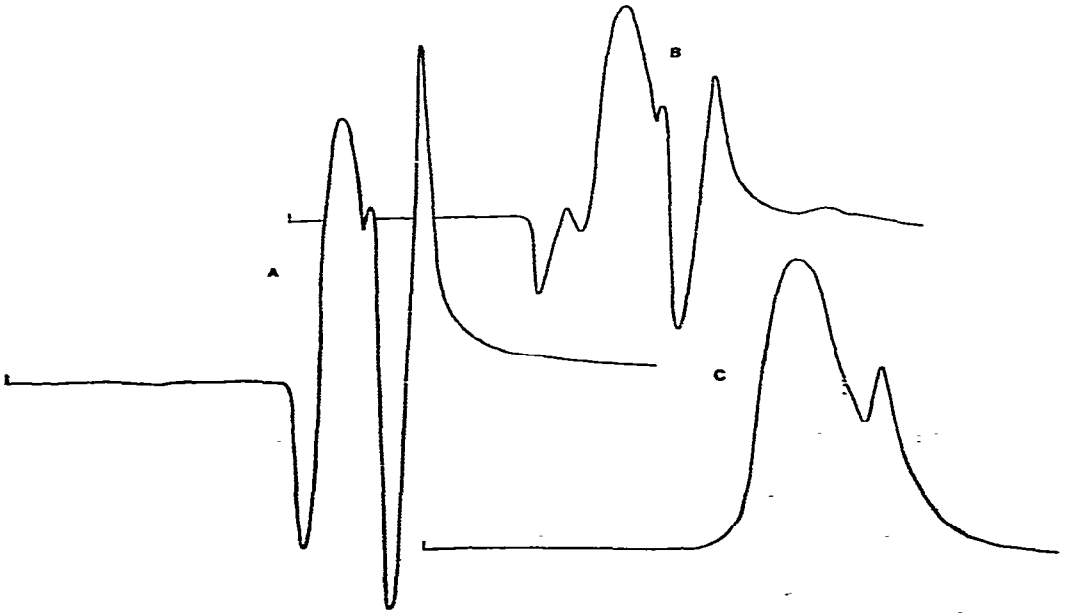


Fig. 2. Chromatograms of $5.2 \cdot 10^3$ MW PDMDAAC in: A, 0.1 *M* sodium citrate, direct solution; B, 0.1 *M* sodium citrate, partial dialysis; C, 0.2 *M* NaCl direct solution. G3000 PW column.

Calibration and efficiency

Fig. 3 shows calibration curves obtained in 0.1 *M* and 0.2 *M* NaCl using PEO standards. In order to employ the light-scattering MW reported for the broad distribution $1 \cdot 10^5$ MW PEO standard, the elution volume corresponding to $\bar{M}_w$ was calculated from the chromatogram in conjunction with a trial calibration curve based on a linear extrapolation from the other PEO data points. This procedure was extended to the $9 \cdot 10^5$ MW PEO sample with a somewhat lesser degree or certainty, reflected in the large error bar for V_e for this datum. The columns exhibited plate counts of 25,000–30,000 plates meter^{-1}, obtained using ethylene glycol or ^2H_2O. These efficiencies were maintained over several months of application.

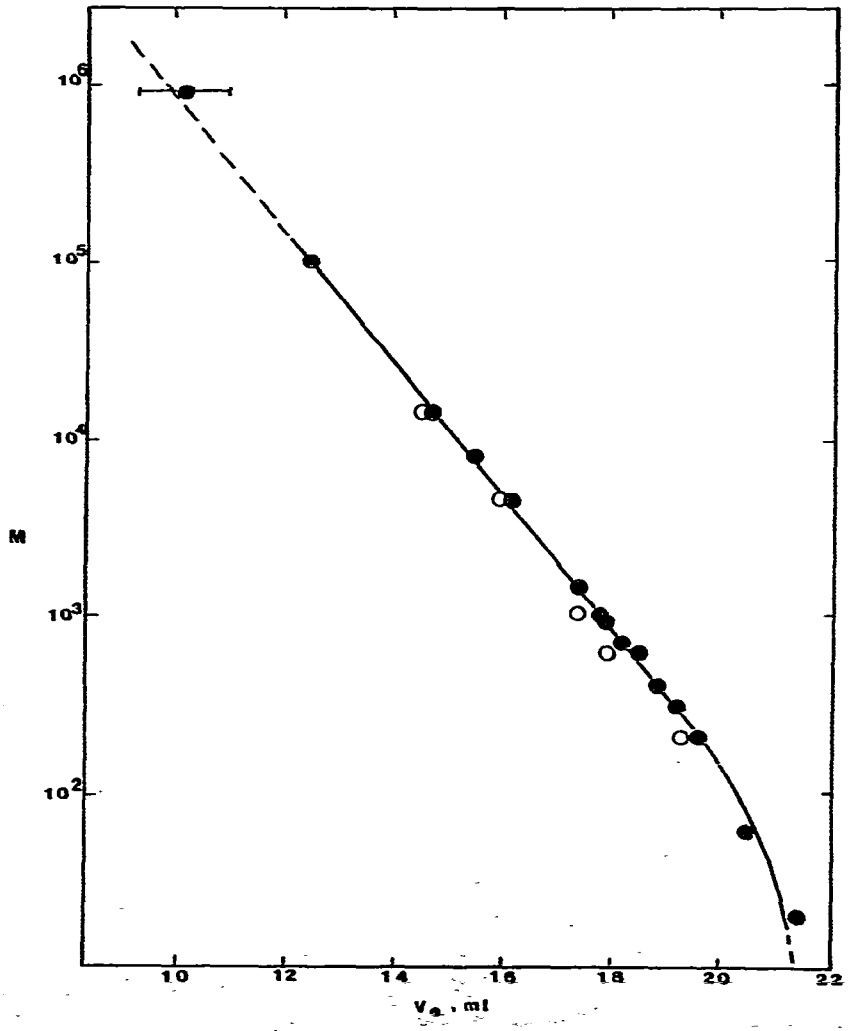


Fig. 3. PEO calibration curves for G5000 PW + G3000 PW columns, in 0.2 *M* NaCl (●) and 0.1 *M* NaCl (○).

Application to cationic polymers

Fig. 4–6 show chromatograms of several strongly cationic polyelectrolytes. All elute with no evidence of adsorption, *i.e.*, with no tailing and peak areas consistent with the sample mass. Table I compares apparent GPC MW values, based on PEO calibration curves, with MWs from other methods for the characterized polymers. With the exception of the result for PVA in 0.2 *M* NaCl, apparent peak MW values are lower than expected MWs by a factor of 2–4 in this solvent, and by a factor of 4–

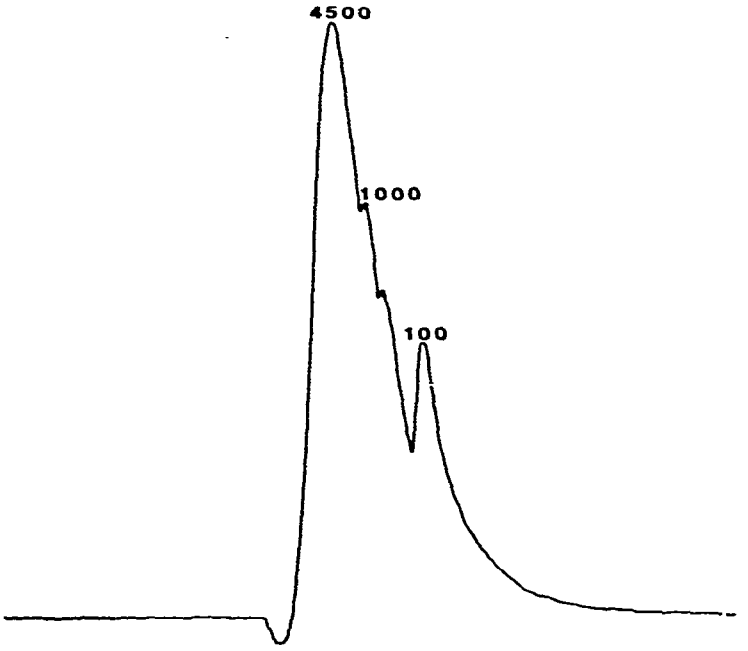


Fig. 4. Chromatogram of 4,4-ionene. Values shown are MWs based on PEO calibration. G3000 PW, 0.1 *M* NaCl.

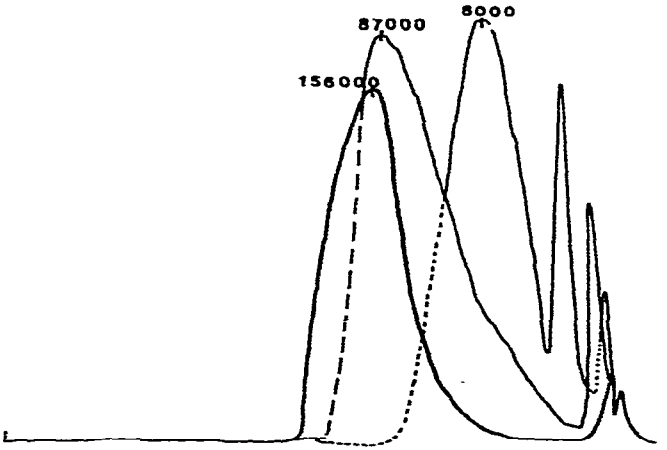


Fig. 5. Chromatograms of PMAPTAC samples of varying MWs. Values shown are peak MWs based on PEO calibration. G5000 PW + G3000 PW, 0.2 *M* NaCl.

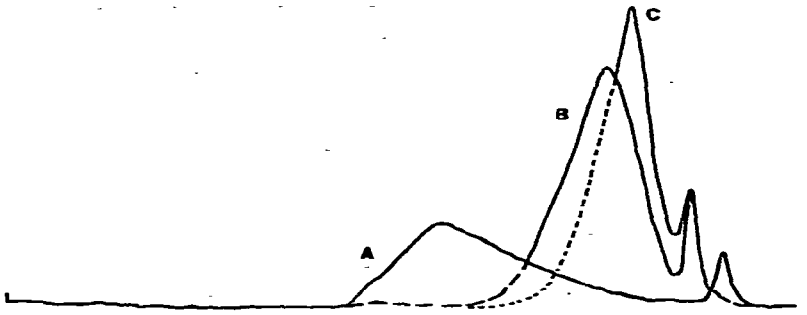


Fig. 6. Chromatograms of PDMDAAC samples. $\bar{M}_w = 4.5 \cdot 10^5$ (A); $5.4 \cdot 10^4$ (B); $5.2 \cdot 10^3$ (C). G5000 PW + G3000 PW, 0.2 *M* NaCl.

10 in 0.1 *M* NaCl. GPC retention times correlate with molecular volumes, as measured for example by $[\eta]M$, rather than molecular weights. Apparent MWs based on PEO elution may be low simply because PEO, primarily by virtue of its large contour length per unit mass, has a small ratio of molecular weight to molecular volume. Comparisons of MWs in Table I are also obscured by the difference between the measured MW, typically close to $\bar{M}_w$ or $\bar{M}_v$, and that of the species eluting at the chromatographic peak, usually intermediate between $\bar{M}_w$ and $\bar{M}_n$. Hence, low apparent GPC MW values do not of themselves provide evidence for adsorption. On the other hand, we observe increased retention relative to PEO (*i.e.*, lower M_p^{PEO}) at the lower ionic strength. This effect, contrary to that expected from changes in polymer dimensions due to electrostatic screening, is suggestive of polymer–substrate interactions. Separation of the effects of molecular dimensions from those of adsorption may best be accomplished by universal calibration methods.

TABLE I

EXPECTED MWs OF CATIONIC POLYMERS AND APPARENT GPC MOLECULAR WEIGHTS (RELATIVE TO POLYETHYLENE)

Sample	*Reported MW (method)*	*Apparent MW, M_p^{PEO}**	
		0.1 M NaCl	*0.2 M NaCl*
PDMDAAC	$4.5 \cdot 10^5$ (viscosity, light-scattering)	$5.0 \cdot 10^4$	$2.0 \cdot 10^5$
PDMDAAC	$1.2 \cdot 10^4$ (viscosity, light-scattering)	$1.2 \cdot 10^3$	$3.0 \cdot 10^3$
PDMDAAC	$5.2 \cdot 10^3$ (viscosity, light-scattering)	$8.0 \cdot 10^2$	$1.6 \cdot 10^3$
PEI	$1.4 \cdot 10^4$ (ultracentrifugation)[9]	$2.0 \cdot 10^3$	$4.5 \cdot 10^3$
PEI	$4.4 \cdot 10^3$ (ultracentrifugation)	$6.5 \cdot 10^2$	$2.0 \cdot 10^3$
PVA	$6.5 \cdot 10^5$ (osmometry and GPC of PVAc)[11]	**	$7.7 \cdot 10^4$

* From peak elution volume and PEO calibration curve.
** No elution.

Universal calibration

It has been amply demonstrated on both theoretical and experimental grounds that the product of intrinsic viscosity and MW determines, for all polymers in a given column–solvent system, the GPC distribution coefficient and hence the elution

volume. Thus, the congruence of $[\eta]M$ *vs.* V_e data for a variety of polymers with differing functional groups is strong evidence for the absence of non-steric effects. MW and viscosity data used for universal calibration plots in 0.1 *M* and 0.2 *M* NaCl are assembled in Table II. For non-ionic polymers such as PEO, PVAc and dextran, we may neglect the influence of salt on viscosity and employ the values of $[\eta]$ found in pure water. This approximation is supported by findings for PEO[17] and amylose[18]. For PEI, viscosity data obtained in 0.1 *M* NaCl were used[10]; in the pH range of the chromatography, 9–10, the change in viscosity with a two-fold increase in ionic strength is negligible[10]. Viscosities of PDMDAAC were measured only in 0.1 *M* NaCl; a small (*ca.* −10%) correction was made for 0.2 *M* NaCl, according to previously established relationships[9]. The intrinsic viscosity of β-lactoglobulin was obtained from a viscosity–MW relationship constructed from data for globular proteins[19].

TABLE II

MW AND VISCOSITY DATA FOR POLYMERS OF THIS STUDY

Polymer	*MW*	$[\eta]$ (dl g^{-1})	$[\eta]M$
PEO	$1.0 \cdot 10^5$*	0.93	$9.3 \cdot 10^4$
	$1.4 \cdot 10^4$**	0.25	$3.5 \cdot 10^3$
	$8.0 \cdot 10^3$**	0.18	$1.4 \cdot 10^3$
	$4.5 \cdot 10^3$**	0.13	$5.7 \cdot 10^2$
	$1.4 \cdot 10^3$**	0.070	$1.0 \cdot 10^2$
PVAc	$2.9 \cdot 10^5$	1.11**	$3.2 \cdot 10^5$
	$1.5 \cdot 10^5$	0.79	$1.2 \cdot 10^5$
	$1.7 \cdot 10^4$	0.25	$4.1 \cdot 10^3$
	$3.0 \cdot 10^3$	0.10	$3.0 \cdot 10^2$
Dextran	$7.3 \cdot 10^4$	0.28***	$2.0 \cdot 10^4$
	$4.0 \cdot 10^4$	0.21	$8.4 \cdot 10^3$
	$9.7 \cdot 10^3$	0.10	$9.7 \cdot 10^2$
PEI	$1.4 \cdot 10^4$	0.14[§]	$2.0 \cdot 10^3$
	$7.2 \cdot 10^3$	0.10[6]	$7.6 \cdot 10^3$
	$4.4 \cdot 10^3$	0.077	$3.4 \cdot 10^2$
β-Lactoglobulin	$1.8 \cdot 10^4$	0.034	$6.2 \cdot 10^2$
PDMDAAC	$4.5 \cdot 10^5$	0.80[§§]	$3.6 \cdot 10^5$
	$5.4 \cdot 10^4$	0.30	$1.6 \cdot 10^4$
	$1.6 \cdot 10^4$	0.14	$2.2 \cdot 10^3$
	$1.2 \cdot 10^4$	0.12	$1.4 \cdot 10^3$
	$5.2 \cdot 10^3$	0.070	$3.6 \cdot 10^2$

* From literature viscosity–MW data in pure water[13–16]. We neglect the influence of NaCl on the viscosity–MW relationship of PEO since 0.1 *M* KCl depresses the viscosity of high-molecular-weight PEO by less than 5% (see ref. 17).

** From viscosity–MW relationship in pure water[12] (see text).

*** Viscosity data in pure water supplied by manufacturer (see text).

§ Measured in 0.1 *M* NaCl at pH 10[10].

§§ Measured in 0.1 *M* NaCl (see text).

The samples of PVAc, PEI, dextran, and all but the high-molecular-weight PEO were fractions of narrow distribution and it was possible to plot $[\eta]M$ *vs.* peak elution volume without regard for the differences between the viscosity- or weight-average MW, and that corresponding to the chromatographic peak. On the other hand, this procedure could not be applied to the PDMAAC samples which were polydisperse with $\bar{M}_w/\bar{M}_n$ ranging from 2 to 10. (Many workers disregard this point and incorrectly identify the measured value of $J = [\eta]M$ —closest to the value of J for the hypothetical species having the viscosity average MW— with the peak elution volume.). An iterative procedure[20] was employed to calculate the elution volumes corresponding to $\bar{M}_w$ from the chromatograms of the PDMDAAC samples; this value, V_{M_w}, is better aligned with $[\eta]M$ than is the peak elution volume.

Universal calibration plots in 0.1 *M* and 0.2 *M* NaCl are shown in Fig. 7A and 7B, respectively. In the latter solvent, the data for PDMDAAC, PEO, dextran, PVAc and the globular protein β-lactoglobulin coincide with a single line, while the data for PEI show progressive deviations towards greater retention with increasing MW. In 0.1 *M* NaCl, the data for PDMDAAC are uniformly displaced to higher elution volumes, while those of the other cationic polymer, PEI, show clear evidence of adsorption with increasing MW.

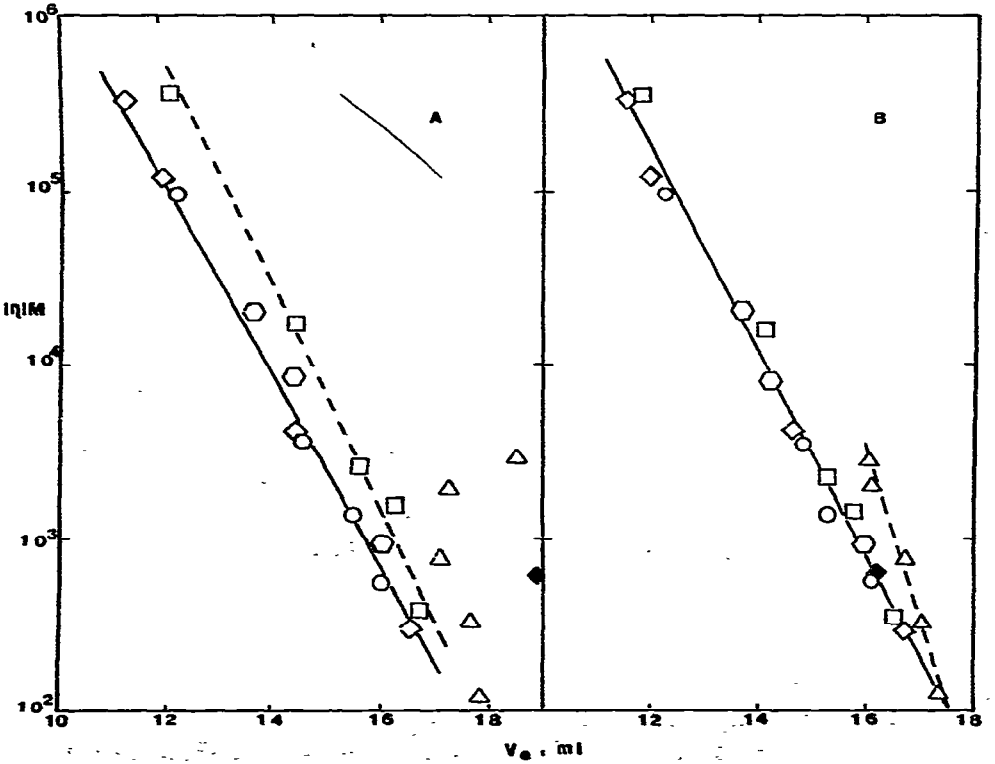


Fig. 7. Universal calibration plots for G5000 PW + G3000 PW in 0.1 *M* NaCl (A), 0.2 *M* NaCl (B). ○, PEO; ○, dextran; ◇, PVAc; □, PDMDAAC; △, PEI; ◆, β-lactoglobulin. V_e for PDMDAAC corresponds to M_w (see text).

The influence of ionic strength on the elution of PDMDAAC samples is illustrated further by the superimposed chromatograms of Fig. 8. As noted above, the shift to larger retention volumes with lower ionic strength cannot be explained by an alteration in molecular dimensions which should yield the opposite result. We may hypothesize that the presence of some anionic functional groups on the gel result in favorable interactions with the cationic polymers which in turn are screened by increased electrolyte concentration. Such an argument, however, is not in accord with the more dramatic retention of PEI, since that polymer should exhibit a positive charge density substantially lower than PDMDAAC. The unusual retention behavior of both PEI and PVA in their nearly un-ionized forms may indicate that hydrophobic interactions are also factors in gel chromatography with PW columns.

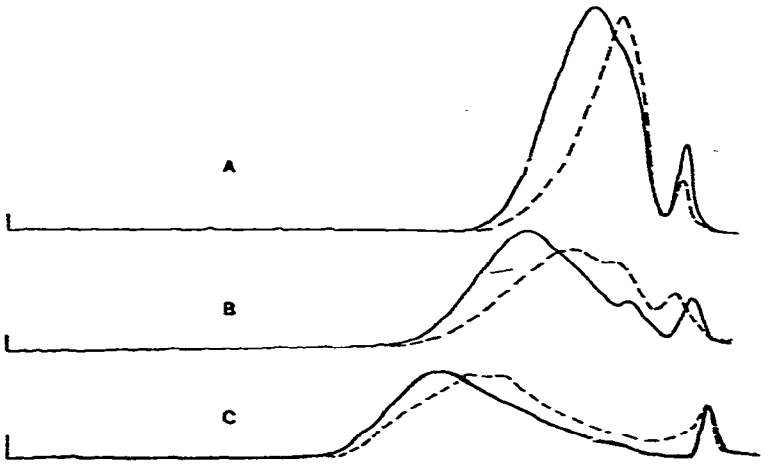


Fig. 8. Chromatograms of PDMDAAC samples in 0.1 *M* NaCl (——) and 0.2 *M* NaCl (—). $\bar{M}_w = 1.2 \cdot 10^4$ (A); $5.4 \cdot 10^4$ (B); $4.5 \cdot 10^5$ (C).

CONCLUSIONS

PW columns may be used for GPC analysis of polycations. Under appropriate ionic strength conditions, universal calibration data reveal no adsorptive effects for strongly cationic polymers. Weak polybases, namely polyethyleneimine and polyvinylamine, display more complex behavior and exhibit retarded elution, particularly at lower ionic strength. While evidence exists for both electrostatic and hydrophobic solute–gel interactions, these factors have yet to be resolved.

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