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Note

Size-exclusion chromatography of cationic polyelectrolytes on Superose gel

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The surfaces of stationary phases used in aqueous size-exclusion chromatography (SEC) often contain polar functional groups which may interact with the macromolecules being chromatographed, leading to elution volumes different from those expected on the basis of size alone. Electrostatic adsorption and exclusion effects are prominent in aqueous chromatography because ionic groups are present in most aqueous SEC packings[1]. Hydrophobic interactions of amphiphilic substances with gel matrices can also occur, and these have been investigated by several workers[2–4].

Because most stationary phases for aqueous SEC bear a negative charge[1], substrate–macromolecule interactions have been especially problematic for polycations, often leading to irreversible adsorption. Such polyelectrolytes are valuable industrial chemicals in areas which involve flocculation, such as water clarification, sewage sludge dewatering and paper processing. Since the molecular weight distribution of polymers plays a central role in these applications, the availability of high-efficiency SEC columns for polycations has major consequences for these technologies.

Several groups have investigated derivatized silica-based packings for SEC of polycations. Talley and Bowman[5] grafted porous silica beads with reagents bearing quaternary ammonium groups and successfully chromatographed poly(2-vinylpyridine) in acidic media. A similar type of packing was investigated by Domard and Rinaudo[6] who observed universal calibration in 0.2 *M* ammonium acetate, indicating that this ionic strength was sufficient to eliminate electrostatic exclusion. A silica-based stationary phase, Fractosil (E. Merck, Darmstadt F.R.G.), was derivatized with dimethylaminoethanol (DMAE) by Stickler and Eisenbeiss[7]. Low-pH conditions provided by 0.1 *M* nitric acid in the eluent were utilized to protonate residual aminosilane groups and to keep any unsilanized hydroxyl groups in the uncharged state. A solvent ionic strength of 0.1 *M* was sufficient to suppress strong coulombic repulsive effects. However, the common calibration standards, dextran and poly(ethyleneoxide) (PEO), were found to adsorb onto the packing, thus requiring calibration using samples of the analyte itself. In addition, these three packings are still sensitive to ionic effects, and, at any rate, are not commercially available.

It is apparent that the use of quaternarized silica-based packings for SEC of polycations has limitations. The intense charge density of cationic bonded phases can exclude cationic polymers from the pores, and the high ionic strengths needed to

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suppress these effects may limit polyion solubility. Also, non-derivatized silanol groups present on the packing appear to be active, as evidenced by the adsorption of non-ionic hydrogen-bond acceptor polymers such as PEO.

Limited studies suggest that the goal of efficient and non-adsorptive SEC of polycations may be better met with organic hydrophilic gels than with derivatized silica. The problems of ionic exclusion and interactions with underivatized silanol groups are not encountered with semi-rigid neutral polymeric gels. Thus, characterization of cationic polymers has been accomplished with the hydrophilic cross-linked polyether PW gel packings[8,9]. Universal calibration studies revealed that ionic effects could be controlled using mobile phases of moderate ionic strength, such as 0.2 *M* sodium chloride[10]. However, even modest reduction in ionic strength led to retention of polycations because of the presence of residual carboxylic acid groups on the packing[11]. It is also important to note that PW gel displays strong hydrophobic interactions with amphilic solutes[12].

Superose (Pharmacia) is a cross-linked, agarose-based medium, recently developed for high-performance gel filtration of biomolecules. While small concentrations of sulfate and carboxylic groups are inherent in agarose, chromatographic studies with proteins and nucleic acids revealed that 0.15 *M* sodium chloride in the mobile phase suppresses ionic interactions between the charged macromolecules and anionic sites on the packing[13].

The current report describes the chromatography of cationic polymers on a commercially available Superose gel column used extensively in high-speed SEC of biomolecules. An aqueous mobile phase of low pH and moderate ionic strength was employed to reduce the adsorption of the solute molecules.

EXPERIMENTAL

Four types of synthetic cationic polymers were studied. Poly(dimethyldiallylammonium chloride) (PDMDAAC) was obtained from Calgon (Pittsburgh, PA, U.S.A.), with nominal molecular weigths of $1 \cdot 10^4$, $3 \cdot 10^4$, $5 \cdot 10^4$, $2 \cdot 10^5$ and $1.5 \cdot 10^6$. Poly(methacrylamidopropyltrimethylammonium chloride) (PMAPTAC) samples, donated by Clairol Research Laboratory (Stamford, CT, U.S.A.), had nominal average molecular weights of $5 \cdot 10^4$, $8.7 \cdot 10^4$, $2 \cdot 10^5$ and $4.3 \cdot 10^5$. Samples of poly(ethyleneimine) (PEI) with average molecular weights of $7 \cdot 10^3$ and $5 \cdot 10^4$ were from Polysciences (Warrington, PA, U.S.A.).

Exclusion chromatography was carried out on an apparatus comprised of a Minipump (Milton Roy), a Model 7012 injector (Rheodyne) equipped with a 100-μl loop, and an R401 differential refractometer (Waters). A Superose-6 column (30 cm × 1 cm O.D.) (Pharmacia) was eluted at 0.52 ml/min.. Column efficiency, determined with ^2H_2O, was at least 12 000 plates per meter.

The selection of mobile phase was based on previous studies utilizing a variable size simplex method[14]. Two variables, the mobile phase pH and ionic strength, were altered simultaneously until separations of proteins exhibited near-ideal behavior. The optimum solvent conditions of pH 5.5 and an ionic strength of 0.38 phosphate buffer were thus determined for SEC of proteins on Superose gel[15]. In this work, 0.4 *M* of sodium chloride–sodium acetate (9:1), (pH 5.5) was employed as the mobile phase. Sodium chloride and sodium acetate were substituted for phosphate because

polycations may precipitate during long-term storage in phosphate buffer, a phenomenon not observed with sodium acetate.

Polymers were dissolved in the mobile phase and filtered (0.20 μm Millipore). The concentration of polymer injected onto the column was approximately 0.3% (w/w), corresponding to a refractive index detector attenuation of 8 ×.

RESULTS AND DISCUSSION

The results of this investigation provide evidence for the non-adsorptive SEC of polycations using Superose gel packing. The chromatograms of PDMDAAC samples are shown in Fig. 1. While it is evident that the polymers are highly polydisperse, the distorted elution profiles characteristic of adsorbed polymers are not observed in these chromatograms. The peak at the high-molecular-weight end, for the two highest-molecular-weight samples, corresponds to the exclusion volume of the column, while the small peak near the low-molecular-weight end of the chromatograms arises from the presence of monomer. The high resolution of these chromatograms is also inconsistent with adsorption.

Adsorptive effects result in elution volumes greater than expected from size exclusion alone. Thus, adsorption vitiates the chromatographic preparation of molecular-weight fractions. Eleven 1-ml fractions were collected during the elution of the $3 \cdot 10^4$ mol.wt. PDMDAAC sample, and four of these fractions were reinjected, with the results shown in Fig. 2. These reinjected samples were found to possess elution volumes identical to the fractionation volumes, which is consistent with separation by molecular weight.

The absence of adsorption effects may be substantiated by demonstrating universal calibration for polymers with widely varying composition and charge state, because uniform dependence of elution volume on $J \equiv [\eta]M$ (where $[\eta]$ = intrinsic

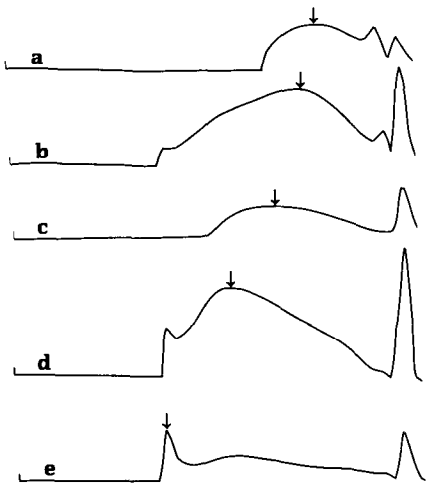

Fig. 1. Chromatograms of PDMDAAC with nominal molecular weight values: (a) $1 \cdot 10^4$; (b) $3 \cdot 10^4$; (c) $5 \cdot 10^4$; (d) $2 \cdot 10^5$; (e) $1.5 \cdot 10^6$. Peak at low-molecular-weight end is salt imbalance peak. Peak elution volumes are indicated by arrows.

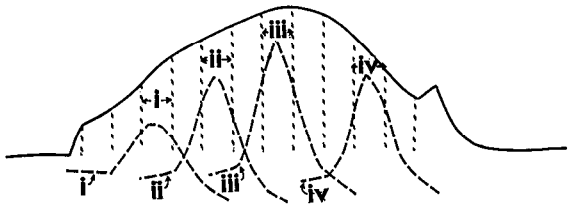

Fig. 2. Fractionation of PDMDAAC with a molecular weight of $3 \cdot 10^4$.

viscosity and M = molecular weight) only occurs if peak migration depends on molecular dimensions alone. For the highly polydisperse polycations of this investigation, universal calibration demands significant data manipulation[16]. Plotting J as a function of peak elution volume in the usual manner neglects differences between the molecular weight of the component eluting at the chromatographic peak (M_p) and the two moments of the distribution that correspond, respectively, to the measured values of $[\eta]$ and M. Thus, the difficulty of assigning a value of J to the species eluting at the chromatographic peak reduces the usefulness of peak elution volumes. Without undertaking the imposing treatment required for universal calibration, we still find evidence for the absence of adsorption effects from comparisons of peak retention volumes and reported molecular weight values.

Calibration plots of molecular weight *vs.* peak elution volume (V_e) are displayed in Fig. 3. Molecular weight values corresponding to the chromatographic peaks of the PDMDAAC samples were obtained by SEC–low-angle laser-light scattering as $2.5 \cdot 10^4$, $2.9 \cdot 10^5$ and $4.3 \cdot 10^5$ for nominal molecular weight values of $1.0 \cdot$

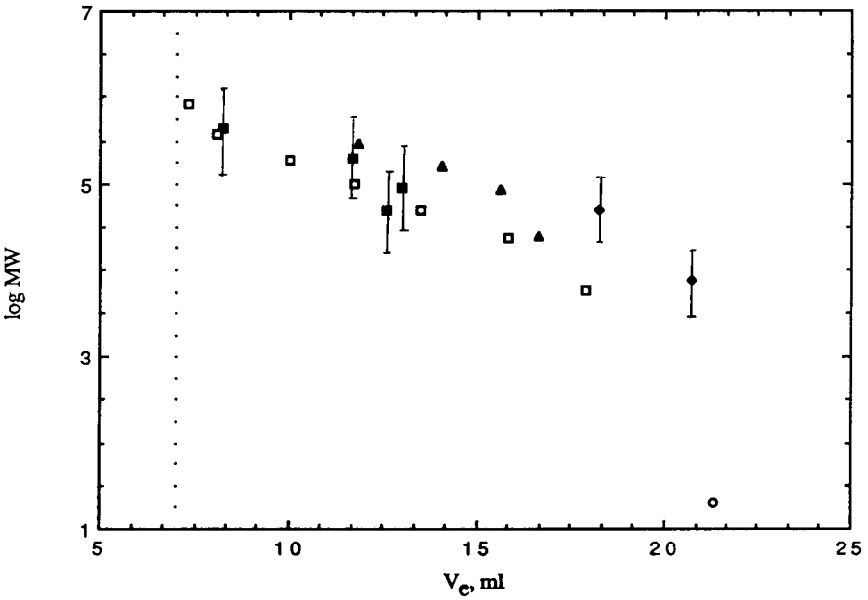

Fig. 3. Dependence of peak elution volume on nominal molecular weight (MW): □ = pullulan; ▲ = PDMDDAC; ■ = PMAPTAC; ◆ = PEI; ○ = ^2H_2O. Dotted line is the column exclusion volume from the elution of blue dextran.

10^4, $2.0 \cdot 10^5$ and $1.5 \cdot 10^6$, respectively[17]. The M_p values of the $3.0 \cdot 10^4$ and $5.0 \cdot 10^5$ samples were interpolated from their elution volumes as $8.5 \cdot 10^4$ and $1.6 \cdot 10^5$. For all other samples, the vertical bars represent the expected maximum difference between the nominal molecular weight and M_p.

In addition to the discrepancy between nominal molecular weight and M_p, the calibration curves for the different polymers would be expected to diverge because of differences in hydrodynamic volumes, *i.e.* J at constant M. Utilizing the relationship of $J = KM^{1+a}$, and literature values for the Mark–Houwink constants in the equation $[\eta] = KM^a$, for pullulan ($K = 1.79 \cdot 10^{-4}$dl/g, $a = 0.67$)[18] and for PDMDAAC ($K = 5.0 \cdot 10^{-5}$dl/g, $a = 0.72$)[19] in 0.4 M ionic strength solvents, the size differences between the two molecular species at various molecular weight values may be calculated. This analysis shows that, as constant J, $M_{\text{PDMDAAC}} \cong 2 \cdot M_{\text{Pullulan}}$, in close agreement with the typical separation of the data points for these two polymers in Fig. 3. Deviations among the calibration curves in excess of the amount expected would suggest adsorption. Instead, Fig. 3 reveals that divergence of the data for PDMDAAC, PMAPTAC and pullulan are within the range expected from considerations of size differences.

The large elution volumes associated with PEI may be due to the structure of the polycation: the extensive branching of PEI results in a greater degree of compactness. Less anomolous behavior of PEI in a methanol–water mobile phase would support these speculations.

CONCLUSION

Superose gel columns may be used for SEC analysis of polycations. Under appropriate ionic strength conditions, elution curves reveal no adsorptive effects for strongly cationic polymers.

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