

Solution properties of polyelectrolytes

IX[☆]. Quantitative dependence on eluent ionic strength of elution volumes in aqueous size-exclusion chromatography

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ABSTRACT

Quantitative evaluation of elution volumes (V_e) of polyelectrolytes in salt-containing eluents was performed, taking into account electric double-layer effects and the effective radius of polyions, and assuming that polyelectrolytes behave as rigid hydrodynamic spheres and that the geometry of gel pores is cylindrical. A polynomial V_e dependence on ionic strength (I) was obtained, namely a second order one in $I^{-1/2}$. The semi-empirical polynomial coefficients depend on column characteristics, namely V_0 (interstitial volume), V_p (total pore volume) and a (mean pore radius), as well as on effective coil radius (R_g) and on k (a parameter related to the electric effects). Fair correlations between predicted and measured elution volumes for uncharged polymers hold, at least for the polyelectrolyte–gel systems tested here: polyelectrolytes poly(L-glutamic acid), sodium poly(styrene sulphonate) and poly(acrylic acid); gels Spherogel TSK PW4000 and Ultrahydrogel 250.

INTRODUCTION

Aqueous size-exclusion chromatography (ASEC) has become a useful analytical and preparative technique for separating macromolecules of different size, shape and charge. However, several problems associated with ASEC, termed non-exclusion or secondary effects, usually interfere with a pure size-exclusion mechanism.

According to Barth [1], secondary effects can be divided into two types, ionic and adsorption effects. Ion-exchange, ion-inclusion, ion-exclusion and intramolecular electrostatic effects are

of the first type, and hydrogen bonding, hydrophobic and attractive ionic interactions are of the second type. A considerable literature on these effects exists [2–4].

Electrolyte-containing eluents are often used for the total suppression or minimization of the ionic effects. However, when the ionic strength of the eluent increases, the elution profile for a given polyelectrolyte becomes broader, so that a poorer resolution is achieved experimentally; the reasons for this behaviour are still unclear [5].

The use of salt-containing eluents affects the solution properties of the polyion as well as its elution volume in ASEC. On the one hand, the intrinsic viscosity of a polyelectrolyte decreases sharply with increasing ionic strength [6–9], as would be expected by the increasing screening of polyion charge. On the other hand, the shrink-

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☆ For part VIII, see ref. 21.

age of the coil with increasing ionic strength is not the only effect responsible for the shifts towards higher elution volumes of polyelectrolyte occurring in ASEC in the presence of extra salts. Other effects, such as the diminution of charge density on a gel surface in the presence of salts, should also be taken into account [10]. However, to date, a quantitative evaluation of each contribution has not been undertaken.

In this context, considerable efforts have been made to quantify the aforementioned ASEC secondary effects, most of them based on experimental evidence obtained with model charged polymers on packing materials of very different nature. The model of Dubin and co-workers [11–13] to predict ion-exclusion effects based on the reduction in the pore volume accessible to polyions deserves mention. They calculate a repulsion volume as a function of an electrostatic potential of the stationary phase. An empirical correlation between the repulsion volume and eluent ionic strength based on the Debye–Hückel theory has also been proposed by Mori [14]. Styring and co-workers [15,16] have established a semi-empirical approach that takes into account exclusively the electrostatic double layer around the polyion and proposes a linear dependence of the elution volumes with the inverse of the square root of the ionic strength (I). Recently, Potschka [17] has reported on the ionic strength dependency of elution in SEC of DNA, proteins and viruses, and has found that chromatographic radii of these macromolecules increase linearly with $I^{-1/2}$. We have also reported an empirical relationship in terms of polymer-support compatibility accounting for solute–matrix attractive/repulsive interactions [10]. It makes use of the thermodynamic formalism previously developed for uncharged polymers [18,19]. Finally, Hoagland [20] has formulated a theoretical treatment on electrostatic interactions of rod-like polyelectrolytes with repulsive, charged surfaces.

In this paper, the dependence on ionic strength of elution volumes of polyions in ASEC is studied. Shifts in elution volumes are quantitatively interpreted in terms of coil dimensions, as defined by hydrodynamic effective radii, mean pore radius of gel and electrical double-layer

effects. Chromatographic data reported in an earlier paper [21], for sodium poly(styrene sulphonate), poly(acrylic acid) and poly(L-glutamic acid) at different ionic strengths, have been used to test the validity of the proposed semi-empirical equation.

EXPERIMENTAL

Chemical and reagents

Dextran samples purchased from Pharmacia (Uppsala, Sweden) with nominal molar masses of 10 000, 17 700, 40 000, 66 900, 83 300, 170 000, 500 000 and 2 000 000 g mol⁻¹, were used as the standards for uncharged polymers. The chromatographic low-molar-mass range was covered by poly(ethylene oxide) (PEO) standards with molar masses 2000 and 4000 g mol⁻¹, from Fluka (Darmstadt, Germany). The polyelectrolytes tested were samples of poly(L-glutamic acid) (PGA) from Sigma (St. Louis, MO, USA), sodium poly(styrene sulphate) (PSS) from Pressure Chemical (Pittsburgh, PA, USA) and poly(acrylic acid) (PAA) from Aldrich (Milwaukee, WI, USA). Their nominal molar masses (in g mol⁻¹) and the code used along the paper were: 13 600 (PGA-1), 43 000 (PGA-2), 77 800 (PGA-3), 1600 (PSS-1), 16 000 (PSS-2), 31 000 (PSS-3), 88 000 (PSS-4), 177 000 (PSS-5), 5000 (PAA-1), 90 000 (PAA-2) and 250 000 (PAA-3). All samples showed polydispersities lower than 1.1.

Solvents used for viscometric measurements and as eluents in SEC were buffers made up from sodium dihydrogenphosphate and disodium hydrogenphosphate for pH 7.0 and from sodium acetate and acetic acid for pH 5.0. Desired ionic strengths were adjusted from 0.005 to 0.20 *M*. Reagents used in the preparation of buffers were analytical grade from Merck (Darmstadt, Germany).

Viscosities

Intrinsic viscosity values [η] for uncharged polymers in pure water at 25.0 ± 0.1°C were evaluated through the viscometric equations [η] = 97.8 · 10⁻³ *M*^{0.50} ml g⁻¹ for dextran [22] and [η] = 2.0 + 0.016 *M*^{0.76} ml g⁻¹ for PEO [23], where *M* stands for molar mass. The effects of

ionic strength and pH on the viscosity of non-ionic polymers were neglected [11].

Viscosity measurements of polyelectrolyte samples at $25.0 \pm 0.1^\circ\text{C}$ were performed with an automatic AVS 440 Ubbelohde-type capillary viscometer from Schott Geräte (Hofheim, Germany). Details of the experimental conditions and procedure have been reported previously [21].

Chromatography

The liquid chromatographic equipment has been described elsewhere [21]. The columns used were an Ultrahydrogel 250 (UHG-250) packed with hydroxylated poly(methacrylate)-based gel of 250 Å nominal pore size and 30×0.78 cm I.D. from Waters (Milford, MA, USA), and a Spherogel TSK PW4000 packed with hydroxylated polyether copolymer of 500 Å nominal pore diameter and 30×0.75 cm. I.D. from Beckman Instruments (Galway, Ireland). Experimental details for obtaining chromatograms of samples and their elution volumes have been reported in an earlier paper [21]. Exclusion volumes, V_0 , and total column volumes, V_T , were 5.48 and 10.46 ml for the UHG-250 column, and 5.15 and 10.40 ml for the TSK one, as determined with blue dextran ($M = 2\,000\,000$ g mol⁻¹) and ²H₂O, respectively.

RESULTS AND DISCUSSION

Intrinsic viscosity, $[\eta]_I$, like other properties of polyelectrolytes in aqueous solution, follows a linear dependence with the inverse square root of the ionic strength:

$$[\eta]_I = [\eta]_\infty + SI^{-1/2} \quad (1)$$

where $[\eta]_I$ stands for the intrinsic viscosity at ionic strength I , $[\eta]_\infty$ is the intrinsic viscosity extrapolated at infinite ionic strength and S is related to the stiffness (or the flexibility) of the macromolecule according to the Odijk's treatment [24]. Plots of eqn. 1 for the polyelectrolyte-gel systems studied in this work have been reported previously [21], and all of them exhibit a good linear correlation.

The hydrodynamic radius or effective radius of

neutral polymers, R_η , and of polyelectrolytes, R'_η , are related to the hydrodynamic volume, $M[\eta]$, through [25]:

$$R_\eta = \left(\frac{3 \cdot 10^{23} M[\eta]}{\pi N_A} \right)^{1/3} \quad (2a)$$

and

$$R'_\eta = \left(\frac{3 \cdot 10^{23} M[\eta]_I}{\pi N_A} \right)^{1/3} \quad (2b)$$

respectively, where M is the macromolecule molar mass and N_A Avogadro's number. Units for R_η are Å if $[\eta]$ is in ml g⁻¹.

On the other hand, Styring and co-workers [15,16] proposed a model to explain the variation in elution volumes of polyelectrolytes with ionic strength. This model, based on the assumption that the polyion electric double-layer thickness in the Gouy-Chapman approximation varies with $I^{-1/2}$, as $[\eta]_I$ does, leads to:

$$R'_\eta = R_\eta + kI^{-1/2} \quad (3)$$

where k is a constant in which the double-layer parameters are enclosed, and the intercept values should correspond to the physical (or geometrical) radius of the uncharged polyelectrolyte due to the screening of charges by the counterions occurring along the chain at high ionic strength, that is when $I \rightarrow \infty$.

Eqn. 3 plots for PGA, PSS and PAA are shown in Fig. 1a–c. As can be seen, all systems show excellent linear dependencies. The slope, k , accounts for the electrostatic repulsive effect, and its value increases with the molar mass of sample (or with the charge of the macromolecule).

The elution volumes in SEC of a neutral polymer, V_e [26], and of a polyelectrolyte, V'_e [11], can be respectively written as:

$$V_e = V_0 + K_{\text{SEC}} V_p \quad (4a)$$

$$V'_e = V_0 + K'_{\text{SEC}} V_p \quad (4b)$$

where V_0 is the interstitial packing volume and V_p is the total pore volume. K_{SEC} represents the partition coefficient when the steric mechanism is the only one accounting for solute separation, and the partition coefficient K'_{SEC} takes into

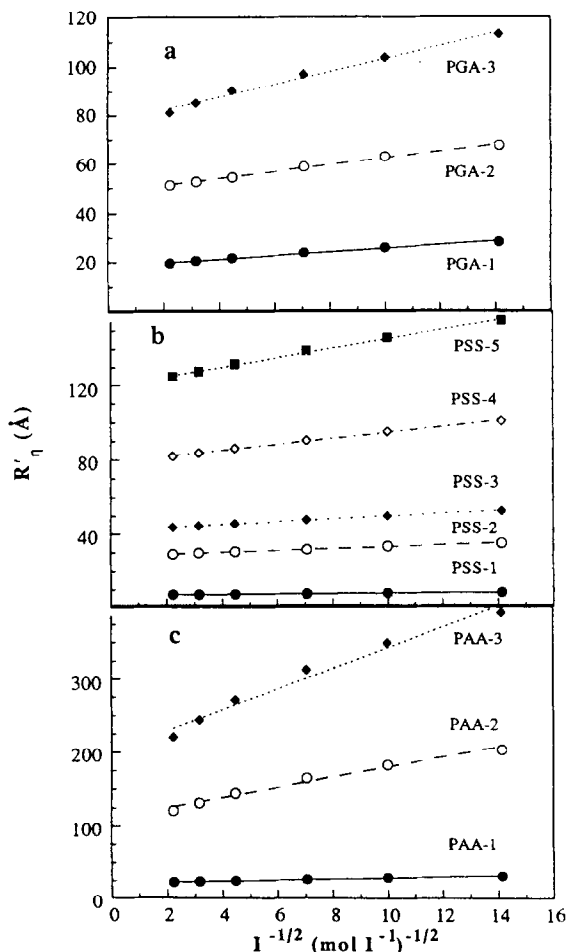


Fig. 1. Effective radius, R'_η , dependence on ionic strength, I , for different polyelectrolytes: (a) poly(L-glutamic acid), PGA; (b) poly(styrene sulphonate), PSS; (c) poly(acrylic acid), PAA.

account steric and other secondary mechanisms. Thus, $K'_{SEC} < 1$ when solute–gel electrostatic interactions become important and $K'_{SEC} > 1$ if adsorption of solute onto the stationary phase occurs. By analogy with the expression of Casassa [27] derived for the permeation of spheres in cylindrical cavities, K_{SEC} and K'_{SEC} can be written as:

$$K_{SEC} = \left(\frac{a - R_\eta}{a} \right)^2 \quad (5a)$$

$$K'_{SEC} = \left(\frac{a - R'_\eta}{a} \right)^2 \quad (5b)$$

with a standing for the mean pore radius of cylinder. By substitution of R'_η given by eqn. 3 into eqn. 5b and further introduction of the obtained K'_{SEC} in eqn. 4b, Styring *et al.* [16] found the following V'_e dependence with I :

$$V'_e = V_\infty - k_{el} I^{-1/2} \quad (6)$$

where V_∞ stands for the elution volume of a polyanion when $I \rightarrow \infty$, *e.g.* when it becomes an uncharged polymer (or when the solute–gel repulsive interactions disappear), and k_{el} is a constant containing double-layer parameters, cylinder pore radius and total pore volume.

V'_e vs. $I^{-1/2}$ plots are depicted in Fig. 2 for PGA in TSK PW4000 (Fig. 2a) and PGA in UHG-250 (Fig. 2b) columns, both at pH 7.0. As can be seen, the expected eqn. 6 dependence is not followed by the systems so far represented, as also occurs with Mori's data on PSS in an FPG(500+170) porous glass column [14], as shown in Fig. 3. Similar results have also been found for the PSS–UHG-250, PAA–TSK

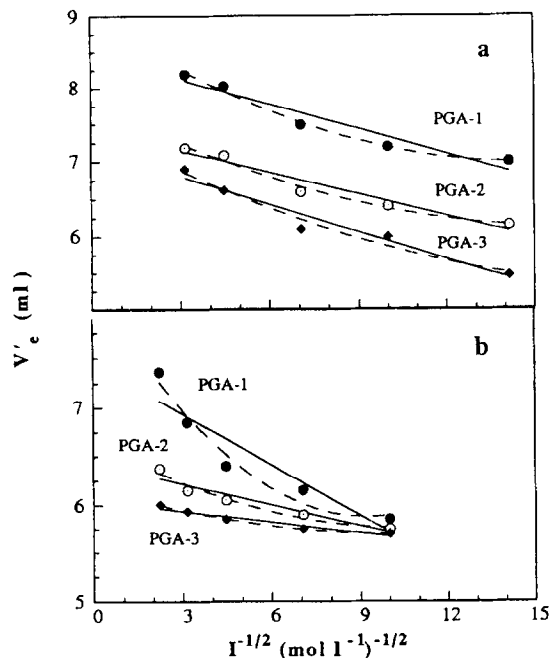


Fig. 2. Elution volume dependence on ionic strength for PGA at pH 7.0 on TSK PW4000 (a) and UHG-250 (b) columns. Points stand for experimental data, solid lines for fits according to eqn. 6 and dashed lines according to eqn. 8.

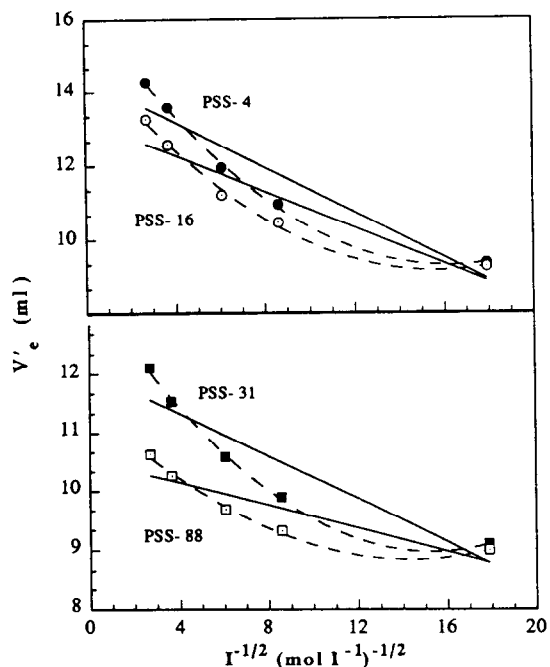


Fig. 3. Elution volume dependence on ionic strength for PSS (4, 16, 31 and 88 refer to molecular masses 4000, 16 000, 31 000 and 88 000, respectively) at pH 8.0 on an FPG(500 + 170) porous glass column (data extracted from Fig. 1 in ref. 14). Symbols as in Fig. 2.

PW4000 and PAA-UHG-250 polyelectrolyte-gel systems here studied and with other systems described previously [12,14,28].

A repetition of the above Styring manipulation, that is substitution of R'_η give by eqn. 3 into eqn. 5b with expansion now of the square term and final substitution of K'_{SEC} into eqn. 4b, leads to:

$$V'_e = V_0 + \left(\frac{a - R_\eta}{a}\right)^2 V_p - 2\left(\frac{a - R_\eta}{a}\right) \frac{k}{a} V_p I^{-1/2} + \frac{k^2}{a^2} V_p I^{-1} \quad (7)$$

or its simplified from (compare with eqns. 4a and 5a):

$$V'_e = V_e - AI^{-1/2} + BI^{-1} \quad (8)$$

where V_e is the elution volume of a polyon when $I \rightarrow \infty$ (equivalent to the V_∞ defined in ref. 16) and A and B are constants accounting for

electrostatic effects (through k) and for the characteristics of packings (through a and V_p).

When an ion-exclusion effect is the main mechanism governing SEC of polyanions, not a first-order V'_e dependence on $I^{-1/2}$, as claimed by Styring *et al.* [15], is obeyed, but a second-order one in agreement with eqn. 8 and the experimental results depicted in Figs. 2 and 3. In order to confirm that predicted V_e values obtained from eqn. 8 are more appropriate than those predicted by means of eqn. 6, in Fig. 4 both sets of V_e values are shown as “universal” calibration plots $\log M[\eta]_\infty$ vs. V_e for the same systems as in Fig. 2, together with the reference curve for dextran (neutral polymer). As can be observed, predicted V_e values from eqn. 8 practically coincide with the reference calibration curve, which seems to indicate that polyelectrolyte becomes a neutral polymer, as expected, and it elutes by a steric mechanism. However, the data predicted by eqn. 6 describe far from ideal SEC behaviour,

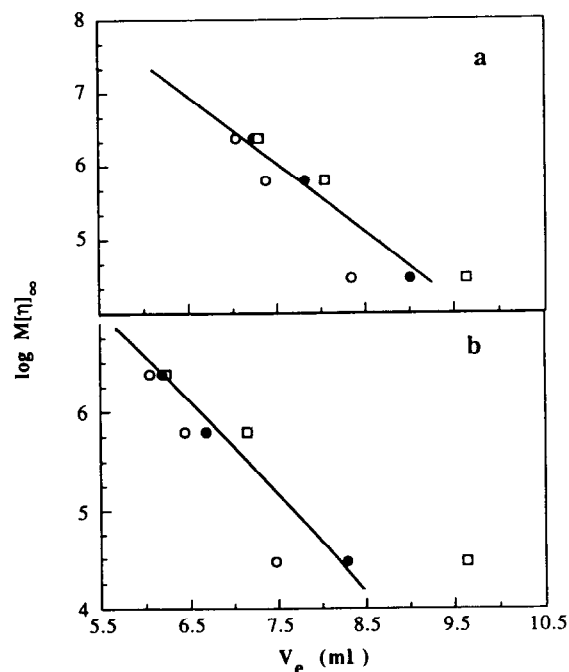


Fig. 4. Universal calibration plots for uncharged polymers. Solid line is the experimental calibration curve (dextran and PEO). Points stand for predicted values from data on PGA-TSK PW4000 (a) and on PGA-UHG-250 (b) systems: \circ = from eqn. 6; \bullet = from eqn. 8; \square = assuming spherical pore geometry.

the more so the lower the molar mass of samples. Universal calibration graphs for PSS in an FPG(500 + 170) column together with the reference pullulan one [14] are shown in Fig. 5. Again, the best prediction of elution volumes when $I \rightarrow \infty$ is achieved through the intercepts of eqn. 8, as is also the case for the other systems tested so far. Therefore, V_e' dependence on ionic strength seems to obey to a second-order polynomial on $I^{-1/2}$, at least in the range of low to moderate ionic strength, whereas electrostatic factors are the dominant ones in the SEC mechanism.

On the other hand, some comments on the assumption made about the geometry of pores deserves to be made. Exponent 2 in eqn. 5 indicates that the most likely pore shape is cylindrical. If a spherical geometry is assumed, the above-mentioned exponent should be 3, and therefore an expression for V_e' dependent on $I^{-3/2}$ should be obtained. When fitting the experimental data to a third-order polynomial on $I^{-1/2}$, the corresponding intercepts yield overestimated values of V_e and theoretical calibration plots appear on the right-hand side of the reference one, as shown, as an example, in Figs. 4 and 5.

As a conclusion, a geometrical model in which the polymers are treated as rigid equivalent hydrodynamic spheres (see eqn. 2) and the pores as well-defined cylinders (see eqn. 5) seems to be

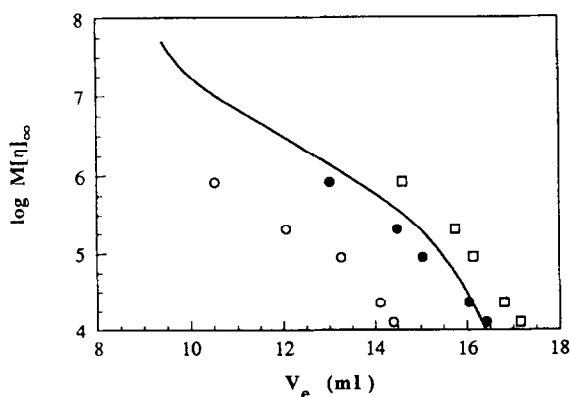


Fig. 5. Universal calibration plots for uncharged polymers. Solid line is the experimental calibration curve (pullulan). Points stand for predicted values from data on a PSS-FPG(500 + 170) system (see ref. 14). Symbols as in Fig. 4.

the most appropriate to describe polyanion elution in aqueous SEC. However, the aforementioned treatment is intended to be a first approximation to the real problem, since it essentially neglects the potential due to the surface of the stationary phase, although this last contribution does not seem to be significant in organic-based hydrophilic gels, as it is the present case. A most rigorous treatment is currently in progress.

ACKNOWLEDGEMENTS

This work was partially supported by Grant No. PB91-0808 from DGICYT (Spain). One of the authors (I.P.) was a recipient of a long-term fellowship from Ministerio de Educación y Ciencia (Spain). We are also grateful to the Secretaría de Estado de Universidades (Spain; Grant No. OP90-0042) and to the Conselleria d'Educació i Ciència (Generalitat Valenciana, Spain) for computer support.

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