

## Solution properties of polyelectrolytes

# X<sup>☆</sup>. Influence of ionic strength on the electrostatic secondary effects in aqueous size-exclusion chromatography

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### ABSTRACT

The retention behaviour of polyelectrolytes in aqueous size-exclusion chromatography (SEC), where electrostatic repulsion is the main secondary effect affecting to the separation mechanism, was investigated. A theoretical treatment was developed in order to establish the influence of the mobile phase ionic strength on calibration graphs, often used for the characterization of polyions by SEC. A master equation, derived in closed form, involving two terms related to the net charge of polyion and the residual surface charge on the gel packing, was derived. The formalism provides the basis for a more detailed analysis of chromatographic retention data in electrostatic interaction systems. Moreover, the introduction of some approximations in the original equation served to obtain an equivalent expression that is easier to use, and in which the functionality with respect to the ionic strength,  $I$ , remains unaltered. Reported data on the elution of sodium polystyrene sulphonate and poly(L-glutamic acid) from both organic and silica-based packings were used to test the goodness of the predictions carried out with the above-mentioned equations.

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### INTRODUCTION

Partially or totally dissociated ionic polymers in aqueous media display electrolyte behaviour. Localized positive and/or negative charges on the lateral groups of the polymer chain confer on polyelectrolytes specific properties different to those of uncharged polymers. In this context, whereas size-exclusion chromatography (SEC) of synthetic polymers in organic solvents is a useful tool for the evaluation of their molecular mass averages and molecular mass distributions, dif-

iculties arise [1] when it is intended to evaluate those properties of polyelectrolytes from aqueous SEC. In order to obtain compatibility between a gel and water, the gels used in aqueous SEC exhibit ionic or strongly polar groups. These groups on the gel surface and the ionic atmosphere surrounding the macroion are the origin of the above difficulties [2]. Although the size-exclusion effect is the main separation mechanism in SEC, when the solute is a polyelectrolyte, interactions between its ionic atmosphere and the polar groups on the inner surface of pores interfere with the SEC process and cause secondary effects.

The distribution of polyelectrolytes inside

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\* For Part IX, see ref. 9.

charged pores is a relatively recent and interesting topic from both the theoretical and experimental points of view. Dubin and co-workers [3–5] developed a model to predict the ion exclusion effect. Their model calculates the pore volume “forbidden” to the polyion, or repulsion volume, as a function of the electrostatic potential of the stationary phase. In this context, Mori [6] proposed an empirical correlation between the repulsion volume and the ionic strength of the eluent. On the other hand, Styring *et al.* [7] focused their attention on the electrostatic behaviour of the ionic atmosphere of polyelectrolytes without paying attention to the residual charge of the gel.

On the other hand, the elution behaviour of synthetic polyelectrolytes and biopolymers has often been treated as if there were an exponential dependence of retention on salt concentration. In this context and following previous work on the SEC of polyanions [8,9], we present here a theoretical treatment of the influence of ionic strength ( $I$ ) on calibration graphs in aqueous SEC. The recently proposed functionality on  $I^{-1}$ , in contrast to that on  $I^{-1/2}$  suggested by other workers [7], is analysed and tested using previously reported chromatographic data [6–10].

## EXPERIMENTAL

### *Samples and solvents*

The standards of uncharged polymers used were dextran samples 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<sup>-1</sup> and poly(ethylene oxide) (PEO) from Fluka (Darmstadt, Germany) with molar masses 2000 and 4000 g mol<sup>-1</sup> in order to cover the chromatographic low-molar-mass range. The polyelectrolytes tested were samples of poly(L-glutamic acid) (PGA) from Sigma (St. Louis, MO, USA) and sodium poly(styrene sulphonate) (PSS) from Pressure Chemical (Pittsburgh, PA, USA). Their nominal molar masses (in g mol<sup>-1</sup>) and the abbreviations used are 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) and 177 000 (PSS-5). All samples showed polydispersities lower than 1.1.

The solvents used for viscometric measurements and as eluents in SEC were buffers made up from NaH<sub>2</sub>PO<sub>4</sub> and Na<sub>2</sub>HPO<sub>4</sub> for pH 7.0 and from NaOAc and HOAc for pH 5.0. The desired ionic strengths were adjusted from 0.005 to 0.10 M. Reagents used in the preparation of buffers were of analytical-reagent grade from Merck (Darmstadt, Germany).

### *Viscosities*

Intrinsic viscosity values [ $\eta$ ] for uncharged polymers in pure water at 25.0 ± 0.1°C were evaluated through their viscometric equations given in refs. 8 and 9. Viscosity measurements on polyelectrolyte samples at 25.0 ± 0.1°C were performed with an AVS 440 automatic Ubbelohde-type capillary viscometer from Schott Geräte (Hofheim, Germany). The details of the experimental conditions and procedure have been reported previously [8].

### *Chromatography*

The liquid chromatographic equipment has been described elsewhere [8]. The columns used were Ultrahydrogel 250 (UHG-250) packed with hydroxylated polymethacrylate-based gel of 250 Å nominal pore size (30 × 0.78 cm I.D.) from Waters Assoc. (Milford, MA, USA), and Spherosgel TSK PW4000 packed with hydroxylated polyether copolymer of 500 Å nominal pore diameter (30 × 0.75 cm, I.D.) from Beckman Instruments (Galway, Ireland). The exclusion and total column volumes were 5.48 and 10.46 ml, respectively, for the UHG-250 column and 5.15 and 10.40 ml, respectively, for the TSK column, as determined with blue dextran (molar-mass = 2 000 000 g mol<sup>-1</sup>) and <sup>2</sup>H<sub>2</sub>O, respectively. Other experimental details were as used previously [8].

## RESULTS AND DISCUSSION

In the SEC of uncharged polymers it has been widely demonstrated that the elution volume data fit well the so-called universal calibration function [11]:

$$\log M[\eta] = a - bV_e \tag{1}$$

where  $M$ ,  $[\eta]$  and  $V_e$  are the molar mass, intrinsic viscosity and peak elution volume of the polymer, respectively, and  $a$  and  $b$  are calibration constants. The slope,  $b$ , can be expressed in terms of column set characteristics as

$$b = \frac{a}{V_0 + V_p + V_a} \tag{2}$$

where  $V_0$ ,  $V_p$  and  $V_a$  are the void volume, the available pore volume and a residual volume specified graphically in Fig. 1, respectively. The insertion of  $b$  from eqn. 2 into eqn. 1 yields

$$V_e = V_0 + V_a + V_p - k_{UC} \log M[\eta] \tag{3}$$

Note that  $k_{UC}$  denotes the inverse of  $b$  according to the terminology used by Styring *et al.* [7].

The same considerations can be extended to charged polymers, so that

$$V'_e = V_0 + V'_a + V'_p - k'_{UC} \log M[\eta]_I \tag{4}$$

where  $V'_e$  is the peak elution volume of the polyion,  $[\eta]_I$  its intrinsic viscosity at a given ionic strength,  $I$ ,  $k'_{UC}$  the inverse of the slope of the particular calibration plot of  $\log M[\eta]_I$  vs.  $V'_e$ , and the remaining parameters are as specified in Fig. 1.

The elution volume of an uncharged polymer can be expressed as a function of the distribution coefficient,  $K_{SEC}$ , through

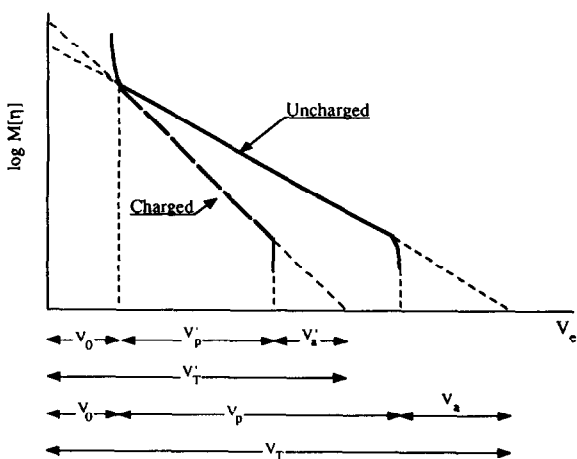


Fig. 1. Depiction of the different chromatographic volumes, appearing throughout the paper, placed on a calibration plot.

$$V_e = V_0 + K_{SEC}V_p \tag{5}$$

and for a polyelectrolyte [10,12] as

$$V'_e = V_0 + K_{SEC}V'_p \tag{6}$$

where  $V'_p = V_p - V_r$  is the virtual pore volume available to the polyelectrolyte, *i.e.*, the difference between the pore volume given by the supplier and the “repulsion volume” impenetrable to the polyion by charge repulsion. The  $V'_p$  value is always lower than  $V_p$  when polymer–gel electrostatic repulsion takes place.

In addition, the elution volumes of polyelectrolytes depend strongly on the mobile phase ionic strength, as has been evidenced experimentally in several contributions dealing with the SEC of polyanions [3–9,10,12–14]. In this context, Styring and co-workers [7,13] explored this effect quantitatively and proposed the following empirical relationship:

$$V'_e = V_\infty - k_{eI}I^{-1/2} \tag{7}$$

where  $V_\infty$  is the elution volume of a polyanion when  $I \rightarrow \infty$ , denoting an ionic strength high enough to screen their charges or to cancel the solute–gel repulsive interactions, and  $k_{eI}$  is a constant accounting for electrical and geometrical features of both the polyion and gel support [7]. However, some discrepancies arose when the above equation was used to fit experimental data. For this reason, we have recently proposed an alternative semi-empirical correlation for the  $V'_e$  dependence on  $I$ , namely [9]

$$V'_e = V_e - k_{eI}I^{-1/2} + K''I^{-1} \tag{8}$$

where  $V_e$  has the same meaning as  $V_\infty$  in eqn. 7, and the coefficient  $K''$  also takes into account polymer–gel electrostatic interactions. For more precise information about this coefficient see eqn. 7 in ref. 9.

So far, we have presented basic equations for the SEC of charged and uncharged polymers and a recently reported empirical correlation of  $I$  with  $V_e$ . We now proceed to combine the above equations in order to obtain an expression that could take into account the influence of ionic strength on the calibration graphs of polyions. For this purpose, the insertion of the expressions

for  $V_e$  and  $V_e'$  given by eqns. 5 and 6, respectively, into eqn. 8, after some rearrangement yields

$$V_p' - \frac{K''}{K_{SEC}} \cdot I^{-1} = V_p - \frac{k_{el}}{K_{SEC}} \cdot I^{-1/2} \quad (9)$$

and inserting into eqn. 9 the  $V_p$  and  $V_p'$  expressions derived from eqns. 3 and 4, respectively, and coupling with some algebra, the following equation can be written:

$$V_e' + \log \left\{ \frac{(M[\eta]_I)^{k_{UC}}}{(M[\eta]_\infty)^{k_{UC}}} \right\} + V_a - V_a' \\ = V_e - \frac{k_{el}}{K_{SEC}} \cdot I^{-1/2} + \frac{K''}{K_{SEC}} \cdot I^{-1} \quad (10)$$

Note that  $[\eta]_\infty$  has been used instead of  $[\eta]$  because eqn. 3 is also valid for polyions when  $I \rightarrow \infty$ , that is, when they behave as uncharged polymers.

A tentative effort to analyse quantitatively the secondary electrostatic effects in aqueous SEC of polyions can be performed by means of eqn. 10. Nevertheless, we must previously proceed to express the above relationship in a more convenient form. First, note that most of the magnitudes involved in this equation are often handled by chromatographers, except the difference  $V_a - V_a'$ , which can be replaced with an equivalent term extracted from Fig. 1 and expressed as  $V_T - V_T' - V_p + V_p'$ . Moreover, recalling eqn. 9 and neglecting the numerical value of the difference  $V_a - V_a'$ , the above relationship could be written as

$$V_e' + \log \left\{ \frac{(M[\eta]_I)^{k_{UC}}}{(M[\eta]_\infty)^{k_{UC}}} \right\} + V_p - V_p' = V_e \quad (11)$$

In the light of this equation, two terms account for specific contributions to the secondary electrostatic effects in aqueous SEC. The first one,  $\log \{(M[\eta]_I)^{k_{UC}} / (M[\eta]_\infty)^{k_{UC}}\}$  takes into account the influence of eluent ionic strength on the  $M[\eta]$  as representative of the shape and size of the polyion. Of course, when  $I \rightarrow \infty$ ,  $[\eta]_I \rightarrow [\eta]_\infty$  and  $k'_{UC} \rightarrow k_{UC}$  and this term will be cancelled out. The second term refers to the pore volume inaccessible to polyions owing to the electrostatic potential of the stationary phase. Its value is  $V_p - V_p' = V_T = A\chi_e$ , where  $A$

is the area of the inner surface of the pores and  $\chi_e$  the width of the electrostatic barrier created by the surface potential [3,10]. In fact, as  $\chi_e$  is proportional to the Debye length, when  $I \rightarrow \infty$ ,  $\chi_e \rightarrow 0$ , and this term will also be cancelled out.

We next proceed to verify the validity of eqn. 11. For this purpose, we selected previously reported chromatographic systems [6–10]. In order to make this equation manageable, a final transformation was carried out, yielding

$$V_e' + \Delta V' = V_e - k'_{UC} \log [\eta]_I \quad (12)$$

where  $\Delta V' = \log \{M^{k_{UC}} / (M[\eta]_\infty)^{k_{UC}}\} + V_p - V_p'$ . Consequently, a plot of  $V_e' + \Delta V'$  vs.  $k'_{UC} \log [\eta]_I$  should allow one to obtain by extrapolation  $V_e$  data that will serve to compare them with the corresponding experimental values.

In Figs. 2, 3 and 4 are depicted the plots of eqn. 12 for the systems: PGA–Spherogel TSK PW4000, PGA–UHG-250 and PSS–UHG-250, respectively, in eluents with different  $I$  and pH values. All data necessary to apply this equation

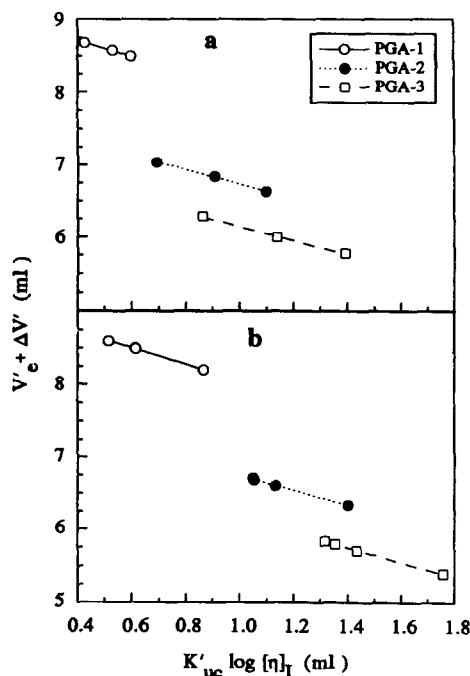


Fig. 2. Plot of eqn. 12 for poly(L-glutamic acid) (PGA)–Spherogel TSK PW4000 in the eluents (a) acetate buffer (pH 5.0) and (b) phosphate buffer (pH 7.0). Ionic strength range, 5–50 mM. Data from refs. 8 and 9.

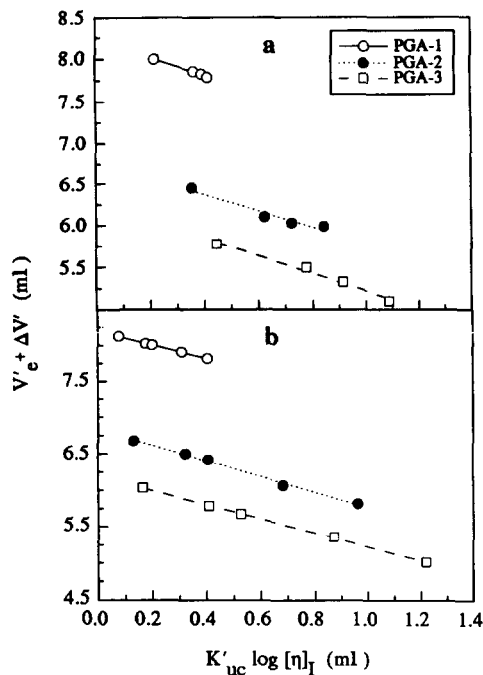


Fig. 3. Plot of eqn. 12 for poly(L-glutamic acid) (PGA)–Ultrahydrogel (UHG-250) in the eluents (a) acetate buffer (pH 5.0) and (b) phosphate buffer (pH 7.0). Ionic strength range, 5–100 mM. Data from refs. 8 and 9.

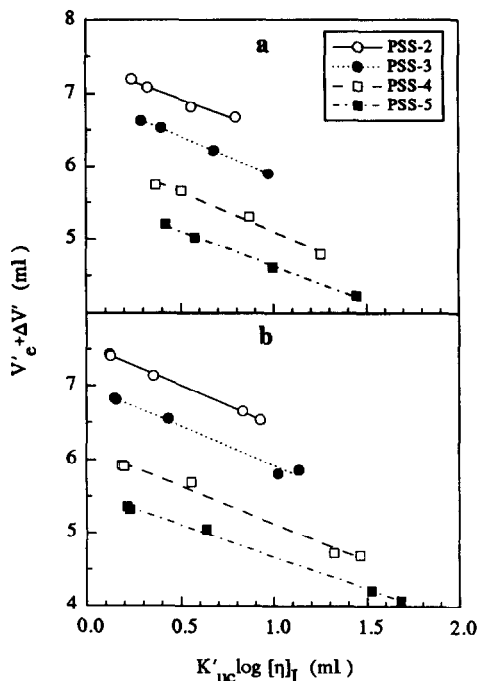


Fig. 4. Plot of eqn. 12 for sodium poly(styrene sulphonate) (PSS)–UHG-250 in the eluents (a) acetate buffer (pH 5.0) and (b) phosphate buffer (pH 7.0). Ionic strength range, 5–50 mM. Data from refs. 8 and 9.

have recently been reported [8,9]. Good linear fits with slope values close to unity are observed in all instances, consistent with the proposed functionality. From a quantitative point of view, the predicted  $V'_e$  values for each sample and system agree very well with those from dextrans, as can be seen in Table I. From the comparison between both sets of  $V'_e$  data, a slight deviation within the experimental error is observed. The same trend has been evidenced for other systems reported by different workers [6,7,10], as can be seen in Figs. 5–7 and Table II.

In spite of the formal importance of eqn. 12, it is scarcely useful in practice because its application demands a knowledge of an excessive number of data from both charged and uncharged polymers. In order to surmount this drawback, an approximate form of the original eqn. 10 was derived assuming that  $k'_{uc} \approx k_{uc}$  and  $V_a - V'_a \approx 0$ :

$$V'_e + \Delta V^* = V_e - \frac{k_{el}}{K_{SEC}} \cdot I^{-1/2} + \frac{K''}{K_{SEC}} \cdot I^{-1} \quad (13)$$

where  $\Delta V^* = k'_{uc} \log ([\eta]_I / [\eta]_\infty)$ . Several comments should be made about this simplified version: (a) the assumptions made to derive eqn. 13 are less drastic as the divergence between the calibration graphs for both charged and uncharged polymers vanishes (see Fig. 1); (b) for practical purposes, this equation is workable using as input data those obtained from charged polymers exclusively; and (c) in addition to the introduced approximations, the functionality of  $V'_e$  on  $I^{-1/2}$  remains unaltered, being a second-order polynomial with respect to  $I^{-1/2}$ , whereas Styring *et al.*'s model [7] predicts a linear dependence expressed as

$$V'_e + \Delta = V_e - k_{el} I^{-1/2} \quad (14)$$

where  $\Delta = k_{uc} \log ([\eta]_I / [\eta]_\infty)$ .

Eqns. 13 and 14 were tested with the same chromatographic systems as mentioned above. As an example, Figs. 8 and 9 depict plots of eqn. 13 (parts a) and eqn. 14 (parts b) for PSS–UHG-250–buffer (pH 7.0) [8,9] and PSS–CPG–buffer

TABLE I

DATA ON ELUTION VOLUMES FOR UNCHARGED POLYMERS [8,9] AND VALUES FOR CHARGED POLYMERS PREDICTED THROUGH EQNS. 12, 13 AND 14

System	Sample	$V_e$ (ml)			
		Eqn. 12	Eqn. 13	Eqn. 14	Uncharged
PGA-TSK-buffer (pH 5.0)	PGA-1	9.14	9.42	10.26	9.12
	PGA-2	7.73	7.99	8.27	7.75
	PGA-3	7.12	7.42	7.87	7.15
PGA-TSK-buffer (pH 7.0)	PGA-1	9.20	9.43	8.56	9.12
	PGA-2	7.76	8.05	7.48	7.75
	PGA-3	7.15	7.20	7.21	7.15
PGA-UHG-buffer (pH 5.0)	PGA-1	8.23	7.81	7.61	8.21
	PGA-2	6.77	6.95	6.54	6.82
	PGA-3	6.26	6.14	6.12	6.21
PGA-UHG-buffer (pH 7.0)	PGA-1	8.20	8.48	7.59	8.21
	PGA-2	6.83	6.80	6.50	6.82
	PGA-3	6.19	6.31	6.14	6.21
PSS-UHG-buffer (pH 5.0)	PSS-1	9.42	9.60	9.29	9.40
	PSS-2	7.40	7.93	7.53	7.51
	PSS-3	6.95	7.01	6.82	6.97
	PSS-4	6.18	6.03	6.23	6.12
	PSS-5	5.57	5.89	5.93	5.55
PSS-UHG-buffer (pH 7.0)	PSS-1	9.36	9.75	9.14	9.40
	PSS-2	7.55	7.99	7.15	7.51
	PSS-3	6.98	7.25	6.58	6.97
	PSS-4	6.14	6.29	6.07	6.12
	PSS-5	5.55	6.01	5.89	5.55

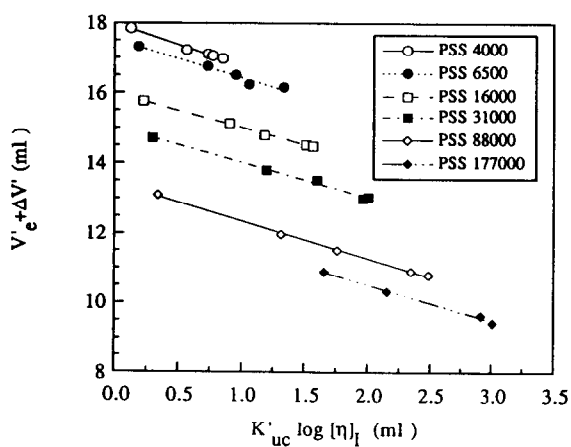


Fig. 5. Plot of eqn. 12 for PSS-FPG(500 + 170) in buffer of pH 8.0 as eluent. Ionic strength covering the range 14–270 mM. Data from ref. 6.

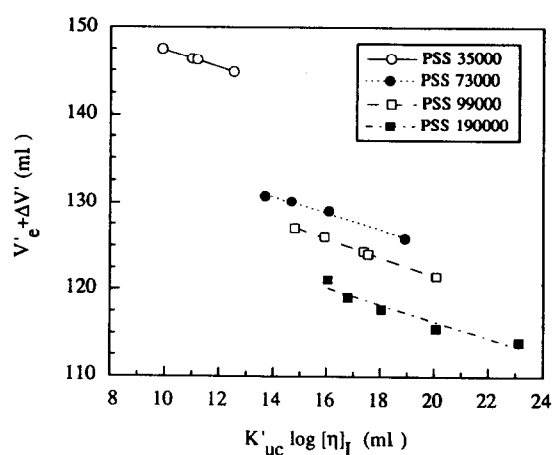


Fig. 6. Plot of eqn. 12 for PSS-glass beads in buffer of pH 6.0 as eluent. Ionic strength covering the range 12–100 mM. Data from ref. 7.

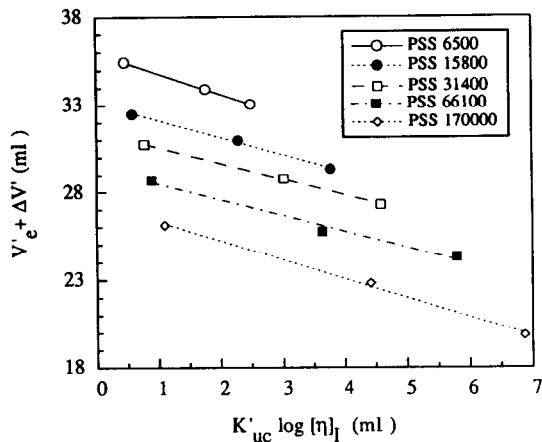


Fig. 7. Plot of eqn. 12 for PSS-CPG in buffer of pH 8.0 as eluent. Ionic strength covering the range 1.2–500 mM. Data from ref. 10.

(pH 8.0) [10] systems, respectively. Good fits of eqn. 13 are clearly observed in all instances, whereas the corresponding fits of eqn. 14 show discrepancies with respect to the linear correla-

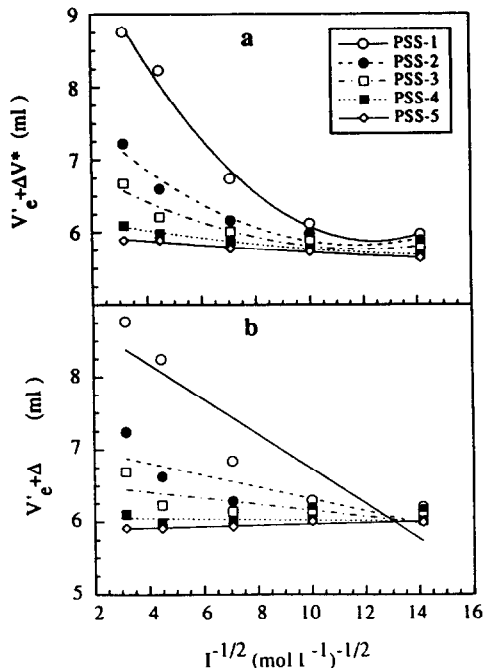


Fig. 8. Comparison between (a) eqn. 13 and (b) eqn. 14 for PSS-UHG-250-buffer (pH 7.0). Ionic strength range, 5–100 mM.

TABLE II

DATA ON ELUTION VOLUMES FOR UNCHARGED POLYMERS [6,7,10] AND VALUES FOR CHARGED POLYMERS PREDICTED THROUGH EQNS. 12, 13 AND 14

System	Sample	$V_e$ (ml)			
		Eqn. 12	Eqn. 13	Eqn. 14	Uncharged
PSS-FPG [6]	PSS 6500	17.51	16.29	14.36	17.49
	PSS 16 000	15.97	16.13	13.50	15.96
	PSS 31 000	15.01	15.77	12.78	15.03
	PSS 88 000	13.43	13.94	11.36	13.44
	PSS 177 000	12.56	12.46	10.76	12.38
PSS-glass beads [7]	PSS 35 000	157.5	156.1	152.1	156.4
	PSS 73 000	144.0	143.7	144.2	144.5
	PSS 99 000	142.9	140.1	139.6	140.9
	PSS 190 000	135.2	134.3	134.6	134.3
PSS-CPG [10]	PSS 6500	35.98	37.70	33.68	35.83
	PSS 15 800	33.12	34.77	29.66	33.11
	PSS 31 400	31.46	33.44	28.74	31.55
	PSS 66 100	29.37	31.70	27.11	29.68
	PSS 170 000	27.38	27.25	24.75	27.10

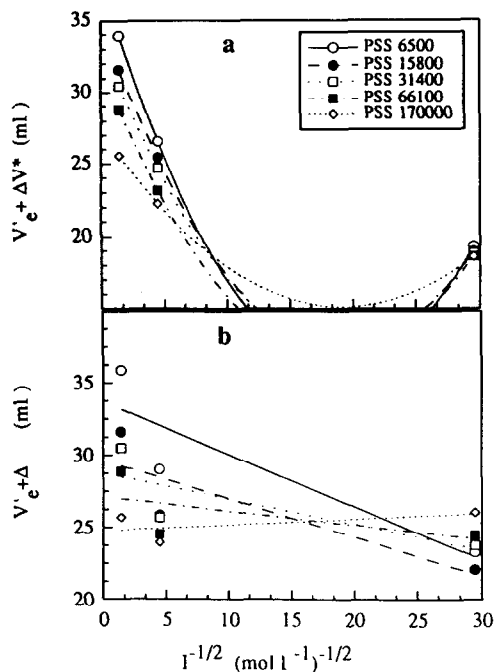


Fig. 9. Comparison between (a) eqn. 13 and (b) eqn. 14 for PSS-CPG-buffer (pH 8.0). Ionic strength range, 1.2–500 mM.

tion, being more pronounced as the polyion molar mass decreases. The extrapolated  $V_e$  values for these systems and for others, not plotted here for simplicity, are also given in Tables I and II. The comparison between  $V_e$  values from eqn. 13 and those for uncharged polymers reveals a good agreement in general, the deviation being *ca.* 4.0% for the most unfavourable cases. This small deviation validates the proposed eqn. 13, at least for the systems selected here, and the assumptions made to derive it. In contrast, the  $V_e$  values predicted through eqn. 14 show poor agreement when compared with the experimental elution volumes for uncharged polymers, the deviation being *ca.* 14% in some instances.

In order to complete our test on the predictions carried out with the proposed equations, we have built up current plots more suitable for the SEC characterization of polymers. Thus, Fig. 10 depicts the comparison between the calibration graph obtained for uncharged polymers (solid line) and those predicted by means of eqns. 12, 13 and 14 (symbols) for (a) PGA-

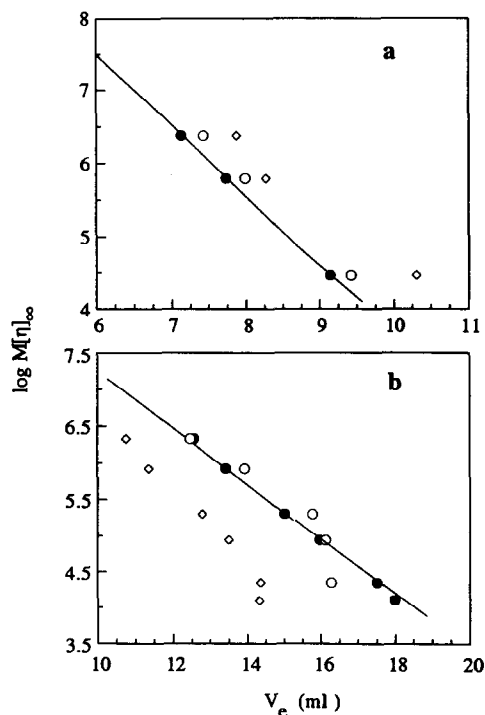


Fig. 10. Comparison between the calibration graphs for uncharged (solid lines) and charged polymers (symbols) predicted with (●) eqn. 12, (○) eqn. 13 and (◇) eqn. 14 for (a) PSS-UHG-250-buffer (pH 7.0) and (b) PSS-CPG-buffer (pH 8.0) chromatographic systems.

TSK-buffer (pH 5.0) and (b) PSS-FPG-buffer (pH 8.0) chromatographic systems. The values of  $[\eta]_\infty$  were determined with the equation  $[\eta]_I = [\eta]_\infty + SI^{-1/2}$  (see eqn. 1 in refs. 8 and 9). As can be seen, the  $V_e$  values predicted through eqn. 12, considered as a closed form, fit the reference calibration graph well, whereas those obtained from eqns. 13 and 14 exhibit deviations, which are pronounced when using eqn. 14.

## CONCLUSIONS

We have developed a formalism to predict the effect of the mobile phase ionic strength on the calibration graphs for the SEC of polyions in aqueous media by means of some fundamental equations combined with a recently proposed semi-empirical correlation between  $I$  and  $V_e$ . It should be emphasized that this treatment infers nothing about the influence of ionic strength on



the elution behaviour of polyelectrolytes when other separation mechanisms, such as adsorption, hydrophobic interactions or hydrogen bonding, take place.

The proposed master equation (eqn. 10 or 12) and the simplified version (eqn. 13) derived from the former making some plausible assumptions, display two terms accounting for the influence of  $I$  on the size and shape of the polyion and on the width of the electrostatic barrier on the gel surface. The simultaneous inclusion of these effects in a unique expression allow the quantitative evaluation of both contributions to the elution volume of a given polyion in aqueous SEC.

The predictions made using eqns. 12 and 13 support the proposed formalism, and comparison with eqn. 14 reveals that at high polymer molar masses both functionalities, on  $I^{-1/2}$  from Styring *et al.* [7] and on  $I^{-1}$  proposed here, work well. However, at low molar masses or when the divergence between the charged and uncharged calibration graphs is more pronounced, the equations reported here yield more accurate predictions.

#### SYMBOLS

$I$	ionic strength
$M$	molar mass
$[\eta]_I$	intrinsic viscosity of a polyion at a given ionic strength
$[\eta]_\infty$	intrinsic viscosity of a polyion at $I \rightarrow \infty$ (high enough to screen its charges)
$V_c$	elution volume of an uncharged polymer
$V'_e$	elution volume of a polyelectrolyte
$V_0$	interstitial or void volume
$V_p$	available pore volume for an uncharged polymer
$V'_p$	available pore volume for a polyelectrolyte at a given $I$
$V_T$	total pore volume for an uncharged polymer
$V'_T$	total pore volume for a polyelectrolyte
$V_a$	$= V_T - (V_0 + V_p)$
$V'_a$	$= V'_T - (V_0 + V'_p)$
$k_{UC}$	inverse of the slope of the uncharged polymer calibration graph

$k'_{UC}$	inverse of the slope of the polyelectrolyte calibration graph
$K_{SEC}$	distribution coefficient accounting for size-exclusion mechanism
$k_{el}$	constant accounting for electrical and geometrical parameters
$K''$	constant accounting for chromatographic packing features
$\Delta V'$	$= \log \frac{M^{k'_{UC}}}{(M[\eta]_\infty)^{k_{UC}}} + V_p - V'_p$
$\Delta V^*$	$= k'_{UC} \log \left( \frac{[\eta]_I}{[\eta]_\infty} \right)$
$\Delta$	$= k_{UC} \log \left( \frac{[\eta]_I}{[\eta]_\infty} \right)$

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