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SOLUTION PROPERTIES OF POLYELECTROLYTES

IV. USE OF A NEW HYDROPHILIC SIZE-EXCLUSION CHROMATOGRAPHIC PACKING FOR THE SEPARATION OF ANIONIC AND CATIONIC POLYIONS

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SUMMARY

The chromatographic behaviour of sodium polystyrenesulphonate in Ultrahydrogel aqueous size-exclusion chromatographic packings was analysed under different experimental conditions. Three types of mobile phases (salt-free water, sodium nitrate solution and buffers of different pH) were investigated in order to characterize the elution mechanisms and to optimize the separation. Several parameters, such as sample concentration, injection volume, eluent pH and ionic strength were considered. Finally, a "universal" calibration graph was obtained under simple, mild, optimized conditions of the mobile phase (0.2 *M* acetate buffer, pH 4.0), which is congruent for both polyanions and polycations and also for uncharged polymers.

INTRODUCTION

The high-performance liquid chromatography of charged macromolecules in aqueous media has undergone a great evolution in the last few years, mainly owing to the advent of commercially available hydrophilic stationary phases for high-performance size-exclusion chromatography (SEC)[1]. There are a number of SEC effects inherent in polyelectrolytes which arise from both ionic interactions between the

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polyion and the packing (*e.g.*, Donnan equilibrium ion inclusion and ion exclusion) and from changes in the dimensions of the polymer molecules in solution, controlled by concentration, pH and ionic strength. These effects and ways of minimizing them have been discussed in detail elsewhere[2–6]. In this context, two recent contributions deserve to be mentioned: Dubin *et al.*[7] reported a study of the resolution of electrostatic and steric factors for sodium polystyrenesulphonate on controlled-pore glass and Potschka[8] eluted a set of native proteins, having well defined structures, on rigid resins in a variety of aqueous buffers differing in composition, pH and ionic strength. It was concluded that the unperturbed dimensions of a polyelectrolyte can be determined by SEC, taking into account the surrounding diffuse double layer and any other interactions of the solvent. However, in practice, the existence of electrostatic interactions between the eluite and the surface of the packing is still problematic, as it causes deviations towards higher or lower elution volumes when the elution volume of uncharged polymers of the same molecular weight is taken as a reference. In order to minimize or even prevent this effect, the use of a support bearing a relatively small number of ionizable groups may be of great interest, in addition to both the design of a suitable composition of the mobile phase (pH and ionic strength) and the optimization of other experimental parameters, such as sample concentration and injection volume.

In this paper, we report a preliminary study of the influence of the aforementioned variables on the elution of sodium polystyrenesulphonate (NaPSS) from Ultrahydrogel[9]. This packing is based on hydroxylated polymethacrylate with a low content of residual carboxylic groups, which confer upon it *a priori* some advantages over the silica-based supports by minimizing the electrostatic interactions with the polyelectrolyte. The elution behaviour was characterized in terms of the log $M[\eta]$ *vs.* elution volume calibration (where M is the molecular weight and $[\eta]$ the intrinsic viscosity) for each of the mobile phases tested. This allowed the selection of optimum experimental conditions for the analysis of NaPSS with these columns.

The recommended conditions were later applied to the chromatography of several polycations, such as poly(2-vinylpyridine) (P2VPy), poly(4-vinylpyridine) (P4VPy) and poly-L-lysine (PLys). According to the symmetry and positions of the peaks in the chromatograms, it appears that the frequently observed secondary adsorption effects do not occur to a significant extent and that the elution mechanism approaches that of pure SEC[10]. Finally, a universal calibration, suitable for uncharged polymers, polyanions and polycations is presented.

EXPERIMENTAL

A Waters liquid chromatograph (Waters Chromatography Division, Millipore, Milford, MA, U.S.A.) equipped with an M-45 solvent delivery system, a U6K universal injector and an R-410 refractive index detector, coupled to a SP 4290 automatic recorder (Spectra-Physics, San José, CA, U.S.A.), was used.

Two Waters Ultrahydrogel columns packed with hydroxylated polymethacrylate-based gel of 250 and 500 Å nominal pore size were used. These columns will be referred to as UHG-250 and UHG-500, respectively. They have been reported to perform over a broad pH range (from 2 to 12) and are compatible with eluents containing a high percentage of organic solvents[9].

Three different types of mobile phases were used: Waters Milli-Q-quality pure water, sodium nitrate solution and acetate and phosphate buffers with several pH and ionic strength values. All salts used were of analytical-reagent grade from Merck (Darmstadt, F.R.G.). The eluents were always filtered and degassed through a 0.45-μm Micro Filtration Systems regenerated cellulose filter (Millipore) prior to use. The columns were equilibrated for at least 12 h before the start of an experiment. The polymer solutions were always prepared with the corresponding mobile phase as solvent. The flow-rate was 0.8 ml/min. Each sample was injected three times as a check on the reproducibility.

The following dextran samples were obtained from Pharmacia (Uppsala, Sweden): 10T, 20T, 40T, 70T, 150T and 500T, having weight-average molecular weights of $10 \cdot 10^3$, $17.7 \cdot 10^3$, $40.0 \cdot 10^3$, $83.3 \cdot 10^3$, $170 \cdot 10^3$ and $500 \cdot 10^3$, respectively. NaPSS standards were narrow-distribution ($M_w/M_n < 1.1$ in all instances), dialysed fractions, purchased from Pressure Chemical (Pittsburg, PA, U.S.A.), with nominal molecular weights of $1.6 \cdot 10^3$, $4 \cdot 10^3$, $16 \cdot 10^3$, $31 \cdot 10^3$, $88 \cdot 10^3$, $177 \cdot 10^3$ and $354 \cdot 10^3$. Poly(ethylene oxide) (PEO) samples (MW 2000 and 4000) were obtained from Fluka (Buchs, Switzerland). Narrow fractions of poly(2-vinylpyridine) (P2VPy) (MW $2.9 \cdot 10^3$, $7.0 \cdot 10^3$, $10.5 \cdot 10^3$ and $28.0 \cdot 10^3$, were from Pressure Chemical. A poly(4-vinylpyridine) (P4VPy) sample was obtained by ultrafiltration of a commercial sample of nominal MW $4 \cdot 10^4$, supplied by Polyscience (Warrington, PA, U.S.A.). Ultrafiltration was carried out with a Millipore 142-mm diameter HI-FLUX UF cell. Finally, two poly-L-lysine (PLys) samples from Sigma (St. Louis, MO, U.S.A.) of average MW $3.8 \cdot 10^3$ and $5.8 \cdot 10^3$ were also used as polycations.

Viscosity measurements were made with a modified Ubbelohde viscometer thermostated at 25.0 $\pm$ 0.1°C. Flow-times were determined to 0.01 s; the flow-time of the pure solvent was always higher than 110 s. Kinetic energy corrections were included in the calculations of specific viscosities. The viscometric equations used for the uncharged polymers in all instances were $[\eta]_{dextran} = 97.8 \cdot 10^{-3} M^{0.50}$ (ref. 11) and $[\eta]_{PEO} = 2.0 + 0.016 M^{0.76}$ (ref. 12) because, as has been previously indicated, the influence of salt on the viscosity of non-ionic polymers, such as those mentioned above, may be neglected[10].

RESULTS AND DISCUSSION

In this paper, the elution volume, referred to as $V(c_p,c_s)$, is a function of two variables: the injected polyion concentration, c_p, and the concentration of salt in the eluent, c_s.

Numerous studies on the SEC of polyelectrolytes have been carried out with derivatized silica gel packings[2,13] in order to minimize the undesirable polymer–support interactions. We used high-performance SEC Ultrahydrogel columns[9], packed with a soft or semi-rigid non-derivatized gel, for the chromatography of different classes of polyions. It is well known that soft or semi-rigid gels must be used at low pressures, and this makes separations more time consuming. Further, the particles are larger and less uniform, causing a slight reduction in column efficiency. However, these apparent drawbacks may be largely compensated for in this instance by the clear advantages of bypassing the need for derivatization and especially the diminished polyelectrolyte–support interactions owing to the low residual charge density in the

gel. Our aim was to determine the extent to which and the conditions under which these advantages can make the use of this packing for the chromatography of polyions particularly appropriate. Different factors and variables that directly influence the SEC separation were investigated and optimized in order to obtain a calibration graph applicable to both polyanions and polycations and also to uncharged polymers by use of a common mobile phase.

It must be pointed out that, in general, the chromatography of polycations has been carried out with derivatized silica gels and eluents specifically designed for this type of polyelectrolyte[13]. We used three different types of mobile phases, in the following order: (a) salt-free water, (b) sodium nitrate solution and (c) phosphate and acetate buffers with different pH and ionic strength values.

Salt-free water

The use of pure water as a mobile phase allowed us to check the extent of the contribution of the residual surface charge density of the support to undesirable interactions with the polymer, and to characterize the behaviour of the system and the influence of several experimental variables under the less favourable conditions for the chromatography of polyions. It is well known that NaPSS in salt-free water adopts a concentration-dependent more or less stretched conformation, owing to the electrostatic repulsion between the charges of the monomeric units (see, for example, the values for the persistence length of this polymer in pure water in Table 4 in ref. 14). In fact, the more dilute the sample is, the more stretched are the chains. Therefore, if the interactions of the polyions with the gel were relatively strong (as is usually the case for other types of supports), a pronounced ion-exclusion effect should be observed,

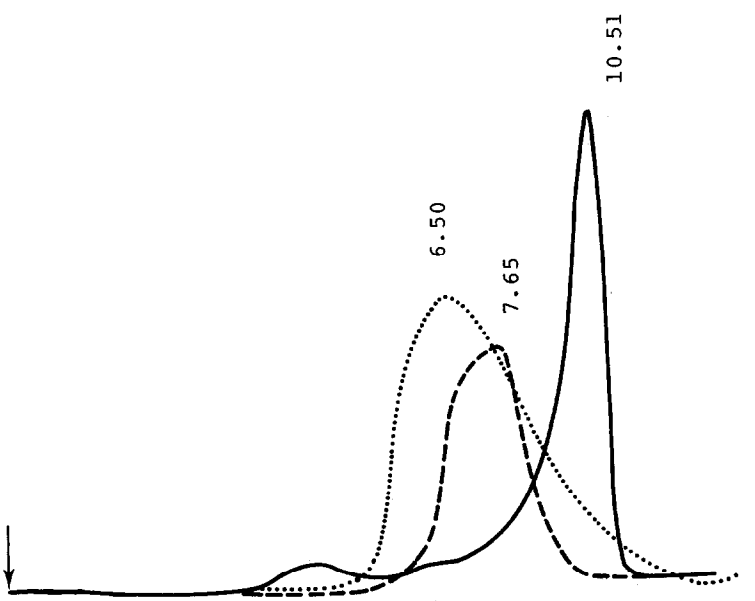

Fig. 1. Elution profiles of NaPSS for UHG-500, eluted with salt-free water. Flow-rate, 0.8 ml/min; injection volume, 100 μl; NaPSS concentration, 10 g/l. NaPSS molecular weight: (· · ·) 177; (– – –) 31; (———) 1.6 kDa.

preventing a reasonable separation of NaPSS samples of different molecular weight. However, as will be shown later in detail, a satisfactory (although evidently not optimized) resolution can be obtained, even with salt-free water as eluent, if certain simple experimental conditions are met. As an example, Fig. 1 shows the separation of three NaPSS samples of MW 177, 31 and 1.6 kDa on UHG-500, eluted with pure water, the elution volumes being 6.50, 7.65 and 10.51 ml, respectively. Moreover, this behaviour may be a real advantage, especially when for any given reason (*e.g.*, to minimize hydrophobic interactions between polymers and gel), salt-containing eluents of very low concentration must be used.

In order to verify these assumptions and to characterize and optimize the elution behaviour of NaPSS with salt-free water, a systematic study was carried out, using UHG-250 and UHG-500 independently, and including c_p and the injection volume V_i, as variables. V_i was considered on the basis of the following assumption: because (i) in pure water the conformation of the polyion is strongly dependent on concentration and (ii) the actual dilution of the sample through the column will increase as the volume injected decreases, it is to be expected that the conformation of NaPSS will vary to a different extent during elution, depending on the volume injected. Thus, for a given c_p value, the lower is V_i the more stretched the polyion chains are and, therefore, the smaller is the observed elution volume. This assumption proved to be correct, as shown in Fig. 2A, where, as an example, $V(c_p, 0)$ on UHG-250 is plotted as a function of V_i at constant c_p (10 g/l) for two different NaPSS molecular weights. Note that the variation is not linear; $V(p_p, 0)$ increases with increasing V_i up to about 50 μl and then the effect is attenuated, especially for the higher molecular weight. This trend was also observed for all the other molecular weights used (not shown). Interestingly, when only 1–5 μl are injected, the dilution of the sample is so dramatic that all NaPSS are eluted close to the column void volume. Under these conditions, the chains are presumably eluted in a very stretched (rod) conformation. Therefore, the range 50–100 μl and, in particular, a V_i value of 100 μl seems to be the most appropriate, because any error in the measurement of V_i will have a much smaller repercussion on $V(c_p, 0)$. On the other hand, significantly higher V_i values, such as 150–200 μl, cannot be recommended because of overloading effects (see below).

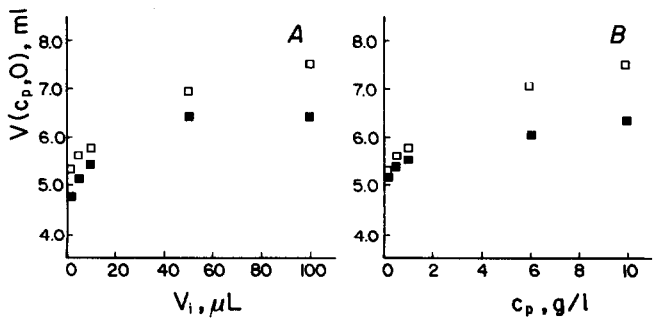

Fig. 2. Dependence of the elution volume, $V(c_p, 0)$, of NaPSS on (A) the injection volume, V_i, at a constant c_p (10 g/l) and (B) polyion concentration, c_p, at constant V_i (100 μl), for UHG-250 and salt-free water as eluent in both instances. NaPSS molecular weight: (A) (□) 1.6 and (■) 354 kDa; (B) (□) 1.6 and (■) 31 kDa.

Fig. 2B shows, as an example, the variation of $V(c_p, 0)$ on UHG-250 as a function of c_p [at constant V_i (100 μl)] for two different NaPSS molecular weights. This dependence also is not linear, similarly to the behaviour of NaPSS with salt-free water and a Waters I-250 protein column[14], as opposed to the behaviour obtained for uncharged polymers with organic solvents[15–18]. A similar trend to that in Fig. 2B was observed for all the other molecular weights tested. However, note that the shift in the elution volume with c_p increases as the molecular weight of the sample decreases, in contrast with the behaviour observed for uncharged polymers in organic media[16], where the concentration effect is almost negligible below 40 kDa. Although the efficiency of this column increases with increasing c_p when pure water is used as the eluent, it is evident that it is still poor.

A similar test was carried out with UHG-500 in order to establish a comparison under the same experimental conditions. Fig. 3 shows the effect of c_p on $V(p_p, 0)$ [at constant V_i (100 μl)] for NaPSS samples of 1.6, 31 and 177 kDa. Again, the functionality observed is similar to that found for UHG-250, but in this instance the separation achieved for $c_p > 2$ g/l is much better. Hence the higher average pore size of the UHG-500 column clearly provides a more appropriate sieve for the stretched NaPSS chains to be permeated in salt-free water. On the other hand, an increase in the shift of the elution volume with increasing c_p can be observed for this packing as the molecular weight decreases, as opposed to the behaviour for uncharged polymers in organic solvents. In a similar way to Fig. 2B, the injection of the polyelectrolyte at very low concentrations causes the resolution to be completely lost, this early elution being due to both coil expansion and charge repulsion. In addition to c_p, the influence of V_i on $V(c_p, 0)$ was also studied; the same trend as in Fig. 2A was observed for all the molecular weights used (not shown). Again, the highest $V(c_p, 0)$ value for a given sample at a given c_p was obtained for $V_i = 100$ μl.

The elution volumes for $V_i = 200$ μl were essentially the same as for 100 μl when the sample was dilute. However, when 200 μl were injected for $c_p > 2$ g/l, overloading effects[3,19,20] occurred, distorting the chromatogram. Hence, of all the conditions tested so far with salt-free water, an injection volume of 100 μl and a polyion

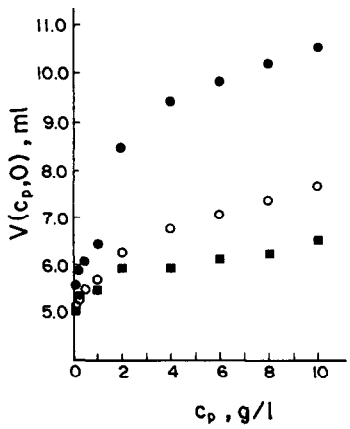

Fig. 3. Dependence of $V(c_p, 0)$ on NaPSS concentration at constant V_i (100 μl) for UHG-500, eluted with salt-free water. NaPSS molecular weight: (●) 1.6; (○) 31; (■) 177 kDa.

concentration of 10 g/l seem to be optimal for both UHG-500 and UHG-250. Further, as far as c_p is concerned, a value of 10 g/l should not be exceeded in the range of molecular weights examined, for two reasons:

(i) in order to prevent the appearance of viscous fingering[19] and macromolecular crowding[20], widely studied for both uncharged and charged polymers in organic and aqueous media respectively; these effects cause a distortion of the chromatogram, making it impossible to obtain any reliable data;

(ii) from a quantitative point of view, the upper threshold for c_p must correspond to the lower limit between dilute and semi-dilute concentrations[21]. Hence, this threshold would be determined by the relationship $c_p[\eta] < 1$ according to Doi's theory[22,23], because for $c_p[\eta]$ values higher than unity entanglement occurs, the polyion chains no longer remaining an individual entity. In this instance, the chromatographic column would not be able to achieve a good separation, and preliminary dilution of the sample would be need to suppress entanglement.

A more detailed analysis of the elution behaviour of NaPSS with UHG-500 and pure water was next carried out in terms of the log $M[\eta]$ *vs.* elution volume calibration for the different experimental conditions tested. This type of plot allows a better understanding and interpretation of the elution mechanisms[14,15,24,25] (including polyelectrolyte–support interactions) and will be used for the study of the remainder of the mobile phases in this work.

Fig. 4 depicts the different log $M[\eta]_{p,c_p}$ *vs.* $V(c_p, 0)$ calibration graphs for NaPSS for salt-free water, obtained as a function of c_p [at constant V_i (100 μl)], for a range of polyion concentrations from 0.1 to 10 g/l. $[\eta]_{p,c_p}$ has been defined previously[14]; it refers to the intrinsic viscosity of the polymer at finite concentration in pure water; its values have been reported elsewhere[14]. The reference calibration for uncharged polymers (dextrans and PEO), obtained at infinite dilution by extrapolation of at least four concentrations, is also included. The general pattern of the variation of the graphs is similar to that corresponding to NaPSS for salt-free water and the Waters I-250 protein column (see Fig. 7 in ref. 14). However, in this instance, the calibration graph at

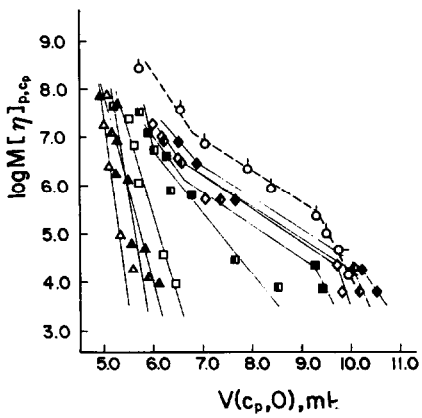

Fig. 4. Calibration graphs for NaPSS on UHG-500, eluted with salt-free water, at constant V_i = 100 μl, as a function of the polyion concentration. c_p: (△) 0.1; (◭) 0.25; (▲) 0.5; (□) 1.0; (◧) 2.0; (■) 4.0; (◇) 6.0; (◆) 8.0 and (◆) 10.0 g/l. Also included is the calibration graph for uncharged polymers: (○) dextran and (○–) PEO.

the highest c_p is much closer to the reference graph and, interestingly, their slopes are almost identical. In addition to the set of graphs in Fig. 4, another set was obtained on plotting log $M[\eta]_{p,c_p}$ *vs.* $V(c_p, 0)$ as a function of V_i [at constant c_p (10 g/l)] for a range of injection volumes from 1 to 100 μl (not shown). A very similar trend was observed, the calibration graphs approaching that for the uncharged polymers as V_i increased.

In order to summarize the results from both plots, Fig. 5 shows the variation of the slopes of the calibration graphs as a function of (A) c_p and (B) V_i. The dotted lines correspond to the slope of the reference calibration graph (dextran and PEO). It is evident that in both instances the slopes show a typical "polyelectrolytic behaviour", which must be basically governed by the conformation-dependent conformational changes of the poly-ion. As the actual concentration of the sample on the column increases, the differences between the slopes for NaPSS and uncharged polymers become smaller. In fact, some interesting conclusions concerning the polymer–substrate interactions arise from the analysis of Figs. 4 and 5. If the reference calibration is taken as that corresponding to a pure SEC elution mechanism, then the calibration graph obtained under the experimental conditions, c_p = 10 g/l and V_i = 100 μl, reveals a satisfactory resolution for the "very inadequate" eluent (salt-free water) used. This, in turn, supports the assumption that the interaction between the polyelectrolyte and the residual charges on the support is indeed diminished when compared with the behaviour reported for other types of hydrophilic gels, where the solute–substrate electrostatic interactions cause considerable secondary effects. In fact, the deviation in Fig. 4 towards higher elution volumes can be attributed to both (i) a not completely reached totally folded (Gaussian) conformation of the chains because

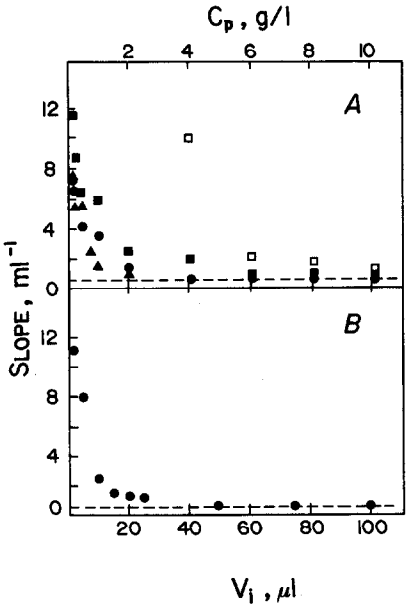

Fig. 5. Variation of the slope of the calibration graphs for NaPSS on UHG-500, eluted with salt-free water as a function of (A) c_p, at constant V_i = (●) 100 μl; (■) 50 μl; (□) 25 μl; and (▲) 200 μl; and (B) V_i, at constant c_p (10 g/l). The dotted lines correspond to the value of the slope for the uncharged polymers in both instances.

the concentration is not high enough and (ii) a residual, but definitely small, repulsive interaction between NaPSS and the carboxylic groups of the gel.

Sodium nitrate solution

UHG-250 was used for all the experiments involving salt-containing mobile phases because its pore size proved to be more suitable for the analysis once the chains approach the Gaussian coil conformation owing to the screening of poly-ion charges by counter ions. The ionic strength of the eluent is an important factor, as it strongly determines the conformation of the polyelectrolytes in solution[5,8,24–26]. Recently, a correlation between eluent ionic strength and size of the diffuse double layer around polyelectrolytes at infinite dilution was established in deriving a theory on the elution mechanisms of SEC of poly-ions[8]. In this way, counter ions in a polyelectrolyte solution can be classified into three categories[27]: counter ions freely moving outside the region occupied by macro-ions, those bound but mobile in this region and those bound to individual charged groups of the macro-ion. The equilibrium between free counter ions and bound but mobile counter ions is most important in determining the conformation or size and shape, the main factor governing SEC. Thus, the poly-ion conformation will now depend on both c_p and c_s. When c_s increases at constant c_p, the Gaussian coil $\rightleftharpoons$ rod simplified equilibrium is shifted to the left as a result of the screening of the macro-ion charges, causing a decrease in the repulsion between different chain segments and facilitating the folding. From the chromatographic point of view, it can be assumed that (i) the charge screening will decrease even further the repulsive interactions between the polymer sulphonate and the residual carboxylic groups on the support and (ii) the accessibility of the NaPSS to the pores will be improved by the chain folding.

Fig. 6 shows the calibration graphs for NaPSS in UHG-250 when either a very dilute (c_s = 0.002 *M*, Fig. 6A) or a moderately concentrated (c_s = 0.1 *M*, Fig. 6B) sodium nitrate solution is used as eluent. The calibrations at two c_p values are shown, corresponding to the maximum and minimum poly-ion concentrations used in the previous section. The calibration graph for uncharged polymers (dextran and PEO) is also included. The values for the intrinsic viscosity of NaPSS at finite poly-ion concentration, at a given salt concentration, $[\eta]_{p,c_p,c_s}$, were obtained from ref. 25 and are summarized in Table I. As can be seen in Fig. 6A, the extent of screening by counter ions and, hence, of chain-folding is too limited for the macro-ions to penetrate the pores of the gel, so that elution takes place mainly through the interstices of the packing, making a chromatographic separation under these conditions impracticable. On the other hand, at this very low c_s, a concentration effect can still be observed on the calibration graphs. In contrast, at a salt concentration of 0.1 *M* (Fig. 6B) a calibration graph almost identical for NaPSS and uncharged polymers is obtained, which indicates that the repulsive polyelectrolyte–gel interactions have been substantially cancelled and that the hydrodynamic volume is now that corresponding to Gaussian coils.

Buffers of different pH values

Another important factor influencing the SEC separations of polyelectrolytes is the pH. It is important in determining whether a solute is ionized or not, and to regulate the degree of ionization of the surface functional groups on the support.

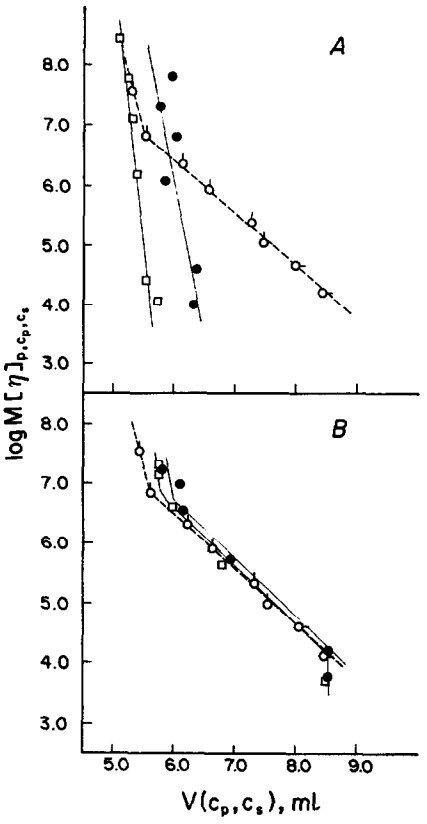

Fig. 6. Calibration graphs for NaPSS on UHG-250, eluted with sodium nitrate solutions. V_i = 100 μl in all instances. c_p: (□) 0.1 and (●) 10 g/l. Sodium nitrate concentration: (A) 0.002 and (B) 0.1 *M*. Also included are the calibration graphs for uncharged polymers: (○) dextran and (○–) PEO.

TABLE I

INTRINSIC VISCOSITY, $[\eta]_{p,c_p,c_s}$(ml/g) OF NaPSS IN SODIUM NITRATE SOLUTIONS[25]

Sodium nitrate concentration · 10^{-3} (g/mol)	c_s = 0.002 *M*		c_s = 0.10 *M*	
	c_p = 10 g/l	c_p = 0.1 g/l	c_p = 10 g/l	c_p = 0.1 g/l
354	176.8	799.1	51.4	54.9
177	108.4	313.7	58.5	81.8
88	68.9	147.6	38.7	45.6
31	36.0	49.0	16.3	14.6
4	11.4	11.9	4.5	3.6
1.6	6.4	7.0	3.8	3.5

Hence the mobile phase must be selected so as to minimize the remaining net charges without undesirable perturbations of the system or damaging of the gel. In addition, operating at lower pH may in some instances[5,7] contribute to a reduction in sample viscosity.

In order to establish the influence of pH on the elution behaviour of NaPSS for UHG-250, the following mobile phases were compared: 0.02 *M* phosphate buffer of pH 7.0 and 5.9 and 0.02 and 0.2 *M* acetate buffers of pH 4.0. As styrenesulphonic acid is strongly acidic, it is to be expected that variations of pH in the range 7.0–4.0 will not significantly affect the degree of ionization of the polyelectrolyte. However, as the pH decreases (approaching 4.0), the degree of protonation of the residual carboxylic groups on the support will increase, thus contributing to further deactivation of the gel surface and to a diminution of the polymer–substrate repulsive interactions. Fig. 7 shows the calibration graphs obtained for NaPSS for all the eluents tested. The corresponding $[\eta]_{p,c_p,c_s}$ values are summarized in Table II. As expected, the graphs approach the calibration for uncharged polymers as the pH decreases. Moreover, it is interesting that, at pH 4.0, even at low salt concentration (0.02 *M*), the resolution is moderately satisfactory. With both a low pH and a moderate ionic strength (0.2 *M*), slight shifts towards higher elution volumes occur. This shift in the elution volume could be attributed to hydrophobic and/or adsorption effects between the poly-ion and the packing. A similar behaviour for NaPSS has been observed at high ionic strength[28]. Conditions where apparently the undesirable secondary effects were therefore selected as being optimal for further study.

Finally, the analysis of the chromatographic behaviour of polyelectrolytes on UHG-250 was extended to the chromatography of polycations. It is well known that the chromatography of polycations presents some particularly difficult problems[29].

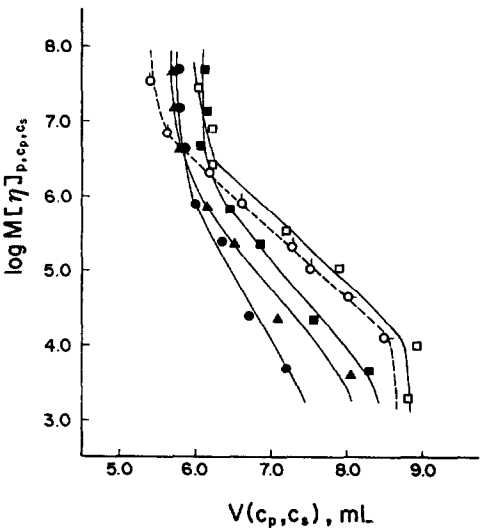

Fig. 7. Calibration graphs for NaPSS on UHG-250, eluted with buffers of different pH and ionic strength values. c_p = 10 g/l and V_i = 100 µl in all instances. Eluents: 0.02 *M* phosphate buffer at pH (●) 7.0 and (▲) 5.9; (■) 0.02 and (□) 0.2 *M* acetate buffer at pH 4.0. (ȯ) Dextran and (○–) PEO.

TABLE II

INTRINSIC VISCOSITY, $[\eta]_{p,c_p,c_s}$ (ml/g) OF NaPSS IN DIFFERENT BUFFERS

c_p = 10 g/l in all instances.

Buffer	*NaPSS molecular weight · 10^{-3} (g/mol)*						
	1.6	*4*	*16*	*31*	*88*	*177*	*354*
0.02 *M* phosphate (pH 7.0)	3.1	5.9	15.6	24.4	52.4	81.5	134.3
0.02 *M* phosphate (pH 5.9)	2.8	5.3	14.2	22.3	49.0	75.9	126.4
0.02 *M* acetate (pH 4.0)	3.0	5.7	13.7	24.0	52.5	79.4	132.1
0.2 *M* acetate (pH 4.0)	1.3	2.6	7.3	11.7	29.9	42.9	77.8

Several mechanisms accounting for non-steric interactions between cationic polyelectrolytes and porous packing materials have been proposed, involving electrostatic adsorption, electrostatic exclusion and hydrophobic partitioning[13]. In order to verify the existence of a common mobile phase for the satisfactory elution of both polyanions and polycations, which would corroborate a "universal" behaviour for this packing, a number of polycations were examined under the above-mentioned seleccted chromatographic conditions (0.2 *M* acetate buffer, pH 4.0). Fig. 8 depicts, as an example, three elution profiles, corresponding to P2VPy, P4VPy and PLys samples with molecular weights of 10.5, 40 and 3.8 kDa, respectively. Also included for comparison is the chromatogram for a 40-kDa dextran sample. Although the solutions injected were prepared from the eluents, "salt" peaks always appeared. This is believed to be a result of ion inclusion[6]. In all instances, the position of these peaks is far enough from the polymer peak not to disturb it. If the polyelectrolyte samples adsorbed on the gel matrix (because of the opposite charge), severely distorted elution profiles (sharp start-up and tailing) would be observed. However, as these features do not appear in any of the chromatograms in Fig. 8, it can be reasonably assumed that all the samples are eluted essentially in terms of their molecular size, without significant secondary

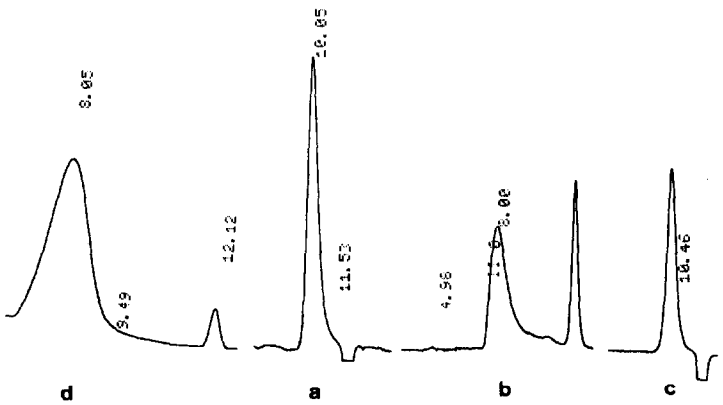

Fig. 8. Elution profiles of several polycations on UHG-250, eluted with 0.2 *M* acetate buffer (pH 4.0). (a) P2VPy, 10.5 kDa; (b) P4VPy, 40 kDa; (c) PLys, 3.8 kDa; (d) also included for comparison is a 40-kDa dextran sample.

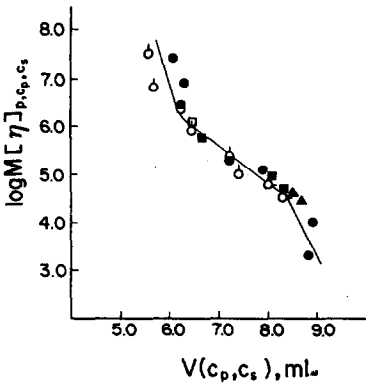

Fig. 9. Universal calibration graphs for both polyanions and polycations and also for uncharged polymers, obtained on UHG-250, eluted with 0.2 *M* acetate buffer (pH 4.0). c_p = 10 g/l and V_i = 100 μl in all instances. (●) NaPSS; (■) P2VPy; (□) P4VPy; (▲) PLys. (◌) Dextran and (○–) PEO.

TABLE III

MARK–HOUWINK CONSTANTS FOR DIFFERENT POLYELECTROLYTES IN 0.2 *M* ACETATE BUFFER (pH 4.0)

Sample	$K \cdot 10^3$ *(ml/g)*	*a*	*Ref.*
NaPSS	0.803	0.940	This work
P2VPy	11.30	0.730	30
P4VPy	22.00	0.687	31
PLys	2.58	0.844	32

adsorption effects. Apparently, the combined effect of a low pH with a moderate ionic strength on the residual carboxylic groups of the support seems to cancel the electrostatic attractive interactions to a satisfactory extent, thus suppressing adsorption. This is further supported by the results in Fig. 9, which shows an optimized, general "universal" calibration graph for UHG-250 under the above-mentioned conditions of pH and ionic strength, including both anionic (NaPSS) and cationic (P2VPy, P4VPy and PLys) polyelectrolytes, and also uncharged polymers (dextran and PEO). The intrinsic viscosity values for the different poly-ions are summarized in Table III.

CONCLUSION

An analysis of the chromatographic behaviour of NaPSS with Ultrahydrogel packing has been carried out under different experimental conditions and an optimized composition of the mobile phase (pH and ionic strength) has been elucidated, allowing us to minimize polymer–substrate interactions. For the range of molecular weights studied, a common, "universal" calibration graph at a finite concentration of sample injected has been obtained for UHG-250 in the chromatography of both polyanions and polycations.

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