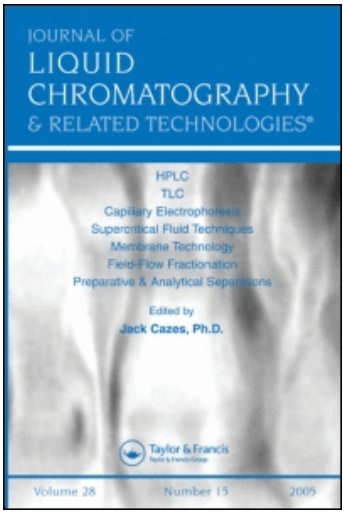

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SOLUTION PROPERTIES OF POLYELECTROLYTES. XI. ADSORPTION EFFECTS IN AQUEOUS SIZE-EXCLUSION CHROMATOGRAPHY OF POLYANIONS

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ABSTRACT

Elution profiles of a series of polymer standards such as sodium poly(styrene sulphonate), poly(acrylic acid) and poly(L-glutamic acid) have been obtained from size-exclusion chromatography experiments using separately two types of hydrophilic supports. A variety of mobile phase compositions have been performed to enhance adsorption effects in order to study how this phenomenon can affect to the chromatographic separation mechanism of polyanions. Distribution coefficient values, in general greater than unity, have served to quantify the adsorption effect, as well as to analyze their dependence on eluent ionic strength, on the ionic groups of the support and on the chemical nature and molar mass of the polyion. The physical basis of the weak polymer-gel attractive interaction have been attributed to hydrogen-bonding and to hydrophobic effects. We present basic equations derived from the Flory-Huggins theory of polymer solutions to explain the adsorption process in terms of preferential interaction, being this description consistent with the expected values assigned to the interaction parameters involved in the above theory.

INTRODUCTION

Aqueous size-exclusion chromatography (ASEC) of water soluble polymers and biopolymers has been an emerging field in the last decade due to the mild and non-destructive character as well as to the short-time consuming of this separation technique (1-4). In contrast with the elution mechanism of polymers in organic media (5,6), the understanding of the separation mechanisms of hydrophilic ionic polymers in aqueous media demands much more theoretical and experimental contributions, mainly due to the diverse nature of the so-called secondary effects referred to as polymer-gel interactions distorting pure ASEC (7-17).

Similarly to the elution of non-ionic polymers in organic media (18,19), polyelectrolytes in aqueous solutions can be early or retarded eluted relative to the elution of an equivalent uncharged polymer at the same experimental conditions. The early elution has been termed as ion-exclusion effect and it is generally assumed to be caused by electrostatic repulsion between the charges along the polyion and the residual charges of the gel packing. Recently, considerable efforts have been devoted to study this secondary effect, including chromatographic and physico-chemical parameters accounting for it, as well as practical recommendations for the total suppression in both rigid and soft gels, by means of the addition of a simple electrolyte to the eluent (8,10,14,20). Simultaneously, most chromatographers involved in biochemical separations have detected the same effect when peptides, proteins and complex macromolecular structures have been eluted using water-compatible porous packing materials (16,17,21).

The opposite effect leads to displacements towards elution volumes higher than those of the non-ionic polymers taken as reference, as a consequence of attractive solute-gel interactions. The nature of these interactions has been treated in depth, being classed as: i) electrostatic attraction, ii) hydrogen-bonding, and iii) hydrophobic interaction (22-24). It is a difficult task to elucidate what of these contributions is responsible for the adsorption effect because more than one type of forces can affect to the separation mechanism. The partial or total prevention of this secondary effect has been directed towards the addition of some components to the mobile phase, such as a few p.p.m. of neutral surfactants (25,26).

The abovementioned displacements of the elution volumes towards lower or higher values have been advantageously employed for separation of biological macromolecules (27). Moreover, it is well-known that one packing can be used for several types of liquid chromatography by a suitable selection of the eluent components. In this context, at present ion-exchange chromatography (IEC) (28) and hydrophobic interaction chromatography (HIC) (29-31) are two important branches of liquid chromatography, being both carried out on a given gel packing at low or high mobile phase ionic strength, respectively. To date, both IEC and HIC techniques are widely implanted in separation of peptides and proteins rather than in the characterization of ionic polymers.

This paper concerns to the adsorption secondary effect evidenced by means of the elution of synthetic polyelectrolytes which differ in chemical nature and molar mass. Two hydrophilic and organic-based conventional packings such as TSK PW4000 and Ultrahydrogel have been used separately, and the obtained results compared between them. The influence of eluent pH and ionic strength has also been considered in order to regulate adequately the intensity of the adsorptive effects. In addition, a semi-quantitative analysis of the SEC results of polyions, when a weak hydrophobic interaction takes place, is developed in the framework of the Flory-Huggins (FH) theory in terms of polymer-gel network compatibility.

MATERIALS AND METHODS

Chemical and Reagents

Sodium acetate, acetic acid, sodium monohydrogen phosphate and sodium dihydrogen phosphate involved in the preparation of buffer solutions were all analytical grade from Merck (Darmstadt, Germany). Water was distilled and deionized in a Milli-Q system (Millipore, Mildford, MA, USA) and its conductivity was daily tested. The non-ionic water-soluble polymers were dextran standards purchased from Pharmacia (Uppsala, Sweden) with molecular weights (MW) of

10, 17.7, 40, 66.9, 83.3, 170, 500 and 2000 (blue dextran) kg·mol^{-1}. The chromatographic low-molar-mass range was covered by poly(ethylene oxide) (PEO) from Fluka (Darmstadt, Germany) with MW of 2 and 4 kg·mol^{-1}. Used polyelectrolytes were narrow-distribution samples (polydispersities lower than 1.1 in all instances) of poly(L-glutamic acid) (PGA) from Sigma (St. Louis, MO, USA) with MW of 13.6, 43 and 77.8 kg·mol^{-1}; sodium poly(styrene sulphonate) (PSS) from Pressure Chemical (Pittsburgh, PA, USA) with MW of 1.6, 16, 31, 88 and 177 kg·mol^{-1}; and poly(acrylic acid) (PAA) from Aldrich (Milwaukee, WI, USA) with MW of 5, 90 and 250 kg·mol^{-1}.

Size-Exclusion Chromatography

All chromatographic measurements were performed on a Waters liquid chromatograph (Mildford, MA, USA) equipped with an M-45 solvent delivery system, a U6K universal injector and an R-401 refractive index detector, coupled to a Yokogawa Electric Works dual-channel recorder (Tokyo, Japan).

Two organic-based column were used in this work. A Spherogel TSK PW4000 (30 × 0.75 cm I.D.) packed with hydroxylated polyether copolymer of 500 Å nominal pore diameter from Beckman Instruments (Galway, Ireland) and an Ultrahydrogel 250 (30 × 0.78 cm I.D.) packed with hydroxylated poly(methacrylate)-based gel of 250 Å nominal pore diameter from Waters. Both columns will be hereinafter referred to as TSK and UHG, respectively. The interstitial packing volume and total volume were 5.15 and 10.40 mL for the TSK and 5.48 and 10.46 mL for the UHG column, as measured with blue dextran and ^2H_2O, respectively.

The mobile phase for non-ionic polymers was distilled and deionized water and for the polyelectrolytes it was made up from NaAc/HAc buffer (pH 5.0) and from Na_2HPO_4/NaH_2PO_4 buffer (pH 7.0) to the desired ionic strength ranging from 0.01 to 0.20 M. In all instances, mobile phases were filtered and degassed through regenerate cellulose 0.45-μm filters from Micro Filtration Systems (Dublin,

CA, USA). All pH measurements were made with a Crison pH-meter model MicropH 2000 (Barcelona, Spain).

All chromatographic measurementss were conducted at room temperature and the columns were equilibrated at least 12 h prior to starting any experiment. Chromatograms were obtained at a flow-rate of 1 $mL \cdot min^{-1}$ in isocratic mode, by injection of 100 μL of 0.1 % (w/v) solute solutions freshly prepared using the corresponding mobile phase as solvent. Flow-rates were measured and found to be constant within ±0.5% by weighting the collected effluent. Each sample was injected three times as a check on the reproducibility.

Viscometry

Measurements were made with an automatic Ubbelohde-type AVS 440 capillary viscometer from Schott Geräte (Hofheim, Germany) at 25.0±0.1 °C. Efflux times were obtained with precision of ±0.01 s as an average of five or six measures. At least five dilutions were obtained by adding the appropriate aliquots of solvent. Kinetic energy corrections were included in the calculation of specific viscosities. Intrinsic viscosity, $[\eta]$, was determined by the usual extrapolation to zero solute concentration. For uncharged polymers, dextran and PEO, $[\eta]$ was evaluated from their viscometric equations (32,33) in pure water because the influence of ionic strength and pH on the viscosity of non-ionic polymers may be neglected (11,13,20).

RESULTS AND DISCUSSION

Let us first present calibration curves $\log M[\eta]_I$ against elution volume (M, molar mass; $[\eta]_I$, intrinsic viscosity at a given ionic strength) for charged and uncharged standards as a function of eluent pH and ionic strength, I; chemical architecture of the macromolecules and some features of the gel packings. Figure 1

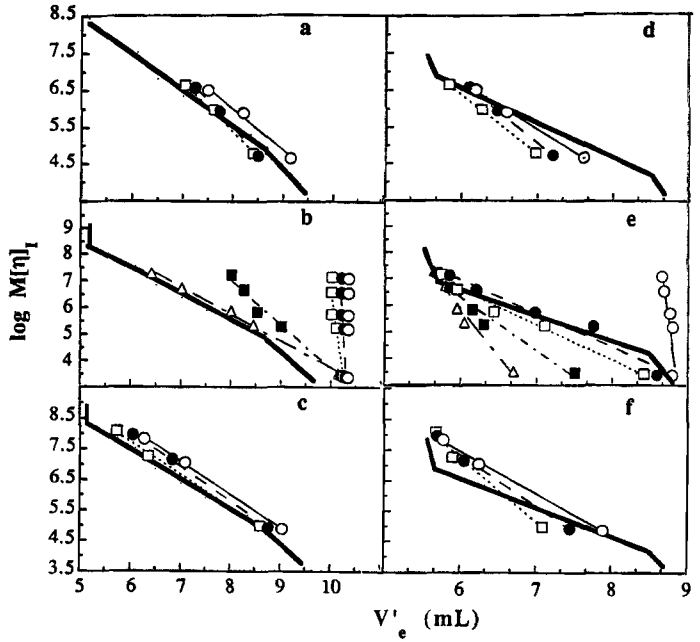

FIGURE 1. Calibration plots, $\log M[\eta]_I$ against retention volume in acetate buffer (pH 5.0) as eluent at diverse ionic strengths: (△) 0.01; (■) 0.02; (□) 0.05; (●) 0.1 and (○) 0.2 M. Solid lines refer to dextran calibrations in pure water. The following polyion/gel packing pairs are depicted: (a) PGA/TSK; (b) PSS/TSK; (c) PAA/TSK; (d) PGA/UHG; (e) PSS/UHG and (f) PAA/UHG.

compiles these calibration graphs for PGA, PSS and PAA (from top to bottom) using TSK and UHG (from left to right) as packing columns in acetate buffer (pH 5.0) with different ionic strength values ranging from 0.01 to 0.20 M. Because this paper is mainly concerned with solute adsorption as a non-exclusion effect in ASEC, in this Figure we have selected suitable experimental conditions that permits to emphasize this particular secondary effect. So that, other experimental conditions allowing an early elution of polyions relative to the non-ionic polymers have been omitted. We see that PGA and PAA calibration curves (parts a and c) follow a similar trend with respect to the reference one (dextran and PEO in the present

work), they are placed slightly on the right-hand side owing to a weak adsorption of the polyion by the gel. In contrast, the corresponding curves for PSS at I≥0.05 M show that all polymer samples are practically eluted at the same elution volume independent of their molar masses, reaching the column total pore volume. Notice that this anomalous behavior is caused by a strong adsorption of the PSS by the TSK gel under the assayed mobile phase compositions. The same measurements have been carried out using UHG column (Figures 1d-f). In general, calibration graphs approach to the reference one with increasing I, cross it, and then diverge on the other side. From the comparison between both sets of curves, for TSK and UHG, seems that the solute retention or adsorption effects are more pronounced when the former is used. However, at I=0.20 M, PSS shows a great retention irrespective the nature of the packing material. The same experiments have been carried out using phosphate buffer (pH 7.0) as mobile phase (not shown here). The results obtained in this case are similar but all curves were shifted to lower elution volumes denoting an increase in ion-exclusion secondary effects.

Basically, the retention volume of an ionic polymer depends on three major variables: mobile phase composition (pH and I), chemical nature of the stationary phase and chemical structure of the analyte, besides other chromatographic variables, not considered here, such as injected volume, sample concentration, flow-rate, etc. that can also interfere the separation process. The influence of the pH and I on the elution of a polyelectrolyte in aqueous media has been extensively studied in the past (11,13,34-36). For this reason, we next proceed to discuss the observed retention differences exclusively on the basis of the chemical nature of both polyion and gel. Figure 2 depicts calibration curves for PSS, PGA and PAA as well as the non-ionic polymers on TSK (part a) and on UHG (part b) in a common buffer solution at I=0.2 M (pH 5.0). In the light of the relative position of the calibration graphs respect to the reference one, it is easy to observe that the intensity of adsorptive effects increase according to the following order: PGA<PAA<PSS in both stationary phases.

Assuming that at pH 5.0, near to the $pK_{a(COOH)}$, the –COOH lateral functional groups of PGA and PAA are partially dissociated, c.a. 50%, both the protonated and dissociated forms will coexist. The protonated species will be

susceptible to bind to the –OH groups strategically located on the network of TSK or UHG via H-bonding, whereas the unprotonated forms will be fully screened by the counter-ions because of the high salt concentration (I=0.2 M) of the eluent. In the light of this argument, the adsorption forces will depend on the density of the –COOH groups by polymer chain. For instance, at a given molecular mass of the polymer, PAA posses twice as much carboxylic groups as PGA since their monomer molar masses are 72 and 147 $g \cdot mol^{-1}$, respectively. For this reason, it can be expected that PAA adsorbs onto the gel stronger than PGA. This behavior is corroborated by the calibration graphs depicted in Fig. 2. In addition, the divergence between both calibration curves is more pronounced in UHG than in TSK, denoting the existence of additional specific interactions between the carbonyl ester group of methacrylate (the monomer base of UHG gel) and the –COOH group of PGA and PAA samples.

Unfortunately, the above arguments cannot be invoked to explain the anomalous retention exhibited by PSS. Under the experimental conditions assayed (pH 5.0; I=0.2 M), the charges of the sulphonic groups are fully screened by the counter-ions, hence the observed adsorption can be exclusively caused by hydrophobic interaction between the hydrocarbon patterns of the PSS and those of the TSK or UHG gel packings. In this case,the polymer-gel attractive interaction becomes more intense due to the unlocated nature of the driving forces involved, which affects to the overall domain of the macromolecule.

In order to analyze more in-depth the influence of the chemical nature of the gel packing, Figure 3 depicts calibration graphs for PSS in both gels. For the sake of comparison, we have selected in the present example a mobile phase ionic strength of I=0.1 M. From the inspection of this figure, it is clearly evidenced that PSS samples are eluted very close to the uncharged polymers when UHG column is used, denoting that the macromolecular separation is carried out according to a pure size-exclusion mechanism. In contrast, PSS suffers a strong adsorption on the TSK column. In the light of this behavior, it can be inferred that UHG gel is innerter than TSK one. However, if one whishes employ advantageously the hydrophobic character of this gel for separation of biomacromolecules, proteins for instance, the TSK packing will be preferred. In conclusion, when the remaining variables (pH, I, type of solute) are fixed, the packing hydrophobicity observed will be TSK>UHG.

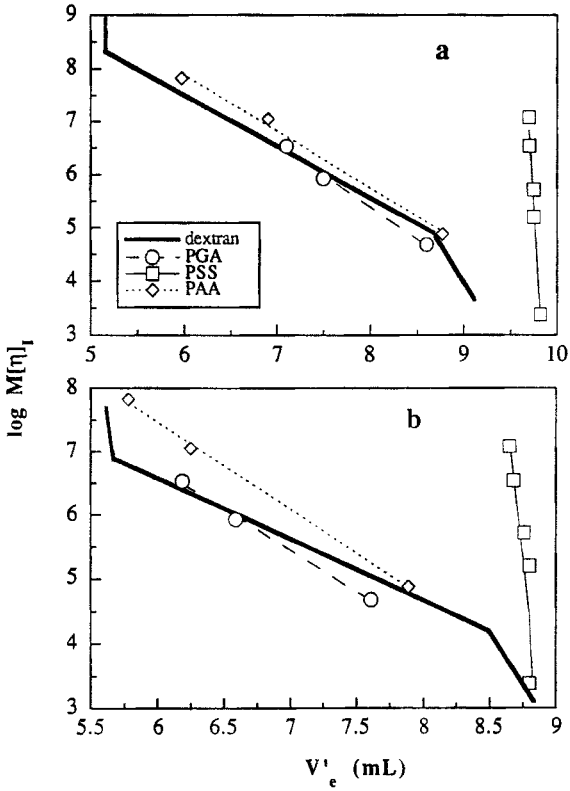

FIGURE 2. Comparison between the calibration plots obtained from charged (symbols) and uncharged (solid line) standards polymers in acetate buffer (pH 5.0) at 0.2 M, using TSK (part a) and UHG (part b) as gel packings.

To date, considerable efforts have been devoted to give a theoretical description of the secondary effects in SEC, including organic, aquoorganic and aqueous mobile phases (4,19,23). Most of them consider a unique force as the origin of the interactions between adsorbent (gel packing) and solute (polyelectrolyte). In this way, for instance, physico-chemical treatments accounting for electrostatic effects (8,12), solid-liquid adsorption and hydrophobic interaction (23,31) can be examples of the abovestated. However, in the particular case of the

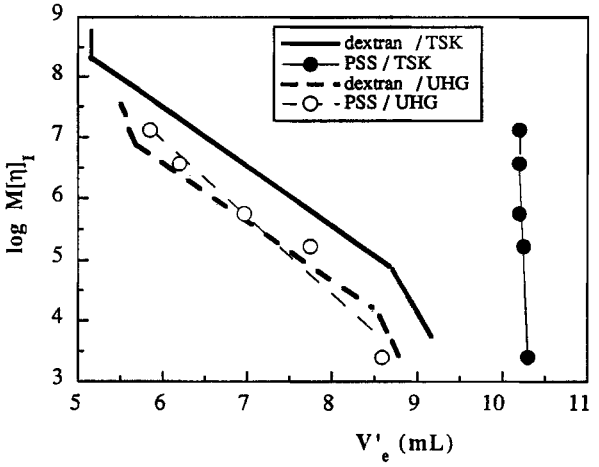

FIGURE 3. Comparison between the calibration curves for diverse polymer/gel packing pairs as specified in the legend plot, using acetate buffer (pH 5.0) at 0.1 M as eluent.

elution of polyanions through the gel packings used here, more than one force resembles to contribute to the overall adsorption effect, being more difficult to perform a theory predicting properly this effect. A temptative to explain, at least semiquantitatively, adsorption in ASEC has been developed in the frame of the Flory-Huggins theory of polymer solutions for multicomponent systems.

The first assumption deals with the fact that adsorption effects can be viewed as a reversible adsorption-desorption equilibrium between solute (S) and gel (G), expressed as follows:

$$S + G \leftrightarrow SG$$

if the activity coefficients corresponding to occupied (SG) and unoccupied (G) sites are supossed to be the same (37), the adsorption equilibrium constant can be defined as:

$$K_{ad} = \frac{[SG]}{[S][G]} \qquad (1)$$

where [G] and [SG] are the molar concentrations of the free and occupied gel active centers, respectively, in the volume of the stationary phase available to the solute, and [S] is the molar concentration of free solute. According to Laurent and Killander (38) and following the development proposed by Janado (23), the elution volume of a polymer can be expressed as:

$$V'_e = V_0 + K_{SEC}\left[1 + \frac{K_{ad}[G]_0}{1 + K_{ad}[S]}\right]V_p \qquad (2)$$

being V'_e, V_0 and V_p, the elution volume of a polyelectrolyte sample, the interstitial packing volume and the pore volume, respectively. $[G]_0$ refers to the total concentration of stationary phase accessible to the solute and K_{SEC} to the chromatographic distribution coefficient when the solute partition is due solely to steric exclusion effect.

On the other hand, when secondary effects in SEC become important, the elution volume of a polymer sample can be defined as (11,39):

$$V'_e = V_0 + K_{SEC}K_pV_p \qquad (3)$$

being K_p the partition coefficient accounting for secondary effects, adsorption in the present case. Comparison of eqns. 2 and 3 leads to:

$$K_p = 1 + \frac{K_{ad}[G]_0}{1 + K_{ad}[S]} \qquad (4)$$

Notice that K_p is a partition coefficient obtained by conventional elution chromatography, $K_p=(V'_e-V_0)/(V_e-V_0)$, whereas K_{ad} refers to an equilibrium constant. Moreover, the physical meaning of the former parameter corresponds to a ratio of polymer concentrations between the mobile and the stationary chromatographic phases, whereas K_{ad} denotes the ratio of molar concentrations of the species involved in a true chemical equilibrium. At this point, when the injected

polymer solution becomes highly diluted, it can be assumed that $[S] \rightarrow 0$, and then eqn. 4 transformed into:

$$K_p = 1 + K_{ad}[G]_0 = 1 + K'_{ad} \qquad (5)$$

since $[G]_0$ is a constant for a given chromatographic gel, $K'_{ad} = K_{ad}[G]_0$. Therefore, K'_{ad} or K_p can be used to quantitatively evaluate adsorption effects. Thus, positive values of K'_{ad} imply $K_p > 1$ (see eqn. 5) or $V'_e > V_e$, in other words, calibration curves for polyelectrolytes shifted towards the right-hand side of the reference one obtained for uncharged polymers.

In Table 1 we present K_p values extracted from calibration curves at pH 5.0 and 7.0 for PSS in both TSK and UHG columns. The eluent ionic strength was 0.1 M in both sets of experiments. In general, the K_p values increase as the molecular weight goes up and pH decreases, according to previous reported data for aqueous and organic SEC (11,18). From the comparison of K_p data obtained in both columns at pH 5.0, it can be observed strong adsorption in TSK whereas in UHG this effect vanishes, yielding K_p values close to unity which denotes that the elution occurs mainly according to a pure size-exclusion mechanism. In the light of this behavior, UHG packings will be more convenient for polymer characterization via conventional SEC, whereas TSK columns seem to be more appropriate to elute solutes according to hydrophobic interaction chromatography (HIC). Similar trends have been found for the remainder polyelectrolyte/gel systems studied here.

In order to investigate the quantitative variation of K_p values within each calibration curve, Figure 4 depicts its dependence on both sample molecular weight and eluent ionic strength, for PSS in TSK at pH 5.0 as an example. Lines connecting points have been drawn to guide the eye. On the one hand, at a given molecular weight, K_p shows a linear dependence on the inverse of the square root of ionic strength, $I^{-1/2}$, in accordance with the functionality exhibited by other variables such as the chromatographic radii of a macromolecule, R (14), the electrostatic repulsion barrier between the polyion and the charged pore, X_e (8), the ion-exclusion or repulsion volume (10), K_{SEC} (40), the persistence length, L_e (41) or the elution volume (36,42) among others. On the other hand, at a fixed ionic

TABLE 1

Values of the Distribution Coefficient, K_p, obtained from SEC Measurements as a Function of the PSS Molar Mass at Different Mobile Phase Compositions. The Ionic Strength of the Buffer Solutions used as Eluents was 0.1 M in all Experiments.

M_2 (kg·mol^{-1})	Column TSK		Column UHG	
	pH 5.0	pH 7.0	pH 5.0	pH 7.0
1.6	1.40	1.24	0.99	0.80
16	1.60	1.41	1.07	0.87
31	1.91	1.68	1.08	0.81
88	2.80	2.46	1.22	0.88
177	4.81	3.60	1.95	1.68

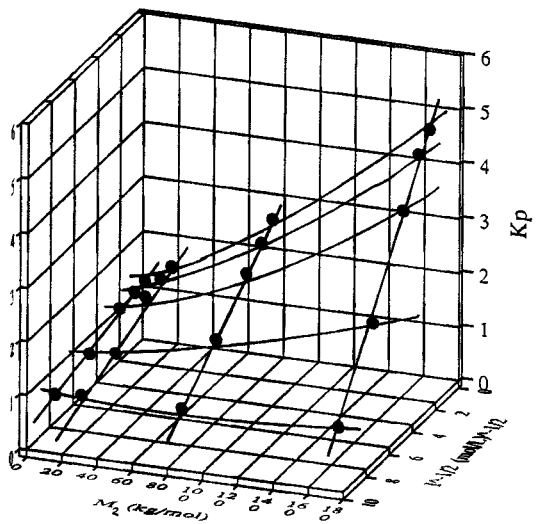

FIGURE 4. Plot of K_p dependence on both M_2 and $I^{-1/2}$ for PSS in a TSK PW4000 column using acetate buffer (pH 5.0) as eluent.

strength, K_p values seem to follow an exponential variation with the molecular weight, mainly at high K_p values. Theoretical prediction of this last dependency has been made in the framework of the Flory-Huggins (FH) polymer solutions theory (43-46). On this regard, some authors consider a ternary system formed by a solvent(1) (the eluent), a polymer(2) (the eluite) and a polymer(3) (the crosslinked polymeric network or gel chromatographic packing) yielding a master equation which correlates K_p with the polymer molecular weight, M_2, through:

$$-\ln K_p = \frac{\phi_3}{\rho_2 V_1}(1 + g_{23} - g_{12} - g_{13})M_2 \qquad (6)$$

being ρ_2 the density of the injected polymer solution, V_1 the molar volume of solvent, ϕ_3 the volume fraction of gel involved in the ternary phase, and g_{ij} the Flory-Huggins interaction parameters.

To date, the validity of the above equation has been exclusively tested for uncharged polymers in organic media (44). For ionic polymers such as polyelectrolytes and proteins in aqueous media, a non-unique dependence has been postulated. Thus, Hjerten (47) proposed the relationship $\log K_p \propto M$ for low molecular weight compounds, whereas for globular and flexible macromolecules the functionality $\log K_p \propto M^{2/3}$ worked better. In previous papers, our group has reported the accomplishment of $\ln K_p \propto M^{-1/2}$ for synthetic polyelectrolytes at low ionic strengths (see Figs.5 and 2 from refs.11 and 13, respectively). However, at moderate and high ionic strengths, we believe that eqn. 6 could be an appropriate functionality to quantitative analyze adsorption secondary effects in ASEC. This aseveration can be supported *a priori* by the fact that at high ionic strength, most charges on the polyion are screened by counterions increasing the chain flexibility and reaching the polymer a random-coil conformation similar to that for synthetic polymers in organic media.

Figure 5 depicts plots of eqn. 6 for: (a) PSS in TSK, (b) PSS in UHG and (c) PAA in TSK using acetate buffer (pH 5.0) at different ionic strengths. The horizontal line drawn on these plots at zero value in ordinates (K_p=1) denotes the

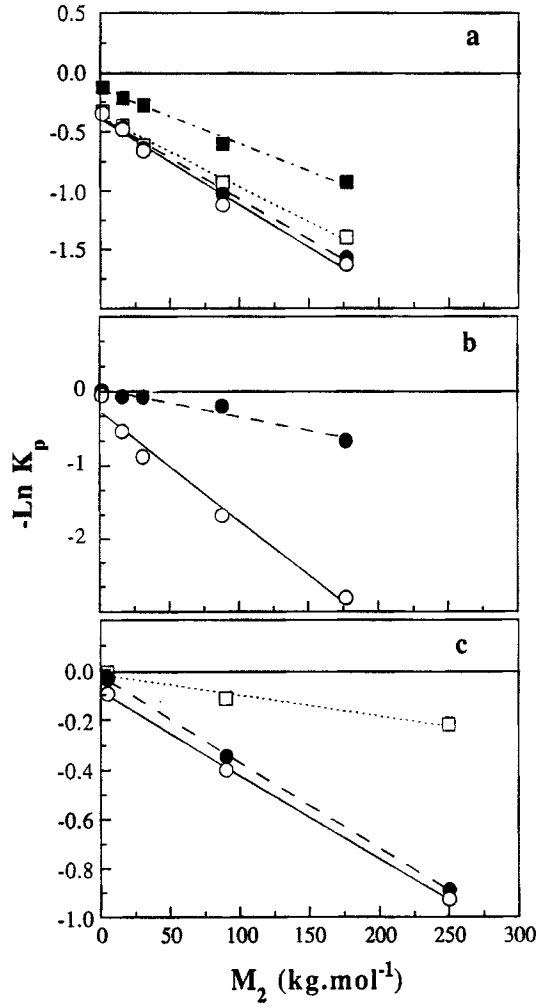

FIGURE 5. Plot of eqn. 6 for the following polyelectrolyte/gel systems: (a) PSS/TSK; (b) PSS/UHG and (c) PAA/TSK. Symbols stand for eluent ionic strength and have the same meaning as in Fig. 1.

location of the pure size-exclusion mechanism, corresponding upper and lower zones to electrostatic polymer-gel repulsion ($K_p<1$) and to polymer-gel adsorption ($K_p>1$), respectively. Firstly, it deserves to be remarked that experimental K_p values fit well to a straight line in all cases in spite of the marked structural differences of polyelectrolytes and gels used. These results support the validity of eqn. 6, at least under the chromatographic conditions and molecular weight range assayed here. Second, the absolute slope values increase as the mobile phase ionic strength does, in accordance with previously reported experiments, where $K_p>1$ were often obtained at high I values (9,48,49). Moreover, the slope values could be regarded as a quantitative measure of how intense becomes the driving forces involved in the adsorption phenomena between the network links on the gel packing and links of the eluted linear polyion.

Figure 6 shows similar plots of eqn. 6 for PSS (part a) and PGA (part b) in TSK using phosphate buffer (pH 7.0) and different I values, as mobile phase. Some comments supporting the selected experimental conditions on this Figure deserve to be made: (i) pH 7.0 was used because most biopolymers display a practical activity in biological fluids streamed in living tissues at a pH values close to neutrality; (ii) the selection of an appropriate I values becomes important in order to minimize secondary effects; (iii) the above experimental conditions can also serve to test the fulfillment of eqn. 6 when size-exclusion and electrostatic repulsion besides adsorption effects take place. From the inspection of Figure 6a, as expected, for $I \geq 0.1$ M the adsorption effects govern the chromatographic elution of PSS whereas at $I \approx 0.01$ M the ion-exclusion seems to be the predominant effect. Elution mechanisms in ASEC are not only controlled by ionic strength but also by the chemical nature and pK_a of the ionic group of the polyelectrolyte. To corroborate this argument, Figure 6b shows plots of eqn. 6 for PGA in the same range of I. In contrast with that displayed in part a, at $I \geq 0.1$ M neither adsorption nor electrostatic repulsion are evidenced, whereas at $I \approx 0.01$ M strong polymer-gel repulsions occur. Again, good linear fits are always obtained corroborating the validity of eqn. 6 for charged polymers in aqueous media.

Following our inspection of Figures 5 and 6, it seems that the slopes of the straight lines depicted are closely related to the binary g_{ij} interaction parameters. On

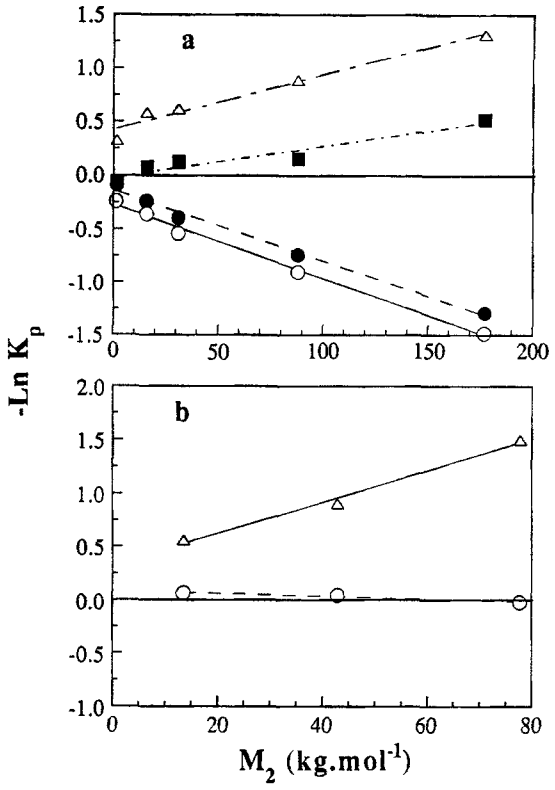

FIGURE 6. Plot of eqn. 6 for the systems: PSS/TSK (a) and PSS/UHG (b) in phosphate buffer (pH 7.0) as mobile phase. Symbols stand for eluent ionic strength and have the same meaning as in Fig. 1.

this regard, recent contributions (50,51) have also analyzed the swelling and collapse of soft gels in polymer solutions (charged or not) in terms of the interaction of components (compatibility) within the FH approximation. Note that for a quantitative interpretation of the elution mechanisms on the basis of polymer-gel compatibility through the slope values of eqn. 6, it is necessary the availability of the g_{ij} experimental values. Owing to the lack of g_{ij} data in the literature for polyions in buffer solutions, we can only speculate about the sign of the slope, directly related to the elution mechanism governing the SEC separation process. In

the particular case that polyelectrolytes are eluted according to the pure size-exclusion mechanism, the slope of eqn. 6 approaches to zero (see Figure 6b). From a thermodynamic viewpoint, this behaviour implies that the g_{23} parameter is close to zero because neither favorable ($g_{23}<0$) nor unfavorable ($g_{23}>0$) polymer-gel interactions are involved in the separation mechanism. However, a more plausible explanation could be given for ionic polymers in hydrophilic gels if one assumes that both favorable and unfavorable interactions exist but cancel each other. Therefore, under chromatographic conditions in which the elution data are slightly shifted from the pure SEC, $g_{23} \approx 0$, and the slope of the above plots will be proportional to $(1 - g_{12} - g_{13})$ and eqn. 6 for these cases can be transformed into:

$$-\ln K_p \cong \frac{\phi_3}{\rho_2 V_1}(1 - g_{12} - g_{13})M_2 \qquad (7)$$

For this particular situation, the entity $(1 - g_{12} - g_{13})$ is coincident with the numerator of the following expression (45):

$$\lambda = \bar{v}_3 \frac{1 - g_{12} - g_{13}}{1 - 2g_{12} - 2(dg_{12}/d\phi_1)} \qquad (8)$$

being λ the preferential solvation parameter, widely studied in polymer-mixed solvent systems (see eqn. 20 from ref.45), and $\bar{v}_3$ the partial specific volume of component 3. Assuming that the denominator of eqn. 8 is always positive (52), valid for random-coil conformations, the sign of the λ parameter and the sign of $(1 - g_{12} - g_{13})$ will be coincident, allowing us a more comprehensive analysis of the secondary effects in ASEC in terms of λ instead of the g_{ij} interaction parameters. First of all, it is important to understand the physical meaning of the sign of λ. On this regard, favorable or preferential eluent-gel interactions are represented by $\lambda>0$ and favorable polymer-gel interactions by $\lambda<0$. These statements can also be associated to the polymer-gel incompatibility and compatibility, respectively. Obviously, when both types of interactions are counterbalanced λ vanishes meaning that macromolecules are eluted according to a pure SEC mechanism. In the light of this formalism, $K_p>1$ corresponds to $\lambda<0$ denoting that the adsorption effect can be

viewed as a polymer-gel compatibility. In contrast, when the main secondary effec is the electrostatic repulsion, $K_p<1$ or $\lambda>0$ closely related to polymer-gel incompatibility.

CONCLUSIONS

It has been selected the partition coefficient, K_p, or its related equilibrium constant K'_{ad} (see eqn. 5), to quantify the intensity of the polymer-gel packing adsorption. The values of this parameter have been extracted from basic chromatographic equations dealing with elution of polyelectrolytes. In most of the experiments reported here, $K_p>1$ denoting that adsorptive effects take place. Some speculations about the driving forces involved in the polymer-gel packing interactions reveal that H-bonding can be the origin of this phenomenon when PAA and PGA are eluted. However, in the case of PSS the adsorption effect can be assigned to hydrophobic interaction between some regions on the surface of the polyelectrolyte and those on the stationary phase. This behavior has been confirmed in the two hydrophilic packings studied here, being the adsorption effects more intense on the TSK column than on the UHG one, for the same polymer eluted under fixed experimental conditions. Therefore, it seems that UHG gel packing is innerter than TSK for size-exclusion separation purposes.

Finally, we have presented in this contribution an attempt to explore adsorption secondary effects in ASEC by means of the Flory-Huggins theory of polymer solutions extended to the eluent(1)/polymer(2)/gel packing(3) ternary system. Some transformations of the original equations allow us to introduce the λ parameter (see eqn. 8) in order to justify the adsorption effects in terms of polymer-gel compatibility. Within the limits of the accuracy of the data, we conclude that linear plots depicted in Figures 5 and 6 support the validity of the functionality obtained in eqn. 6 through the FH theory.

Additional experimental and theoretical contributions, beyond the chromatographic scope, must be done, mainly to evaluate the g_{ij} parameters for ionic species.

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