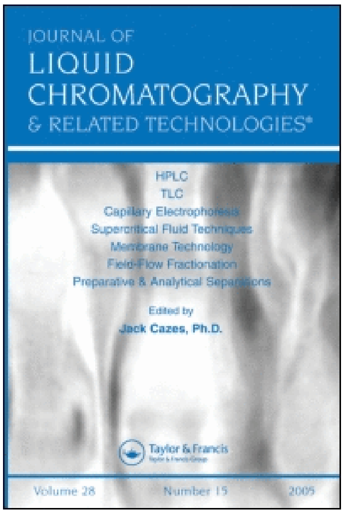

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GEL PERMEATION CHROMATOGRAPHY OF POLYELECTROLYTES

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ABSTRACT

The mechanisms are discussed which control the GPC elution of ionic solutes, both the polyelectrolytes and the low molecular salts. The processes involved are quite general and valid in organic and in aqueous solvents. The conclusion is that gel permeation chromatography is a powerfull method to characterize polyelectrolytes ; and it is shown that the correct data on molecular weight distribution can be obtained when the ionic content in the eluent is larger than 5.10^{-2} M and when the concentration injected is lower than the critical overlapping concentration. The interpretation of chromatograms can be performed using the universal calibration and a viscosimetric detector.

INTRODUCTION

This paper deals with the mechanism of gel chromatography on ionic polymers in organic and aqueous solvents. We have previously shown that the processes involved were controlled by electrostatic mechanisms whatever is the solvent (1 - 4). One will propose a general treatment for characterization of polyelectrolytes by gel permeation chromatography (GPC). Much papers concern GPC on synthetic neutral polymers ; it is well established that the steric exclusion is controlled by the hydrodynamic volume $[\eta]$ M of the polymers (5). When the chromatography of ionic solutes is performed, even with low molecular weight solutes electrostatic exclusions are observed (2, 3, 6, 9). As consequence the volume of elution depends on the ionic concentration of the solute injected but also on that of the eluent. On polyelectrolytes, universal calibration has been validated for polystyrenesulfonate and polyacrylate sodium salts (9).

One wants : 1/ to dissociate electrostatic and steric processes as soon as the secondary adsorptions on the gel are avoided ;
2/ to propose best conditions to obtain steric exclusion mechanism on ionic polymers such as to get their molecular weight distribution.

MATERIAL AND METHODS

The porous silica gels are Spherosil from Rhône-Poulenc ; a set of columns (height = 147 cm ; inner diameter 0.8 cm) are filled up with gels of following average pores diameters 70, 150, 300, 500, 1250, 3000 and 5000 Å.

The solvents are dimethylformamide (DMF) or water freshly distilled and degazed. With H_2O, the tank of solvent is maintained at 60°C during elution. Constant flow-rate is maintained with a Milton Roy pump (11 ml/h in DMF - from 12 to 60 ml/h in water). The detectors are adapted on line : a differential refractometer R 401 Waters, a conductivity cell built up in our laboratory and connected to a Wayne Kerr B 642 Conductimeter and an automatic viscosimeter from Fica filled with constant effluent volume delivered by a special buret built up in the laboratory (4). The signals of the refractometer (Δn) and of the conductimeter ($\Delta \chi$) are associated to get informations 1/ on the purity, osmotic coefficient and charge distribution of polyelectrolytes, (10)
2/ on electrostatic exclusion of low charge solutes or polyelectrolytes.

The differential refractometer signal (Δn) is coupled with the automatic viscosimeter data (η) when steric exclusion is investigated. All the concentrations are expressed in equivalent ionic charge per unit volume. The standards used to calibrate the set of columns are : polystyrenes in DMF, dextrans and sodium polystyrenesulfonates in water ; the total volume V_T of the system is determined with D_2O, whatever is the eluent.

RESULTS AND DISCUSSION

A/ Electrostatic exclusion on simple salt

First, we shall discuss the results obtained with a low molecular weight solute generally NaCl in water (11) and $NaNO_3$ in DMF (10).

Dependence with the ionic concentrations

When a constant volume (v) of a given electrolyte at a constant concentration C_s is injected, the elution volume V_e decreases when the ionic concentration of the eluent (C_e) decreases.

At a given ionic concentration of the eluent, the elution volume V_e decreases to a limit called V_{ex} when the ionic concentration (C_s) injected decreases. The figure 1 gives a schematic representation of this behaviour.

In addition, the form of the peaks eluted is modified when the ionic content varies ; they are symetrical as soon as their elution volume reaches V_{ex} for very dilute solution and V_T for high concentration (figure 2).

The total volume (V_T) of the set of columns is given wathever is the solvent by D_2O used as reference ; the volume of elution of D_2O is independent on the ionic concentration of the eluent. On the contrary, when samples of H_2O are injected, one obtains an elution peak at a limiting volume of exclusion V_{ex} depending only on C_e.

The set of results recalled in this paper is directly related to electrostatic exclusion ; the conclusion is that over 5.10^{-2}M

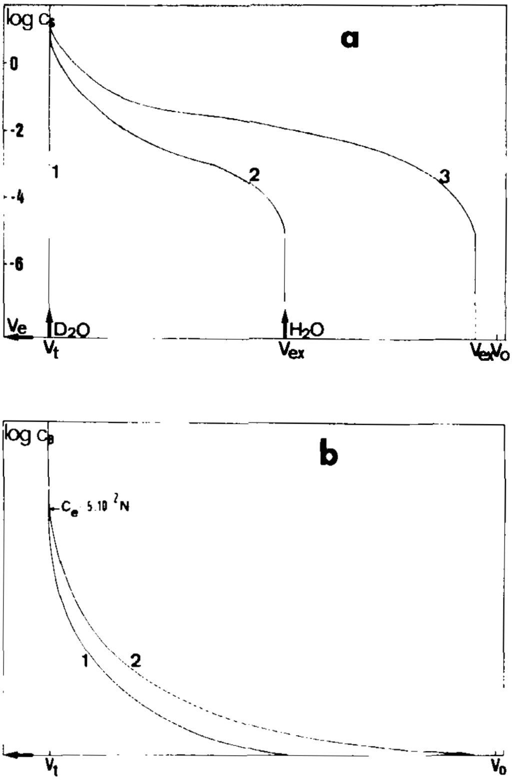

Figure 1 : Schematic dependence of the ionic concentration on the elution volume (V_e) for a constant volume (v) of electrolyte injected.

(a) influence of C_s at constant C_e
1. $C_e = 10^{-1}$ N ; 2. $C_e = 10^{-4}$ N ; 3. water

(b) influence of C_e at constant C_s
1. $C_s = 10^{-2}$ N ; 2. $C_s = 10^{-4}$N.

or better 10^{-1}M for the ionic concentration of the eluent, the repulsions are screened (Figure 1b).

Dependence with the ionic content injected.

As previously mentionned, the elution volume is dependent on the concentration of the solute injected. In fact, ones demonstra-

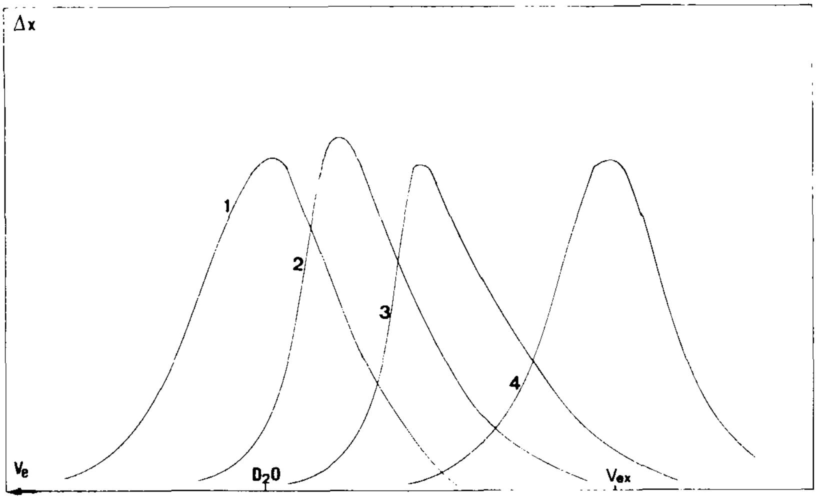

Figure 2 : Conductimetric traces for various concentrations C_s injected and eluted by water (v = 50µl).
1. $C_s = 1$ M - sensitivity x 1 ; 2. $C_s = 4 \times 10^{-2}$M - sensitivity x 20
3. $C_s = 8 \times 10^{-3}$M - sensitivity x 100 ; 4. $C_s = 2 \times 10^{-6}$M - sensitivity x 5000.

te that V_e is imposed by the product (volume x concentration) (11); as the volume of solution (V_S) in which a given salt is eluted is approximatively constant, one proposes to plot the dependence of average salt concentration $C_S = \frac{VC_S}{V_S}$ as a function of the partition coefficient $K = \frac{V_e - V_o}{V_T - V_o}$ (V_0 is the void volume determined from the elution of a very high molecular weight polymer). When the eluent is an electrolyte, the area of the peak as well as its position is imposed by the excess of electrolyte $\Delta\overline{C_S} = (C_S - C_e) \times \frac{V}{V_S}$ (C_e is the electrolyte concentration of the solvent). For each eluent, a only curve correlates the average excess salt concentration $\Delta\overline{C_S}$ to the elution volume expressed by K.

In addition, it is shown that 1) the curve is dependent on the average pore diameter if the elution volume of NaCl is tested on a column filled up with one type of gel (one compares Φ = 300 Å and 5000 Å (Figure 3); 2) the curve obtained for elution of Na_2SO_4 in water is nearly identical to that obtained with NaCl. Each curve is characterized by 3 parameters :

- [A] is the higher average salt concentration necessary to screen the electrostatic repulsion ($\Delta \overline{C}_S >$ [A] $\rightarrow K = 1$). [A] depends on the porosity of the gel.
- [B] is the lower average concentration under which the elution volume remains constant ($\Delta \overline{C}_S <$ [B], $K = \text{const} = K_{ex}$) ; [B] depends on the ionic content of the eluent.

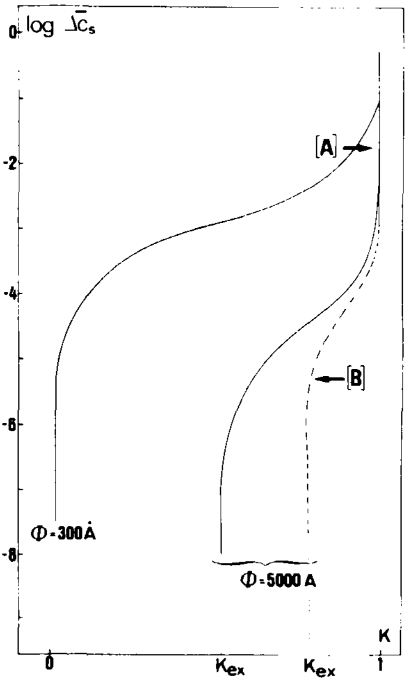

Figure 3 : Influence of the pore size (Φ = average pore diameter) on the volume of elution of NaCl expressed by the partition coefficient K ——— eluent H_2O ; – – – – – eluent NaCl 10^{-4}M.

- K_{ex} is the limiting partition coefficient corresponding to the electrostatic exclusion with $0 < K_{ex} < 1$. K_{ex} depends on the porosity of the gel, on the ionic content of the eluent but not on the valence of the ions. One proposes to interpret qualitatively this set of results by introduction of the screening length of Debye-Huckel $\delta \sim \mu^{-1/2}$ (μ is the ionic strength) compared with the pore size Φ (Φ is the average pore diameter) :
For the ionic concentration B, when $\delta_{[B]} > \Phi$, one gets $K_{ex} = 0$; when $\delta_{[B]}$ is of the same order than Φ, K_{ex} corresponds to the volume fraction of the pores whose diameters are larger than δ ; it implies a sort of steric exclusion without dependence with the net charge of the ions. In the range of porosity investigated, the limit ionic concentration [B] is imposed by the ionic content of the eluent ; it is around 10^{-4} in NaCl 10^{-4} M and 10^{-6} in water.
At last, the ionic concentration [A] is attained when $\delta_{[A]}$ is lower than the lower pore diameter in the gel (3).

B/ Electrostatic exclusion on polyelectrolyte.

When a polyelectrolyte is injected and eluted by pure solvent (water or DMF), a only peak is obtained if the polymer is free of low molecular weight salt. It is a good way to test the purity. The ratio of conductimetric and refractometric peak gives an information on the charge distribution on the polymer ; this point has been previously discussed (1-2).

When two samples of sodium polystyrenesulfonate (M_w = 21.000 and 145.000 respectively) are injected at various concentration C_p and eluted by water , results like that given in figure 3 with NaCl are obtained ; the limit K_{ex} depends on the molecular weight of the polymer (3). This result is interesting and means that for very low concentration ($C_p < C_e$) the chromatograms should be interpreted in terms of molecular weight distribution even if electrostatic exclusion is not at all screened ; the curve $\Delta\chi/\Delta n$ (V_e) gives the charge density as a function of the molecular weight distribution.

When an electrolyte is used as eluent, two peaks are observed in the chromatogram due to Donnan equilibrium previously discussed (1-12). One peak corresponds to elution of the polymers and the second to that of low molecular weight solute contained in the solvent and excluded by the polyelectrolyte. Both peaks separate as a function of the concentration of polymer injected (Fig. 4a).

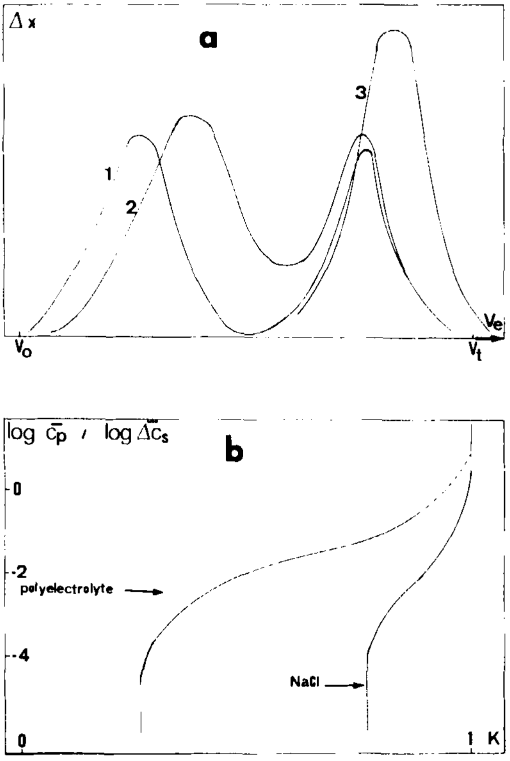

Figure 4 : Influence of the polyelectrolyte concentration injected on the chromatogram (eluent NaCl 10^{-4}M)

(a) Chromatograms obtained for variable polyelectrolyte concentration (C_p) dissolved in the same solvent (NaCl 10^{-4}M) (v = 50 µl) 1. $C_p = 10^{-1}$ eq.l^{-1} ; 2. $C_p = 5.10^{-3}$ eq.l^{-1} ; 3. $C_p = 10^{-3}$ eq. l^{-1}

(b) Schematic dependence of the volume elution for NaCl and the polyelectrolyte injected separately.

In the figure 4b, one gives the volume of elution of NaCl and polyelectrolyte separatly as a function of the average ionic concentration ; it demonstrates how to interpret the evolution of chromatogram (Figure 4a). The area of the peak eluted to higher elution volume allows to calculate the osmotic coefficient Φ of the polyelectrolyte in presence of external salt.

The number of equivalent of salt Q_s under this peak is correlated to that of the polyelectrolyte injected (Q_p) by the relation :

$$Q_s \simeq \frac{\Phi}{4} Q_p = \frac{\Phi \, C_p \, v}{4}$$

By GPC, the coefficient Φ can be obtained as a function of the ionic strength (C_e) and of the polymer concentration (C_p) ; when eluent is water, the conductimetric signal allows to deduce Φ directly (1-2) but also the mobility of the polyelectrolyte. The dependence of Φ as a function of the polymer concentration obtained by GPC has been previously compared with direct measurements (2).

C/ Steric exclusion.

One demonstrates that the same calibration of a set of silica gels is valid in water and organic solvent (4-11) ; the calibration in DMF was performed with standards of polystyrene and in water with dextrans (using 1 % ethyleneglycol in the eluent to avoid adsorption (13))and sodium polystyrenesulfonates. Coupling refractometric and viscosimetric detectors, one interprets the chromatograms obtained for a polyelectrolyte for different eluents. It is very important to control the nature of the counterions and to adopt an univalent electrolyte with same ion than the counterions of the polyelectrolyte. Using the universal calibration ($[\eta]$ M (V_e)) one deduced an apparent molecular weight which decreases to a constant value as the ionic content of the eluent increases (Table I). This effect corresponds to the screening of the electrostatic exclusion of the polyelectrolyte.

TABLE I

Influence of the ionic concentration in eluent ($NaNO_3$ in DMF) on the apparent molecular weight obtained from GPC experiments.

$[NaNO_3]$	M_W [I]	M_W [II]
$5 \times 10^{-3}M$	450,000	350,000
$1.5 \times 10^{-2}M$	350,000	290,000
$5 \times 10^{-2}M$	250,000	200,000
$10^{-1}M$	230,000	170,000
$2 \times 10^{-1}M$ *	250,000	170,000

* Light scattering measurements.

[I] and [II] are two samples of poly(acrylonitrile-co-metalylsulfonate) sodium salt. Ionic content : 0.6 x 10^{-3} and 1,2 x 10^{-3} equivalent $-SO_3^-$ per gram of dried polymer respectively.

When $C_e > 5.10^{-2}M$, the molecular weight M_w obtained by GPC is equal to that obtained by light scattering (1-10).

In addition as the ionic content of the eluent increases over $5.10^{-2}M$, the peak is still displaced to higher elution volume due to decrease of the classical hydrodynamic volume of the polyelectrolyte.

In the Table II, an exemple for the characterization of polystyrenesulfonates in aqueous solvent is given ; the agreement between molecular weight obtained by GPC and direct measurements is good ; the intrinsic viscosities given by the viscometric detec-

TABLE II

Characterization of polystyrenesulfonates (sodium salt form) by GPC ; comparison with direct measurements.

Samples	M_w Light scattering	GPC			$[\eta]$ Static ($ml.g^{-1}$)
		M_w	M_n	$[\eta]$ ($ml.g^{-1}$)	
I	23,400	25,000	18,000	15.2	15.2
II	188,000	194,000	109,000	102	102
III	646,000	651,000	303,000	301	303

Solvent : H_2O - 0.1M NaCl.

tor is also very comparable to that determined in a separate experiment with an Ubbelohde viscometer.

At end, it is important to control the polymer concentration C_p injected ; in fact, one observes a dependence of the elution volume with C_p : up to a critical concentration C^*, V_e is constant; over C^*, it increases (4). This concentration corresponds to the transition between dilute to semi-dilute regime (14). It is a quite general behaviour obtained with neutral and ionic polymer ; so, it is essential to determine the elution volume extrapolated to infinite dilution to plot the calibration and it is necessary to inject a polymer at a initial concentration $C_p \leqslant C^*$. This should be a limitation for the GPC characterization of very high molecular weight polymers.

CONCLUSION

This paper is a synthesis of our contribution to the gel permeation chromatography on polyelectrolytes ; results are quite identic in organic and aqueous solutions. The dependence of ionic concentration of the solute injected, of the eluent, of the porosity of the gel on the elution volume has been discussed.

The electrostatic exclusion from the gel is suppressed with eluent with salt content higher than 5.10^{-2}M at least for gels whose pores diameter is larger than 50 Å ; then the mechanism of steric exclusion is the only one which controls the elution and the universal calibration is convenient to analyze chromatograms. Nevertheless, it is shown that the concentration of the polymer injected must remain lower than the overlapping concentration C^*.

All the results are established on anionic polymers ; it is clear that up to day with partially negative charged gels, it is impossible to elute polycations. The GPC on polyelectrolytes seems to be a very important technic of characterization 1/ to determine the purity of a sample, the ionic charge distribution on the polymer, the osmotic coefficients when the eluent is free of external salt or at a low salt content, 2/ to establish the molecular weight distribution based on steric exclusion using the universal calibration.

REFERENCES

(1) Domard A., Rinaudo M., Rochas C. J. Polym. Sci. Polym. Phys. Ed. 17 673 (1979).

(2) Rochas C., Domard A., Rinaudo M. Eur. Polym. J. 16 135 (1980)

(3) Rinaudo M., Desbrières J. Eur. Polym. J. 16 849 (1980).

(4) Desbrières J., Mazet J., Rinaudo M. J. Polym. Sci (to be published).

(5) Benoit H., Grubisic Z., Rempp P., Decker D., Zilliox J.G. J. Chim. Phys. 63 1507 (1966).
Grubisic Z., Rempp P., Benoit H. J. Polym. Sci. Polym. Sci. Polym. Lett. Ed. 5 753 (1967).

(6) Neddermeyer P.A., Rogers L.B. Analyt. Chem. 40 755 (1968) ; 41 94 (1969).

(7) Cooper A.R., Matzinger D.P., in "Chromatography of Synthetic and Biological Polymers" edited by R. Epton. Vol. I, chap. 28 Ellis Horwood Publ. 1978.

(8) Cooper A.R., Matzinger D.P. ; J. Appl. Polym. Sci. 23 419 (1979).

(9) Spatorico A.L., Beyer G.L. ; J. Appl. Polym. Sci. 19 2933 (1975).

(10) Rochas C. Thesis, Grenoble 1978.

(11) Desbrières J. Thesis. Grenoble 1980.

(12) Lindström T., de Ruovo A., Soremark C. ; J. Polym. Sci. Polym. Chem. Ed. 15 2029 (1977).

(13) Le Page M., Beau R., De Vries A.J. ; J. Polym. Sci. C 21, 119 (1968).

(14) Daoudi S. , Brochard F. ; Macromolecules 11 741 (1978).