European Polymer Journal, Vol. 16, pp. 135 to 140
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0014-3057/80/0201-0135$02.00/0

AQUEOUS GPC OF ELECTROLYTES AND POLYELECTROLYTES

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(*Received* 3 *July* 1979)

Abstract—The gel permeation chromatography (GPC) of polyelectrolytes is discussed. The electrostatic exclusion is demonstrated using simple electrolytes with different eluents. The data for aqueous free salt solutions of polyelectrolytes are interpreted. The osmotic coefficient is deduced and its dependence on molecular weight and polymer concentration reported. In salt eluents, the Donnan exclusion is discussed. The universal calibration obtained with different standards in aqueous solution is shown to be valid and identical to that obtained in an organic solvent using polystyrene standards.

INTRODUCTION

Few papers have been published on gel permeation chromatography (GPC) of polyelectrolytes. The main effects are the same, independent of the chemical composition of the gel. First, one observes an electrostatic exclusion of simple electrolytes as well as polyelectrolytes. This process has been described by Neddermeyer and Rogers [1] for simple electrolytes, and by Cooper and Matzinger [2] and Rochas *et al.* [3] for polyelectrolytes. Secondly, a Donnan equilibrium is established on the gel as recently described by Lindstrom *et al.* [4], Rochas *et al.* [3] and Neddermeyer [1]. Thus one observes, in a salt eluent, a salt exclusion eluted at the same volume as for a simple electrolyte injected alone.

In this paper the variation of the elution volume of a simple electrolyte is investigated as a function of the eluent ionic-strength and of the solute concentration. Then, the elution of polyelectrolyte in salt-free and in ionic eluent is discussed to demonstrate that GPC is a convenient technique to characterize a given polyelectrolyte. Then a universal calibration is established using neutral and ionic polymers as standards.

EXPERIMENTAL

Silica gels were Spherosil with 75, 150, 500, 1250 and 3000 Å porosities. They were mixed in equal parts by weight to fill a 147 cm long glass column (1.5 cm i.d.) connected to a Milton-Roy pump in order to maintain a 22 cm^3/hr flow rate, corresponding to the best resolution. The plate count (1700) was relatively low owing to the high viscosity of the solvent and the nature of the gel. The detectors were a differential refractometer R 401 (Waters), and a conductimetric cell constructed in our laboratory with 9.4×10^{-2} cm^3 volume and a cell constant of 2.64 cm connected to a Wayne Kerr B 642 conductimeter. The conductimetric cell was kept at 25.000 ± 0.005°. The refractive index difference Δn and the conductivity difference $\Delta\chi$ were continuously and simultaneously recorded. The small shift due to the dead volumes between detectors was corrected at the recorder in order to have the same origin for each trace. Elution volumes were determined by using a flowmeter from Sefram. The standards used for calibration are listed in Table 1.

The poly(sodium glutamate salt) $(Glu, ONa)_n$ was previously prepared by Domard [5]. The maltohexaose [$\alpha(1 \rightarrow 4)$ oligomer of D-glucose] was obtained by Heyraud [6] using preparative GPC. The poly(styrene sulphonate sodium salt) (PSS Na) was prepared by sulphonation of polystyrene fractions according to the Carrol and Eisenberg method [7]. The PSS Na was purified by ultrafiltration on Amicon membranes (UM 05 and UM 10). The molecular weights of PSS Na were calculated from the starting polystyrene molecular weight and verified by light-scattering for PSS Na 1 and 2. The dextrans were dextrans T 500, T 70, T 40 and T 10 from Pharmacia Fine Chemicals. Dextrans FDR 858, IV A1, I A1 and FDR 301 FR3 were kindly supplied by Dr Granath (Pharmacia, Uppsala).

RESULTS AND DISCUSSION

GPC of simple electrolytes. Dependence on the ionic strength of the eluent

When aqueous solutions of $NaNO_3$ of various concentrations are eluted in pure water, the position and the form of the peak change with the solute concentration. The beginning of elution corresponds to a

Table 1. Polymer characteristics in 0.1 M $NaNO_3$ as solvent

Polymer standards	M_n	M_w	25° $[\eta]$ (ml × g^{-1})
	Poly(sodium glutamate salt)		
DP 10	1377	1377	4.3
DP 30	3920	4000	15.0
DP 320	37,700	45,500	90.4
	Dextrans		
Glucose	180	180	2.0
Maltohexaose	990	990	3.0
T 10	5500	9400	9.22
FDR 858	26,000	41,000	19.0
T 40	25,900	40,000	20.5
T 70	41,100	64,400	26.0
IV A1	88,000	118,000	34.3
I A1	117,000	221,000	40.0
T 500	142,600	478,000	53.5
FDR 301 FR3	—	1,400,000	67.3
	Poly(styrene sulphonate sodium salt)		
1	19,500	22,500	11.3
2	215,000	255,000	101
3	526,000	862,000	189

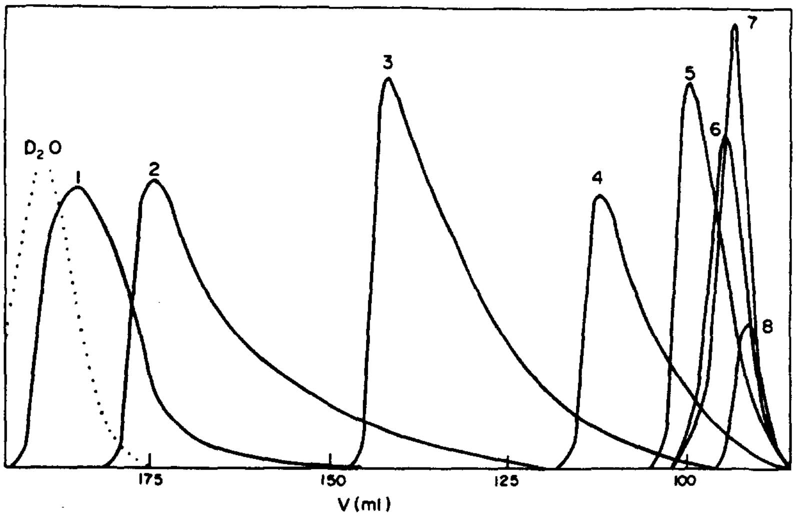

Fig. 1. Conductimetric trace for various concentrations of $NaNO_3$ eluted in pure water (D_2O detected by refractometry) 1: 10^1 M, sensitivity × 1; 2: 1 M × 10; 3: 10^{-1} M, × 100; 4: 10^{-2} M, × 1000; 5: 10^{-3} M, × 4000; 6: 10^{-4} M, × 10,000; 7: 10^{-5} M, × 50,000; 8: 10^{-6} M, × 100,000 (constant volume injected 250 μl).

constant volume V_{el}, depending on the gel but near the void volume V_0 of the column. V_{el} corresponds to the electrostatic exclusion of ionic solutes on the gel. As the ionic concentration increases, the elution position tends to the total volume of the gel V_T. V_T, given by elution of D_2O, is quite independent of the ionic effects. Owing to the high sensitivity of conductometric detection (as low as 10^{-8} eq l^{-1}), it is clear from Fig. 1 that the salt is completely excluded from the gel giving a Gaussian peak with maximum position V_{el} in the very low concentration range. The shift of the peak with solute concentration is attributed to the screening of electrostatic repulsions by the solute itself when its concentration increases.

When the eluent is a low ionic strength solution (e.g. 5 × 10^{-4} M $NaNO_3$) and when the solute concentration is $< 10^{-3}$M, the concentration under the peak is equal to or lower than the ionic concentration of the eluent. The elution volume is constant and the Gaussian peaks are located at a well defined volume characteristic of the ionic strength of the eluent. Owing to the screening effect when the solute concentration in the column becomes $> 5 \times 10^{-4}$ M, the peak shifts to the volume V_T.

When the eluent is highly concentrated (e.g. 0.1 M $NaNO_3$), the elution peak of a simple salt with an initial concentration up to 1 M is eluted at volume V_T. The elution volumes of $NaNO_3$ salt solutions

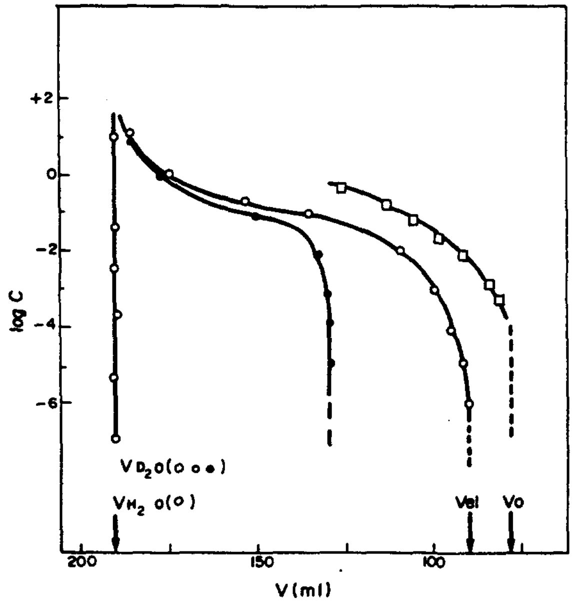

Fig. 2. Dependence of the elution volume for $NaNO_3$ and PSS Na 1 with the injected concentration (C) in various eluents (constant volume injected 250 μl) □□□ PSS Na 1, eluent H_2O; ooo $NaNO_3$, eluent H_2O; ••• $NaNO_3$, (5.10^{-4} M $NaNO_3$) eluent; OOO $NaNO_3$, (10^{-1} M $NaNO_3$) eluent.

injected at various concentrations are plotted in Fig. 2 for the three eluents described (H_2O, 5×10^{-4} M $NaNO_3$ and 0.1 M $NaNO_3$). Electrostatic screening is the only process indicated in these experiments even although these results are qualitative.

GPC of polyelectrolytes

Salt free eluent: purity and osmotic coefficient of the polyelectrolyte. The GPC chromatograms produced by refractometry ($\Delta\eta$) and conductimetry ($\Delta\chi$) can be used to test the purity of the sample. If some low molecular weight salt still remains in a polyelectrolyte, the chromatogram will present a large conductimetric peak for a large elution volume. The ratio $(\Delta\chi/\Delta\eta)_{\text{polyelectrolyte}}/(\Delta\chi/\Delta\eta)_{\text{salt}}$ is generally found to be about 1/20 as previously discussed [3]. In salt free solution, the conductimetric data are related to the polyelectrolyte concentration and charge density by the relation given for each elution volume V_i:

$$\Delta\chi_i = 10^3 z_i C_{pi} \phi_i \Lambda_p$$

in which z_i is the ionic capacity of the polyelectrolyte (equivalent $\times$ g^{-1}), ϕ_i the osmotic coefficient, and C_{pi} the concentration of the polyelectrolyte (g $\times$ l^{-1}). Λ_p is the equivalent conductivity of the polyelectrolyte (characteristic of a fraction of polymer chain carrying an ionic site). The refractometric signal is directly proportional to the weight fraction of polymer by the relation:

$$\Delta n_i = kC_{pi}$$

if to a first approximation the refractive increment (dn/dc) is assumed independent of the ionic capacity.

From the $\Delta\chi_i$ and Δn_i relations, one deduces that $\Delta\chi_i/\Delta n_i = Kz_i\phi_i$.

The distribution Δn_i (V_i) is not directly correlated to the molecular weight distribution M_i (V_i) due to electrostatic exclusion, but the chromatogram can be used to test the homogeneity of the chemical structure of the polymer if the dependence $\Delta\chi_i/\Delta n_i$ (V_i) is considered. If the chemical structure and particularly the ionic charge distribution are constant, $\Delta\chi_i/\Delta n_i$ must be constant over the whole range of V_i. Some experimental results are given in Fig. 3 for various PSS Na.

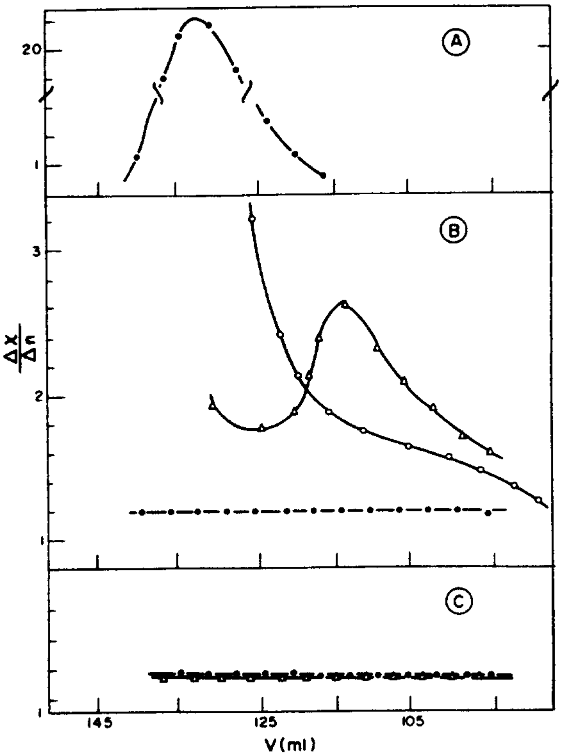

Fig. 3. Tests of purity of poly(styrene sodium sulphonate salt). ○○○ PSS Na 1; ●●● PSS Na 2; △△△ PSS Na 3. Ⓐ before ultrafiltration, Ⓑ ultrafiltration on UM 05, Ⓒ ultrafiltration on UM 10.

The polymer isolated after sulphonation contains a large amount of sodium sulphate; due to the presence of salt and to the difference between the elution volume of the neutral salt and that of the polymer, the ratio $\Delta\chi/\Delta n$ is large (up to 20) and varies with V_i (Fig. 3A). When the polymers are well purified, $\Delta\chi/\Delta n$ is constant as obtained for PSS Na 2 ultrafiltered on UM 05 or PSS Na 2 and PSS Na 3 ultrafiltered on UM 10 (Fig. 3C); on the contrary, when the purification is incomplete as for PSS Na 3 on UM 05 due to

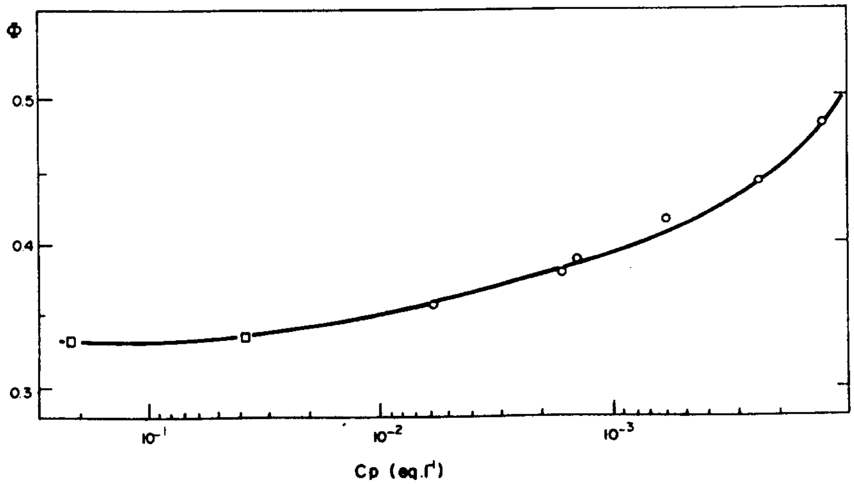

Fig. 4. Variation of osmotic coefficient ϕ of PSS Na 1 with the concentration of polymer. □□□ Vapour phase determination; ○○○ GPC determination.

its high viscosity, $\Delta\chi/\Delta n$ varies with V_i (Fig. 3B). With PSS Na 1, $\Delta\chi/\Delta n_i$ is never constant; as found by Oman *et al.* [10, 11], ϕ_i should increase when M_i decreases corresponding to an increase of $\Delta\chi_i/\Delta n_i$ particularly in the range of low molecular weights.

For high molecular weight monosulphonated PSS Na, the osmotic coefficient can be deduced from the constant value obtained for $\Delta\chi_i/\Delta n_i$. The following method was used: samples 1 and 3 were eluted under Na^+ and K^+ salt forms. Using the relation:

$$\Delta\chi/\Delta n = K\,\phi\,(\lambda p^- + \lambda c^+)$$

one obtains two relations from which λp^- and ϕ are obtained for each polymer. In the hypothesis of independence of ϕ with the nature of counterions, one obtains:

$$\left.\begin{aligned} \lambda p_1^- &= 37.9\,\Omega^{-1}\,\mathrm{cm}^2\,\mathrm{eq}^{-1} \\ \phi_1 &= 0.42 \end{aligned}\right\} \text{PSS Na 1}$$

and:

$$\left.\begin{aligned} \lambda p_3^- &= 38.3\,\Omega^{-1}\,\mathrm{cm}^2\,\mathrm{eq}^{-1} \\ \phi_3 &= 0.28 \end{aligned}\right\} \text{PSS Na 3}$$

The conductivity λp^- is in good agreement with those given by Kwak *et al.* [9].

For the osmotic values, $\phi_3 = 0.28$ is equal to the value deduced from vapour-phase osmometry and agrees with the value found by Oman [10]. For the low molecular weight polyelectrolyte, the agreement is less with $\phi_1 = 0.33$ obtained by vapour-phase osmometry on 0.22 M polymer solution. This difference may be due to the larger dependence of ϕ upon concentration of polymer at lower masses.

The variation of ϕ with the concentration C_p has been deduced from the ratio of the area of the $\Delta\chi$ and Δn traces. The results are plotted as a function of the mean concentration of the polyelectrolyte in the GPC column (PSS Na 1—Fig. 4); the values obtained from vapour-phase osmometry are given and found to agree satisfyingly.

The interest in GPC to test the dependence of ϕ (C_p) is that, using both detectors, one can go to very low concentration only limited by the sensitivity of the refractometer. The range 10^{-3}–5×10^{-3} M has been tested by Oman and Dolar [11] by direct activity measurement and confirms the slight decrease of ϕ when C_p increases.

Donnan equilibrium in eluent with low salt concentrations. As previously discussed [3], when a polyelectrolyte is dissolved in a salt eluent, e.g. 5×10^{-4} M $NaNO_3$, the chromatogram presents two peaks: the first corresponds to the polyelectrolyte elution; the second, with position exactly the same as for a simple salt, can be attributed to an Donnan salt exclusion established on the gel (Fig. 5). We have previously shown [8] that the concentration C_s of a Donnan salt exclusion is:

$$C_s = \frac{\dfrac{\phi Q_p m_s}{V_p}}{4m_s + \phi\dfrac{Q_p}{V_p}}$$

with: ϕ the osmotic coefficient of the polymer

Q_p the amount of injected polymer (expressed in equivalent)

m_s the salt concentration (mol/l) of the eluent

V_p the volume in which the polymer is eluted.

The purpose of GPC is to measure ϕ of the polyelectrolyte in the presence of various concentration of simple electrolyte (m_s).

In the column as $4m_s \gg \phi\dfrac{Q_p}{V_p}$, the lack of salt under the polymer distribution is:

$$C_s = \frac{\phi Q_p}{4V_p}$$

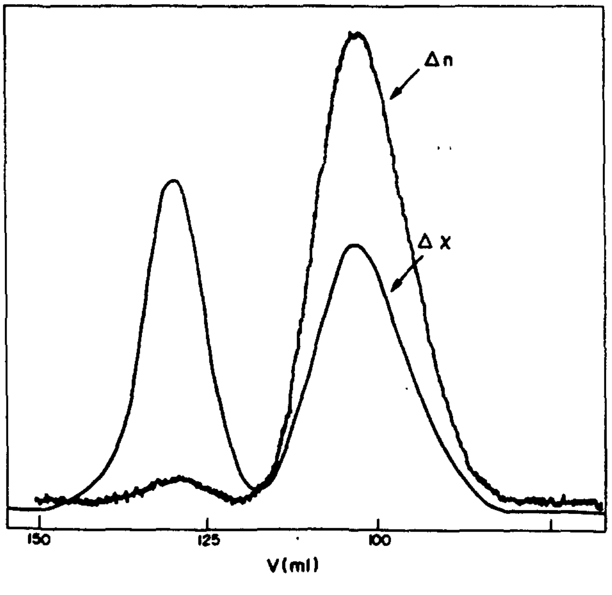

Fig. 5. Conductimetric and refractometric traces corresponding to 1.5 mg PSS Na 1 dissolved and eluted in 5×10^{-4} M $NaNO_3$ and showing the salt exclusion from Donnan equilibrium.

Table 2. Donnan equilibrium interpretation in 5×10^{-4} M $NaNO_3$ eluent with polystyrene sulphonate 1 (the values of ϕ are taken from Fig. 4).

Q_p (eq) injected	Salt reject Q_s (equivalent) Calculated	Salt reject Q_s (equivalent) Determined from calibration
1.98×10^{-5}	1.93×10^{-6}	1.94×10^{-6}
1.00×10^{-5}	1.00×10^{-6}	0.98×10^{-6}
0.69×10^{-5}	0.66×10^{-6}	0.65×10^{-6}
0.17×10^{-5}	0.22×10^{-6}	0.22×10^{-6}

and the amount of salt excluded given by:

$$Q_s = \frac{\phi Q_p}{4} \text{ (expressed in equivalent)}$$

Table 2 shows the values of Q_s using a calibration obtained from known salt quantities injected and by the relation between Q_s and Q_p; there is a good agreement between the sets of data.

An example of a chromatogram is given in Fig. 5. When the polyelectrolyte solution is too concentrated, the chromatogram is perturbed due to the broadening of the excluded peak of polyelectrolyte toward large elution volumes which fall approximately at the same position as the Donnan salt exclusion at the given ionic strength.

Universal calibration in 0.1 *M* $NaNO_3$ *eluent*. As previously pointed out, the electrostatic exclusion disappears for ionic strength > 5×10^{-2} M [3]. Calibration of the set of columns had been established using PSS Na, dextrans and (Glu, O Na)$_n$. Their characteristics are given in Table 1.

The elution volume corresponding to the top of the peak is plotted as a function of the product [η]M corresponding to the hydrodynamic volume as generally used. All the points are clearly on a single calibration curve as recently found by Spatorico [11] except for dextrans with molecular weight < 60,000 probably due to interaction with the polar silica gel. Moreover, one demonstrates that this calibration curve is quite identical to that obtained in DMF and calibrated with polystyrene (Fig. 6).

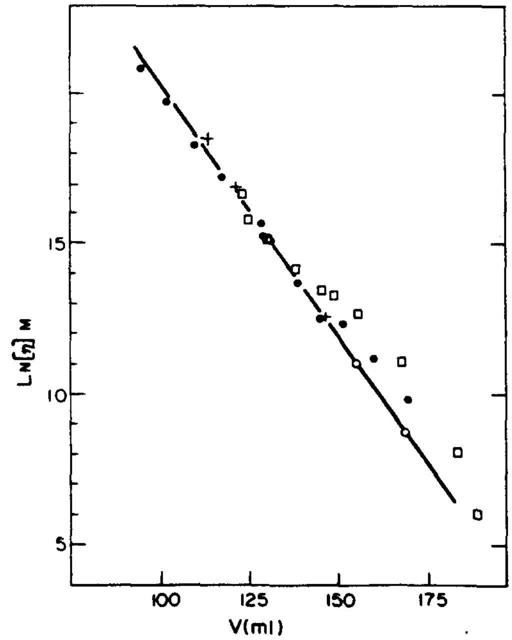

Fig. 6. Relationship between hydrodynamic volume [η]M and the elution volume for various eluents. ●●● Polystyrene eluted in DMF; □□□ Dextran; ○○○ (Glu ONa)$_n$; + + + PSS Na, eluted in 0.1 M $NaNO_3$.

CONCLUSION

In this paper, the main effects encountered in the use of aqueous gel permeation chromatography for water-soluble polymers have been discussed. First, whatever is the support and even with low molecular weight salt, it is observed that the form of the elution peak and its position depend on the concentration of the sample injected and the ionic strength of the eluent. Also, it is observed that, as soon as the eluent concentration is > 5×10^{-2} M [3], the elution peak of a simple salt is Gaussian and eluted at a volume equal to the total porous volume of the column (V_T).

For elution of polyelectrolytes in salt free solution, there is an exclusion process identical to that observed with simple salt. Nevertheless, investigation on the chromatograms obtained using two connected detectors (refractometer and conductimeter) suggests that the purity of the sample may be tested and that the osmotic coefficient ϕ of the polyelectrolyte may be determined. The dependence of ϕ on molecular weight and polymer concentration injected is discussed.

The homogeneity of the charge density of the various molecular weight chains is directly obtained. This characterization should be very useful for water-soluble polymers.

In salt eluent, there is a peak due to salt excluded at a volume corresponding to simple salt elution; it is interpreted in terms of a Donnan equilibrium established on the column. From this treatment it becomes possible to obtain the osmotic coefficient of a polyelectrolyte in the presence of external salt. The universal calibration procedure, expressed as the dependence of the hydrodynamic volume [η]M upon the elution volume, was tested for the column of silica gels. With a set of polymer standards, previous results from Spatorico [12] concerning the validity of the universal calibration are confirmed. The universal calibration is valid whatever the ionic strength as soon as the ionic strength of the eluent exceeds 5×10^{-2} M in order to screen the electrostatic repulsion with polyelectrolytes. Moreover, the calibration curve is the same as that obtained with polystyrene standards in an organic solvent (DMF).

REFERENCES

1. P. A. Neddermeyer and L. B. Rogers, *Analyt. Chem.* **40**, 755 (1968); **41**, 94 (1969).
2. D. P. Matzinger and A. R. Cooper, *J. appl. Polym. Sci.* **23**, 419 (1979).
3. A. Domard, M. Rinaudo and C. Rochas, *J. Polym. Sci. Polym. Phys. Ed.* **17**, 1173 (1979); C. Rochas, Thesis, Grenoble (1978).
4. T. Lindstrom, A. de Ruovo and C. Soremark, *J. Polym. Sci. Polym. Chem. Ed.* **15**, 2029 (1977).

5. M. Rinaudo and A. Domard, *Biopolymer* **14**, 2035 (1975).
6. A. Heyraud and M. Rinaudo, *J. Chromat.* **166**, 149 (1978).
7. W. R. Carrol and H. Eisenberg, *J. Polym. Sci.* **1**, 599 (1966).
8. C. Rochas, A. Domard and M. Rinaudo, *Polymer* **20**, 76 (1979).
9. J. C. T. Kwak, C. F. Morphy and E. J. Spiro, *Biophysic. Chem.* **7**, 379 (1978).
10. S. Oman, *Makromolek. Chem.* **175** 2141 (1974); S. Oman and D. Bratko, *Makromolek Chem.* **178**, 1773 (1977).
11. S. Oman and D. Dolar, *Z. Physick Chem.* **56**, 1 (1967).
12. A. L. Spatorico and C. L. Beyer, *J. appl. Polym. Sci.* **19**, 2933 (1975).