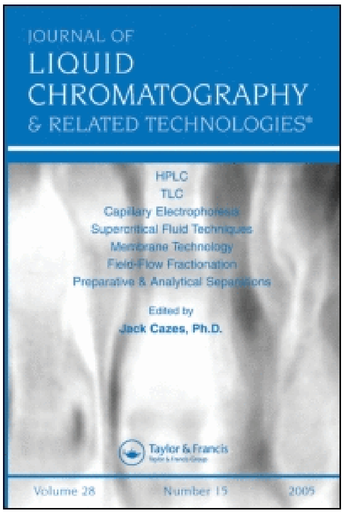

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Gisele Volet[a]; James Lesec[a]
[a] Laboratoire de Physico-Chimie Macromoléculaire de, l'Université Pierre et Marie Curie, Paris Cédex, France

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NON-EXCLUSION EFFECTS IN AQUEOUS SEC: BEHAVIOR OF SOME POLYELECTROLYTES USING ON-LINE MASS DETECTORS

GISÈLE VOLET AND JAMES LESEC*
Laboratoire de Physico-Chimie Macromoléculaire
de l'Université Pierre et Marie Curie
(Paris VI) - C.N.R.S. URA n° 278
E.S.P.C.I. - 10 rue Vauquelin - 75231 Paris Cédex 05 - France

ABSTRACT

Aqueous Size Exclusion Chromatography (SEC), coupled with both a home-made viscometer and a low angle laser light scattering detector (LALLS) is a very valuable analytical tool for the characterization of polyelectrolytes. The great interest of this dual mass detection is that molecular weights can be calculated by two different and independent ways. When comparing the two sets of results, it is possible to check whether every chromatographic parameters has been optimized or non exclusion effects occur in addition to the size exclusion mechanism. Two sets of polyelectrolytes were used in this study : a set of sodium polystyrene sulfonate (NaPSS) standards, as anionic polymers and a family of copolymers of acrylamide and N,N,N-trimethylaminoethyl chloride acrylate (AM/CMA) synthetized in our Laboratory as cationic polymers. Important non-exclusion effects, repulsion and adsorption have been encountered in their study. The results are discussed as a function of the ionic strength of the mobile phase and the nature of the salt that was added and interpreted through polymer-packing interactions. The strong influence of the nature of the column, and especially their history is described.

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* To whom correspondence should be addressed

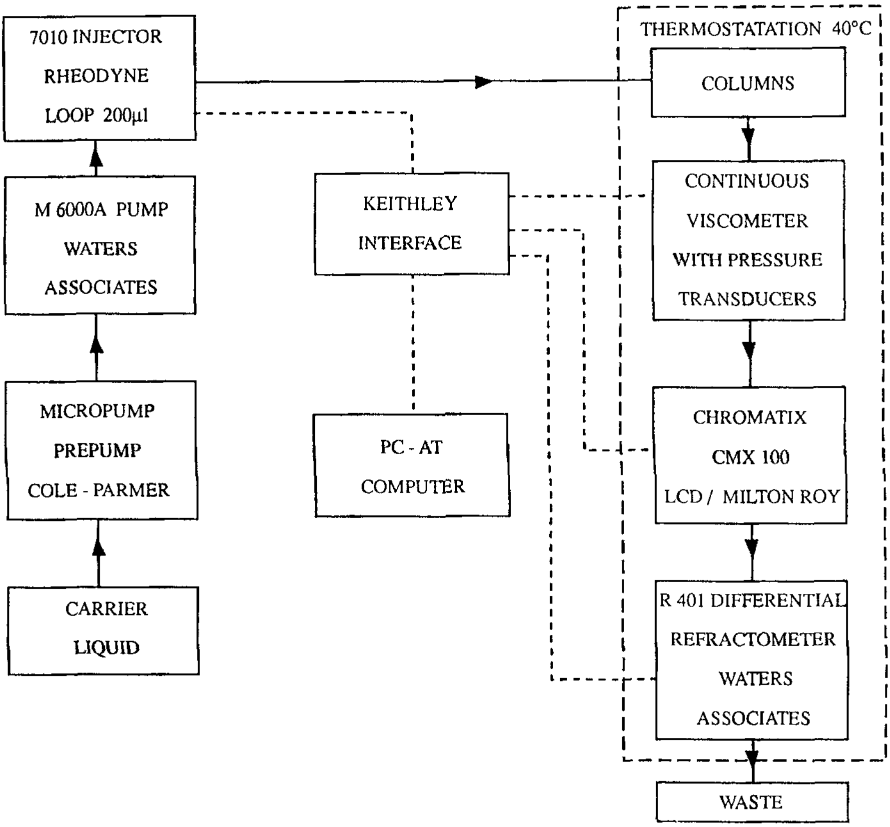

Figure 1 : General scheme of the SEC instrument.

INTRODUCTION

Polyelectrolytes represent a major class of very useful water-soluble polymers. These polymers have ionic groups along their macromolecular chains that involve electrostatic interactions. Then, most applications of polyelectrolytes depend on their viscosity-modifying and surface activity properties. Therefore, polyelectrolytes find uses as viscosity-modifying, gelling or flocculating agents in many areas : water treatment, oil industry, paints, detergents, paper and food industry, etc... Reliable determination of average molecular weights is essential to predict end-use properties of these polymers.

Size Exclusion Chromatography, with a concentration detector and coupled to two on-line mass detectors, appears to be the most efficient method for the determination of molecular weights. This triple coupling allows the calculation of real molecular weights by two different and independent ways. However, the interest of this triple detection is especially important in the case of polyelectrolytes. The presence of ionic groups on the macromolecular chain may induce non-exclusion effects : intramolecular electrostatic interactions and electrostatic interactions between polymer and packing such as ion exclusion (1-2), ion inclusion (3-6) or adsorption (7). The overlay of these non-exclusion effects to the separation mechanism of SEC leads to very erroneous results. Therefore, the possibility of comparing molecular weight values from two different ways becomes of major interest when checking whether the SEC system runs properly with well-controlled parameters or an abnormal behavior of the polymer occurs.

In this paper, we report the behavior of two different families of polyelectrolytes : anionic polymers (sodium polystyrene sulfonate = NaPSS) and cationic copolymers (acrylamide and N,N,N-trimethylaminoethyl chloride acrylate = AM/CMA) chromatographed in mobile phases with increasing ionic strengths. Our experiments were performed with a Waters Associates (Milford, Ma.) modular room temperature instrument equipped with a R 401 refractometer and two mass detectors : a single capillary viscometer and a low angle laser light scattering detector (LALLS) (8). The viscometer allows the determination of intrinsic viscosity versus molecular weight, leading to "universal" molecular weight calculation through universal calibration (9), and gives information on long-chain branching. Simultaneously, the light scattering detector provides absolute molecular weights. The purpose of this work was to investigate the influence of experimental parameters in order to avoid non exclusion effects and to get an accurate characterization of polyelectrolytes using the triple detection.

EXPERIMENTAL

INSTRUMENTATION

The SEC system is described in Figure 1 and is composed of several components described previously (8,9) :

- a micropump prepump (Cole - Parmer, Chicago., Ill.)
- a M 6000A pumping system (Waters Associates, Milford, Ma.)
- a 7010 injector with a 200 µl loop (Rheodyne, Calif.)
- a R 401 differential refractometer (Waters Associates)
- two mass detectors
- and a column set.

Mass detectors

The specific nature of this chromatograph is that both mass detectors are inserted in series between the outlet of the column set and the inlet of the differential refractometer in this sequence : a home-made continuous single capillary viscometer and a light scattering detector. Every component is kept at a well-controlled temperature of 40°C.

Viscometer : This vicometer is made with a 3 meter long Teflon capillary (0.3 mm I.D.) connected to two Sedeme (Paris, France) pressure transducers (CMAC 5 range: 5 bars) at both ends.

LALLS : The light scattering detector is a Chromatix CMX 100 (LDC Analytical). This instrument uses a He - Ne laser (632.8 nm) and measures the scattered light between 5 and 6°.

The great interest of this dual mass detection is that one detector (LALLS) provides absolute molecular weights independent of elution volume. Conversely, molecular weights calculated from universal calibration and viscometry are very dependent on elution volumes since they refer to a calibration curve and may be calculated wrong when elution is disturbed, ie., when non exclusion effects occur.

Column sets

Two sets of columns were used : Ultrahydrogel 500, 1000 and 2000 Å (Waters Associates, Milford, Ma.) and Shodex OH-pak B 803, B 804, B 805 and B 806 (Showa Denko, Tokyo, Japan). Both column sets contain hydrophilic cross-linked gels based on methacrylic copolymers. The real nature of these gels is not described, but according to the suppliers, the gels of these column sets are similar but not identical.

MOBILE PHASES

Mobile phases were water containing various salts ($LiNO_3$, $NaNO_3$ or Na_2SO_4) at various concentrations (0.1 M to 0.75 M). The presence of salt is necessary to screen out the charges on polyelectrolytes in order to avoid polyelectrolyte effects in solution. In every case, 400 ppm of NaN_3 were added to the mobile phase to prevent biological degradation. Every solution was vacuum filtered through 0.45 µm membranes. The flow rate of the mobile phase was 1 cm^3/mn.

DATA ACQUISITION

Injector and detectors were connected to an on-line microcomputer (PC - AT) through a Keithley interface : 199 scanner - multimeter (Cleveland, Ohio). An

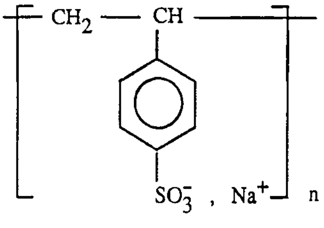

Figure 2 : Sodium polystyrene sulfonate (NaPSS).

Table I : Molecular weights of sodium polystyrene sulfonate standards as quoted by Polymer Laboratories Ltd (Shrosphire, England).

sample number	Nominal molecular weight		Mw / Mn <
	Polystyrene sulfonate ion	Sodium polystyrene sulfonate	
PS 1	31 000	35 000	1.10
PS 2	88 000	100 000	1.10
PS 3	195 000	220 000	1.10
PS 4	354 000	400 000	1.10
PS 5	690 000	780 000	1.10
PS 6	1 060 000	1 200 000	1.10

appropriate personal software "Multidetector GPC Software" (8) allows acquisition and data treatment and leads to reliable interpretations of chromatograms. A detailed description of data acquisition and data handling procedure was reported elsewhere (8).

CALIBRATION

The treatment of viscometric data requires a preliminary calibration of the column set. Two sets of standards were used : polyethylene oxides (20,000 - 850,000) from Toyo Soda (Japan) and pullulan polysaccharides (7,000 - 900,000) from Showa Denko. The universal calibration procedure and all the calibration curves obtained in the different mobile phases are reported in a previous paper (9).

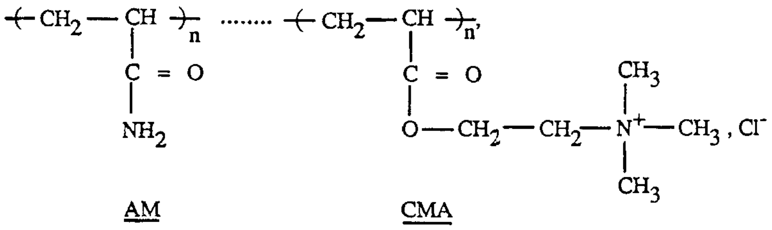

Figure 3 : Copolymer of acrylamide and N,N,N-trimethylaminoethyl chloride acrylate (AM/CMA).

MATERIALS

Two series of polyelectrolytes, representing anionic and cationic polymers, were used in this study (10). Anionic polymers (sodium polystyrene sulfonate standards) are represented in Figure 2. They were obtained from Polymer Laboratories Ltd (Shrosphire, England).

These polymers have a very narrow distribution and contain, a priori, 100% of anionic groups. Their properties, as quoted by the manufacturer, are summarized in Table I.

Cationic copolymers (AM/CMA) were synthetized in our laboratory by radical polymerization (11 - 12) with different degrees of cationic character between 0% and 100% and various molecular weights (Figure 3).

RESULTS AND DISCUSSION

CHARACTERIZATION OF NaPSS

These 100% anionic polymers were studied with both the Ultrahydrogel column set and the Shodex column set. The mobile phases were water containing $LiNO_3$, $NaNO_3$ or Na_2SO_4 with various concentrations between 0.1 M to 0.5 M.

- NaPSS behavior on Ultrahydrogel columns :

NaPSS were completely retained on this packing. Strong interactions occur between anionic polymers and Ultrahydrogel columns, probably due to the presence of charges on the packing surface, opposite to the polyanion.

Table II : Results of sodium polystyrene sulfonates.
$Mw_{univ.}$ and Mw_{LALLS} : average molecular weights calculated from universal calibration and by LALLS detector, respectively.

sample number	Eluent		Ve (cm^3)	[η] ($cm^3.g^{-1}$)	$Mw_{univ.}$	Mw_{LALLS}
PS 1	$NaNO_3$	0.1 M	28.55	18	110 000	42 000
	$LiNO_3$	0.1 M	28.42	19	100 000	40 000
	Na_2SO_4	0.1 M	29.76	16	31 000	40 000
	$LiNO_3$	0.5 M	30.05	12	31 000	36 000
PS 2	$NaNO_3$	0.1 M	27.31	47	160 000	120 000
	$LiNO_3$	0.1 M	27.10	44	190 000	110 000
	Na_2SO_4	0.1 M	28.09	39	90 000	110 000
	$LiNO_3$	0.5 M	28.28	32	90 000	110 000
PS 3	$NaNO_3$	0.1 M	26.16	77	290 000	220 000
	$LiNO_3$	0.1 M	25.69	74	400 000	210 000
	Na_2SO_4	0.1 M	27.02	67	170 000	210 000
	$LiNO_3$	0.5 M	27.31	55	160 000	200 000
PS 4	$NaNO_3$	0.1 M	24.72	150	590 000	470 000
	$LiNO_3$	0.1 M	24.51	150	760 000	500 000
	Na_2SO_4	0.1 M	25.33	130	440 000	440 000
	$LiNO_3$	0.5 M	25.50	87	510 000	410 000
PS 5	$NaNO_3$	0.1 M	24.15	190	650 000	650 000
	$LiNO_3$	0.1 M	24.17	180	850 000	640 000
	Na_2SO_4	0.1 M	24.64	170	570 000	680 000
	$LiNO_3$	0.5 M	24.90	120	510 000	590 000
PS 6	$NaNO_3$	0.1 M	23.11	340	$1.2\ 10^6$	$1.5\ 10^6$
	Na_2SO_4	0.1 M	23.52	280	$1.2\ 10^6$	$1.3\ 10^6$
	$LiNO_3$	0.5 M	23.79	190	$1.1\ 10^6$	$1.1\ 10^6$

- NaPSS behavior on Shodex columns :

On Shodex columns, NaPSS are eluted without any significant adsorption; the results are reported in Table II. Nevertheless, the polyelectrolyte behavior varies with the ionic strength of the mobile phase. The first observation is that the weight average molecular weights Mw_{LALLS} calculated by light scattering are approximately constant and independent of the ionic strength, whatever the molecular weight. The second observation is that elution volumes increase when ionic strength increases, and, at the same time, intrinsic viscosity decreases for every sample. Intrinsic viscosities, which are about the same in $NaNO_3$ and $LiNO_3$ 0.1 M, decrease in Na_2SO_4 0.1 M and decrease again in $LiNO_3$ 0.5 M. The deviation is about 60% between the sets of values measured in $LiNO_3$ 0.1 M and 0.5 M. This variation may be explained by the presence of salt according to the law (13) :

$$[\eta] = A + \frac{B}{\sqrt{C_s}}$$

where C_s is the salt concentration and A , B are constants. The higher the salt concentration , the more screened out the electrostatic interactions and, therefore, the smaller the hydrodynamic volume. This is qualitatively confirmed by the variations of elution volumes that increase with ionic strength in the order :

$$\underset{0.1\,M}{Ve_{NaNO_3}} = \underset{0.1\,M}{Ve_{LiNO_3}} < \underset{0.1\,M}{Ve_{Na_2SO_4}} \leq \underset{0.5\,M}{Ve_{LiNO_3}}$$

Ionic strength of Na_2SO_4 0.1 M is equivalent to a salt concentration of 0.3 M with a monovalent salt according to the relationship :

$$I = \frac{1}{2}\Sigma\, C_i\, z_i^2$$

where C_i and z_i are the ion concentration in $mol.l^{-1}$ and the number of charge, respectively.

However, the weight average molecular weights $Mw_{univ.}$ calculated using the universal calibration curve are not constant in Table II, but increase when ionic strength decreases; this cannot be explained by the change in hydrodynamic volume since universal calibration takes this effect into account. Accordingly, the decrease of elution volumes is an overlay of two phenomena. When ionic strength decreases, in addition to the increase of hydrodynamic volume and, consequently, the decrease of elution volumes, interactions occur between polyelectrolytes and negatives charges of the packing surface that prevent the polyion from freely diffusing into the pores of the gel matrix. These repulsive interactions between anionic groups on the polyelectrolyte chain and negative charges on the gel surface are more important for low molecular weight compounds (Figure 4). When the ionic strength of the mobile phase is not strong enough to screen out the anionic sites of the packing, these repulsions reduce the effective pore volume available to the polymer. As a result, peaks elute earlier than expected for a neutral polymer with the same size and lead to an overestimation of molecular weights calculated with the universal calibration curve (Table II), especially for low molecular weight polymers that can usually penetrate a larger porous volume, and therefore, a larger specific surface area. In $LiNO_3$ 0.5 M and Na_2SO_4 0.1 M, $Mw_{univ.}$ values are in good agreement with Mw_{LALLS} values that is not the case with mobile phases at 0.1 M salt concentration. $Mw_{univ.}$ are calculated using a universal calibration curve obtained with pullulan standards in the same mobile phase (9).

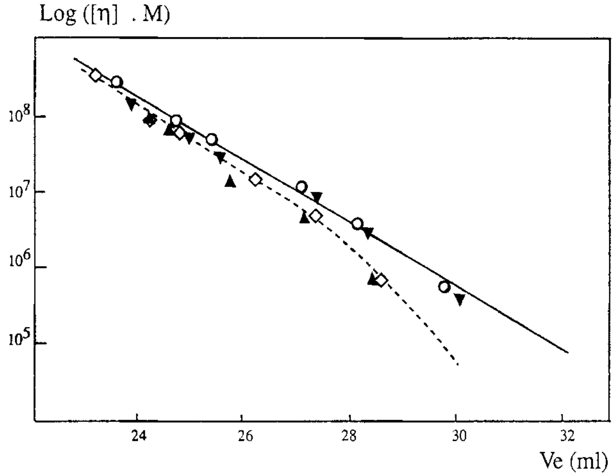

Figure 4 : Universal calibration curves corresponding to NaPSS.
Eluents : (◇) $NaNO_3$ 0.1 M, (▲) $LiNO_3$ 0.1 M, (○) Na_2SO_4 0.1 M, (▼) $LiNO_3$ 0.5 M
(—) pullulan universal calibration curve at 0.5 M,
(---) NaPSS curve at 0.1 M.

As a conclusion of these experiments, an ionic strength corresponding to $LiNO_3$ 0.5 M is required to avoid electrostatic repulsive interactions and to allow an accurate elution of these anionic polymers.

CHARACTERIZATION OF AM/CMA COPOLYMERS

These experiments were performed with the Shodex OH-pak column set. As previously discussed, Shodex packing contains anionic groups on its surface. When a cationic polyelectrolyte, which is oppositely charged, approaches the packing surface, electrostatic attractions may occur, resulting in delayed elution or even, in total adsorption. Therefore, the concentrations of AM/CMA copolymers were measured accurately using the surface area of the refractometric response to prevent erroneous results due to a wrong concentration value. This concentration correction can be made only when the refractive index increment (dn/dc) is known. The variations of dn/dc data are described by the relationship (11) :

$$\left(\frac{dn}{dc}\right)_{\tau} = (1-\tau)\left(\frac{dn}{dc}\right)_{PAM} + \tau\left(\frac{dn}{dc}\right)_{PCMA}$$

where the subscripts PAM, PCMA denote polyacrylamide homopolymer and 100% cationic polymer, respectively, and τ is the cationic rate. The values of specific refractive index increment of PAM and PCMA 0.176 and 0.154 cm^3/g, respectively, were calculated using the refractometer response. The "Multidetector GPC software" allows the correction of every concentration used in every molecular weight calculation ("universal" and LALLS) of the results described later.

For these experiments, we studied AM/CMA copolymers with different levels of cationic character (0 to 100%) and with different molecular weights ranging from 1.2 10^5 to 4.5 10^6 g/mol. The mobile phase was water containing the salt $LiNO_3$ at various concentration from 0.1 M to 0.75 M. With regard to the great number of results, we have chosen to report only a few representative data in Tables III to VIII.

- AM/CMA behavior in terms of cationicity evolution :

For low cationic rates (≤ 5%), the behavior of polyelectrolytes is relatively insensitive to the ionic strength (from 0.1 M to 0.75 M), taking experimental errors into account (Table III). Molecular weights determined from LALLS are constant in the different mobile phases. Molecular weights calculated from viscosity and universal calibration curve are in good agreement with those from LALLS. Moreover, intrinsic viscosities, but also elution volumes, remain constant whatever the salt concentration of the mobile phase. An ionic strength corresponding to $LiNO_3$ 0.1 M is strong enough to allow a good characterization of these polymers.

When the copolymer cationicity is around 6 %, an abnormal elution of the polyelectrolyte is observed in $LiNO_3$ 0.1 M (Table IV). The elution profile is shifted toward high elution volumes (strong increase of elution volume) and molecular weight by LALLS is calculated much smaller than for higher ionic strengths. At the same time, intrinsic viscosity $[\eta]$ is also calculated too small; this demonstrates the absence of high molecular weight molecules. At this ionic strength, the copolymer is partially adsorbed onto the packing; this is confirmed by the measurement of the surface area of the refractometric profile which is smaller than expected. Moreover, the strong decrease of intrinsic viscosity $[\eta]$ and Mw by LALLS shows that the highest molecular weight part of the distribution is preferentially adsorbed. Conversely, for this cationicity, ionic strengths of 0.25 M and higher lead to a correct elution of the copolymer.

A similar behavior is observed with 9 to 14% cationicity copolymers (Table V). The results in $LiNO_3$ 0.1 M are not reported since copolymers are totally adsorbed. At 0.25 M, intrinsic viscosities and Mw by LALLS are underestimated, leading to the same interpretation as for the previous copolymers. The increase of solvent ionic strength to 0.5 M and 0.75 M progressively screens out the charges, allowing a normal elution of the copolymer.

Table III : Results of some AM/CMA copolymers with low cationicity rates.
C_{LiNO_3} : $LiNO_3$ salt concentration
Mw $_{univ.}$ and Mw $_{LALLS}$: average molecular weights calculated from universal calibration and by LALLS detector, respectively.

sample number	% CMA	C_{LiNO_3} (mol.l^{-1})	Ve (cm^3)	[η] (cm^3.g^{-1})	Mw $_{univ.}$	Mw$_{LALLS}$
12 (PAM)	0	0	23.58	250	980 000	880 000
		0.1	23.76	250	850 000	830 000
		0.25	23.79	270	830 000	860 000
		0.5	23.82	250	820 000	750 000
		0.75	23.70	260	860 000	790 000
23	1	0.1	24.14	190	630 000	570 000
		0.25	24.24	190	610 000	590 000
		0.5	24.26	200	670 000	540 000
		0.75	24.24	200	600 000	580 000
38	3	0.1	23.42	250	$1.1\ 10^6$	$1.3\ 10^6$
		0.25	23.09	270	$1.2\ 10^6$	$1.4\ 10^6$
		0.5	23.05	300	$1.1\ 10^6$	$1.2\ 10^6$
		0.75	23.30	290	$1.3\ 10^6$	$1.4\ 10^6$
41	5	0.1	24.24	170	510 000	680 000
		0.25	24.39	150	480 000	540 000
		0.5	24.08	180	590 000	590 000
		0.75	24.10	180	630 000	610 000

Table IV : Results of a 6.4% cationic AM/CMA copolymer.
C_{LiNO_3} : $LiNO_3$ salt concentration
Mw $_{univ.}$ and Mw $_{LALLS}$: average molecular weights calculated from universal calibration and by LALLS detector, respectively
* : partial adsorption of polymer on columns.

sample number	% CMA	C_{LiNO_3} (mol.l^{-1})	Ve (cm^3)	[η] (cm^3.g^{-1})	Mw $_{univ.}$	Mw$_{LALLS}$
52	6.4	0.1	27.18*	76*	54 000*	200 000*
		0.25	23.84	170	730 000	740 000
		0.5	23.98	180	650 000	730 000
		0.75	23.87	190	840 000	820 000

<u>Table V</u> : Results of some AM/CMA copolymers with medium cationicities.
C_{LiNO_3} : $LiNO_3$ salt concentration
$Mw_{univ.}$ and Mw_{LALLS} : average molecular weights calculated from universal calibration and by LALLS detector, respectively
* : partial adsorption of polymers on columns.

sample number	% CMA	C_{LiNO_3} (mol.l^{-1})	Ve (cm^3)	[η] (cm^3.g^{-1})	$Mw_{univ.}$	Mw_{LALLS}
61	9.5	0.25	24.62	96*	380 000*	470 000*
		0.5	24.41	140	490 000	670 000
		0.75	24.24	140	540 000	610 000
64	13.8	0.25	22.83*	270*	1.6 10^6*	2.1 10^6*
		0.5	21.98	400	3.2 10^6	3.2 10^6
		0.75	21.70	470	3.0 10^6	3.4 10^6

At a cationicity of 24% and above, partial adsorption always occurs, mainly for the highest part of the distribution, even when the salt concentration increases to 0.75 M (Table VI). It is impossible to accurately characterize these polymers. Moreover, at a cationicity of 30% and above, polyelectrolytes are totally retained on the columns at an ionic strength of 0.5 M and are only partially eluted at 0.75 M. For chromatographic reasons, it was not realistic to increase the ionic strength beyond 0.75 M to improve elution of high cationicity copolymers.

- <u>Influence of molecular weight</u> :

The set of AM/CMA copolymers we analyzed was comprised of polymers with different cationicities and various molecular weights ranging from 1.2 10^5 to 4.5 10^6 g/mole. In terms of cationicity, the same behavior was observed whatever the molecular weight (Table VII and VIII). Table VII shows the results of 1% CMA copolymers, similar results are obtained at a salt concentration of 0.1 M and 0.5 M for molecular weights ranging from 100,000 to 4 10^6 g/mole.

In Table VIII, AM/CMA copolymers with cationicity around 7% are compared for three different molecular weights : 300,000 , 800,000 and 2.10^6 g/mole. In every case, results are consistent for ionic strength of 0.25 M and above. Also, a systematic decrease of intrinsic viscosity and Mw by LALLS and an increase of elution volumes are observed when ionic strength decreases to 0.1 M that can be interpreted by the adsorption of the highest molecular weight part of the distribution. Adsorption being a function of molecular weight, it is interesting to point out that Mw_{LALLS} values are systematically calculated around 200,000, whatever the real molecular weight of the copolymer. As Mw_{LALLS} is the weight

Table VI : Results of some AM/CMA copolymers with high cationicities.
C_{LiNO_3} : $LiNO_3$ salt concentration
Mw $_{univ.}$ and Mw $_{LALLS}$: average molecular weights calculated from universal calibration and by LALLS detector, respectively
χ : amount of eluted polymer (% recovery).

sample number	% CMA	C_{LiNO_3} (mol.l^{-1})	Ve (cm^3)	[η] (cm^3.g^{-1})	Mw univ.	Mw$_{LALLS}$	χ (%)
70	24	0.5	25.58	61	260 000	960 000	65
		0.75	24.68	110	280 000	630 000	75
82	32	0.5		not	possible		
		0.75	24.17	140	510 000	790 000	72
92	49.5	0.5		not	possible		
		0.75	24.23	180	460 000	1.1 10^6	74
101	100	0.5		not	possible		
		0.75	26.51	74	380 000	530 000	78

Table VII : Results of some AM/CMA copolymers with 1% cationicity at various molecular weights.
C_{LiNO_3} : $LiNO_3$ salt concentration
Mw $_{univ.}$ and Mw $_{LALLS}$: average molecular weights calculated from universal calibration and by LALLS detector, respectively.

sample number	% CMA	C_{LiNO_3} (mol.l^{-1})	Ve (cm^3)	[η] (cm^3.g^{-1})	Mw univ.	Mw$_{LALLS}$
21	1	0.1	27.15	64	120 000	120 000
		0.5	27.17	62	130 000	110 000
22	1	0.1	24.56	160	470 000	460 000
		0.5	24.53	170	490 000	460 000
23	1	0.1	24.14	190	630 000	570 000
		0.5	24.26	200	670 000	540 000
27	1	0.1	21.54	630	3.6 10^6	3.7 10^6
		0.5	21.55	570	3.5 10^6	3.4 10^6

Table VIII : Results of some AM/CMA copolymers with a cationicity about 7% at various molecular weights.
C_{LiNO_3} : $LiNO_3$ salt concentration
Mw $_{univ.}$ and Mw $_{LALLS}$: average molecular weights calculated from universal calibration and by LALLS detector, respectively
* : partial adsorption of polymer on columns.

sample number	% CMA	C_{LiNO_3} (mol.l^{-1})	Ve (cm^3)	[η] (cm^3.g^{-1})	Mw $_{univ.}$	Mw$_{LALLS}$
51	7.5	0.10	27.58*	57*	47 000*	150 000*
		0.25	26.65	98	270 000	310 000
		0.50	26.56	93	260 000	270 000
		0.75	26.58	97	280 000	290 000
52	6.4	0.10	27.18*	76*	54 000*	200 000*
		0.25	23.84	170	730 000	740 000
		0.50	23.98	180	650 000	730 000
		0.75	23.87	190	840 000	820 000
53	6.7	0.10	25.07*	160*	270 000*	>270 000*
		0.25	22.71	320	1.6 10^6	1.9 10^6
		0.50	22.93	350	1.8 10^6	1.6 10^6
		0.75	23.02	320	1.5 10^6	1.6 10^6

average of a broad distribution, it can considered that macromolecules with higher molecular weights than 300,000 - 400,000 are completely adsorbed and only smallest macromolecules, having a molecular weight under this limit, are eluted.

- <u>Diagram of AM/CMA copolymers behavior</u> :

All the results are summarized in Figure 5 where we have reported the behavior of polyelectrolytes in terms of copolymer cationicity and ionic strength of the mobile phase. Every experiment is represented by :

○ when the polymer elution is normal, in other words, when molecular weights calculated from universal calibration agree with those measured by LALLS detector,

■ when the polymer is partly retained, in other words, when Mw $_{univ.}$ < Mw $_{LALLS}$ and when most of high molecular weight molecules are adsorbed on the columns,

● when polymers are completely adsorbed on the columns.

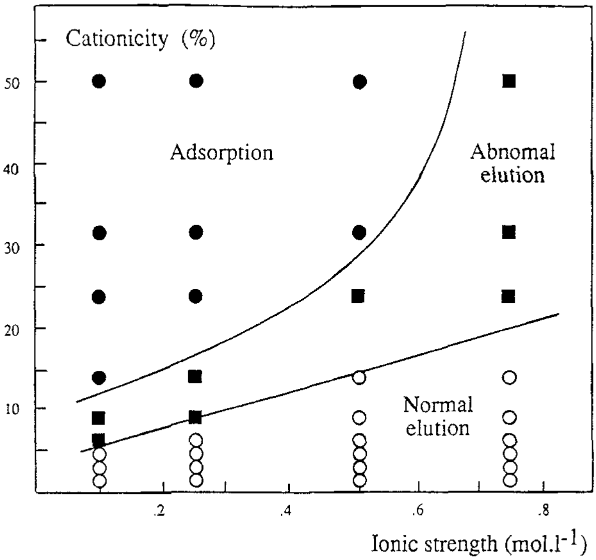

Figure 5 : Diagram of polyelectrolytes AM/CMA behavior versus the ionic strength of the mobile phase.

● total adsorption of polymer on the columns,

■ partial adsorption (Mw $_{\text{univ.}}$ < Mw $_{\text{LALLS}}$),

○ agreement between Mw $_{\text{univ.}}$ and Mw $_{\text{LALLS}}$.

Consequently, the diagram of behavior comprises three areas : normal elution, partial elution and total adsorption. When cationicity increases, it is necessary to increase the ionic strength of the mobile phase to get an accurate characterization of the copolymers. Beyond a cationicity of 20%, it is impossible to characterize these polymers correctly, although the ionic strength value of 0.75 M is above the upper limit of 0.5 M in monovalent salt recommended by the supplier of columns.

It is also very important to note that the diagram shown in Figure 5 corresponds to experiments performed with columns in a given state of use. As an example, beyond 20% of cationicity, the behavior of polymers varies according to the moment when they were injected and elution profiles of the same copolymer may vary. Figure 6 shows the first injection of a 31.5% cationicity copolymer and Figure 7 shows the same copolymer injected some days later. The only difference between the two experiments is that several injections of polymers with the same nature were performed between the two injections. The first profile (Figure 6) could correspond to the profile of a polymer whose highest molecular weights are

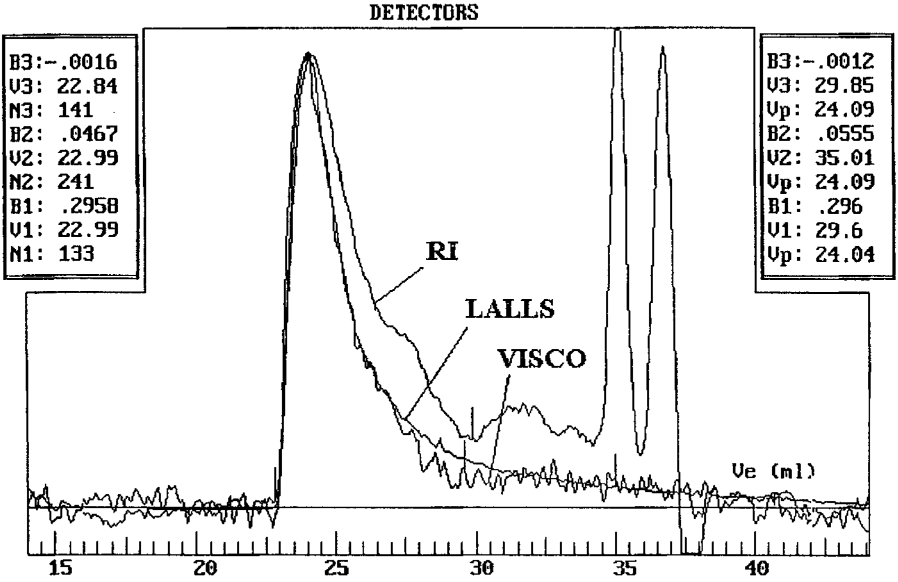

Figure 6 : Chromatograms of a 31.5% cationic copolymer eluted at the beginning of the study.

excluded from the gel because the three profiles (VISCO, LALLS, RI) are beginning in the same time, but the beginning elution volume is 3 cm^3 beyond the exclusion volume of the packing. Therefore, there is no exclusion and this profile corresponds, rather, to a polymer whose highest molecular weights are adsorbed on the columns and other molecular weights eluted later and causing peak tailing.

Moreover, the first profile (Figure 6) corresponds to a percentage of recovery of 36% while the second experiment (Figure 7) with the correct profile corresponds to a recovery of 87%. Obviously, the gradually adsorbed polymers modify the properties of columns by neutralizing the anionic groups on the packing surface. In these experiments, the column performances were drastically improved between the first and the last injection.

This phenomenon, clearly observed for copolymers with cationicity beyond 20%, obviously occurs at lower cationicity but is less important. Therefore, the same experiments, performed on another column set, even with the same nature, could provide a different diagram of behavior, depending on the column history. Before the characterization of such polymers, it could be a good practice to inject some of them until the packing surface becomes stabilized.

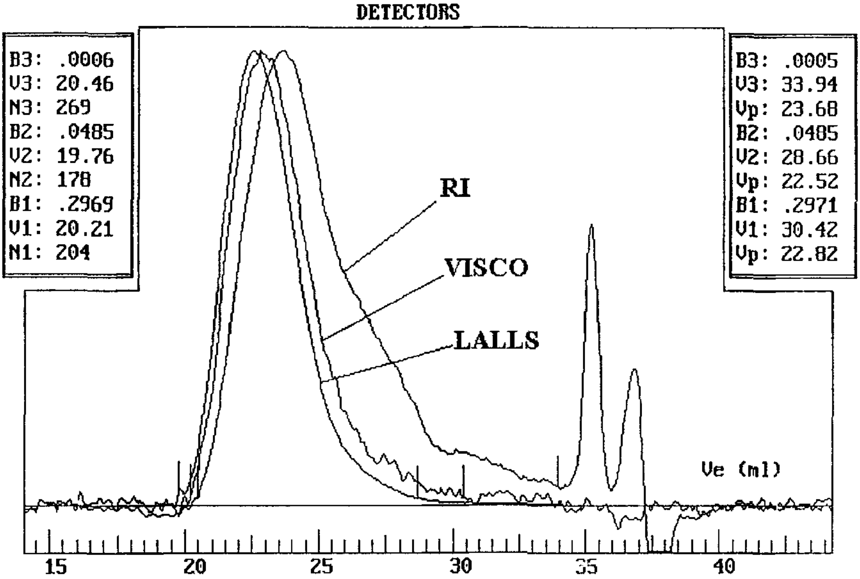

Figure 7 : Chromatograms of a 31.5% cationic copolymer eluted at the end of the study.

CONCLUSION

This study has demonstrated that an accurate characterization of 100% anionic polymers like sodium polystyrene sulfonate can be obtained when a high ionic strength (0.5 M in $LiNO_3$) is used as the mobile phase. This high ionic strength is necessary not simply to avoid the polyelectrolyte effect and to keep NaPSS in a coil-like shape but mainly to screen out repulsive forces between the negative functions of the polyelectrolyte and the negative charges on the packing surface. These forces make a decrease of the available porous volume leading to an apparent decrease of elution volumes, especially in the low molecular weight region, small molecules having access to a larger specific surface.

Conversely, the presence of negative charges on the packing surface disturbs elution of cationic copolymers depending on their cationicity rate. For very low cationicities (around 1%) an ionic strength of 0.1 M in $LiNO_3$ is enough to screen out all the forces and to get a normal elution. For low cationicity (below 6-7%), partial adsorption occurs with mobile phase $LiNO_3$ 0.1 M and it is necessary to increase the ionic strength to 0.25 M to screen out attractive interactions between the polyelectrolyte and the negative charges on the packing surface, allowing a right elution of the copolymer AM/CMA. When the copolymer cationicity is between 9 and 14%, the same effect occurs but at a higher ionic strength. In that case, $LiNO_3$ 0.5 M is necessary to obtain a right elution of the copolymers. Above a 20% cationicity, partial adsorption always occurs, especially for the highest molecular weights of the distribution, even if the ionic strength of the mobile phase is increased to 0.75 M, that is above the upper limit of salt content for the mobile phase. It has never been possible to run a correct experiment in our conditions and to get the right answer for copolymers with 20% cationicity and above.

Properties of ionic groups of the packing surface of Ultrahydrogel and Shodex columns are different. For example, NaPSS are adsorbed on Ultrahydrogel but elute on Shodex columns.

However, the results obviously depend on experimental conditions : temperature, flow-rate and nature of the mobile phase, nature of columns but also on the age and the history of columns whose packing surface may gradually be modified by adsorption of various solutes.

The conclusions of this sudy on the behavior of some polyelectrolytes in SEC, were possible only because viscometric, light scattering and refractometric detections were used simultaneously. The checkings of refractometric surface area and elution volume, intrinsic viscosity and average molecular weight from LALLS and from universal calibration were essential to understand the behavior of the studied samples.

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