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GEL PERMEATION CHROMATOGRAPHY OF SOLUBLE POLYELECTROLYTES

I. PECULIARITIES OF THE ELUTION BEHAVIOUR OF SYNTHETIC POLYELECTROLYTES IN AMIDE SOLVENTS

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SUMMARY

The elution behaviour of some aliphatic and aromatic polyacids and their derivatives in N,N′-dimethylformamide and N,N′-dimethylacetamide has been investigated by gel permeation chromatography on Styragels and macroporous glasses. An abnormally high dependence of the shape of the chromatographic peak and the velocity of the polyelectrolyte zone on the polyelectrolyte concentration in the sample and on the column length has been found. The phenomenon of stable association of macromolecules of oligoacids in a pure solvent up to a concentration of 0.005 g/dl was established. The mechanism of the formation of the polyelectrolyte zone and the possibility of applying gel permeation chromatography to the investigation of conformational transformations in poly-ions are discussed.

INTRODUCTION

Owing to recent advances in experimental procedures, gel permeation chromatography (GPC) has become the most widely used method for investigating the polydispersity of electrically neutral polymers[1,2]. GPC is used not only for investigations of synthetic polymers but also for fractionation and analysis of macro-ions of biological origin[3], such as proteins, peptides, nucleic acids and their fragments.

However, the GPC of synthetic polyelectrolytes has not yet been investigated in detail. The development of the GPC of polyelectrolytes is necessary because they are very widely used[4] as prepolymers in the preparation of plastics of high thermal stability; such as polyimides and polyaryleneimides. The development of methods for the analysis of molecular mass distributions of synthetic polyelectrolytes makes it possible to solve the main problems of controlling their synthesis.

Cha[5] and Cappola *et al.*[6] have shown that the elution behaviour of sulphonated polyacrylonitriles and other polyacids is profoundly affected by the addition of lithium halides to the eluent. These authors have found that the chromatograms of polyelectrolytes in a 0.1 *M* solution of LiBr or LiCl in N,N′-dimethylformamide

(DMFA) have a symmetrical shape and are eluted at much higher retention volumes than in pure DMFA. Similar effects distorting the contribution of polydispersity to the width and shape of chromatograms were observed in the GPC of condensed polyphosphates[7], polysaccharides[8] and legnosulphonates[9] in aqueous eluents. Richard and Gourdenne[10] have also shown that an increase in the column temperature, or addition of LiBr to DMFA, decreases the chromatographic mobility of polyamido acids and their ethyl esters.

Recently[11] we have briefly reported the main relationships of the GPC of polyamido acids (PAA) obtained from pyromellitic dianhydride and 4,4-diaminodiphenylic ester and developed in pure DMFA and in PMFA with the addition of low molecular mass electrolytes. We have shown[12] that the molecular mass analysis of PAA in pure DMFA is impossible because the migration rate and the concentration profile of the polyelectrolyte zone are governed by the concentration of polyelectrolyte in the sample and by the length of the column system. It was also found that association of poly-ions is possible and that the fractionation of PAA in pure DMFA is impossible.

This series of papers, with the general title "Gel permeation Chromatography of Soluble Polyelectrolytes" deals with the following aspects of the GPC of macro-ions:

(1) The nature and significance of effects governing the chromatographic behaviour of polyacids in GPC in pure aprotic amide solvents, the determination of experimental features concerning the appearance and observation of these effects and the discussion of the analytical possibilities of the GPC of poly-ions in pure solvents.

(2) The description of methods for the suppression of polyelectrolytic effects exceeding the contribution of synthetic polyelectrolytes to the chromatograms and of criteria for the determination of their complete suppression.

(3) The use of GPC for the analysis of the molecular mass inhomogeneity of polyelectrolytes in aprotic amide solvents.

This comprehensive investigation is necessary because literature on this problem is virtually non-existent. The investigation is also of practical interest because the molecular mass distribution determined by GPC is the characteristic that makes it possible to establish the relationships between the process of preparation of the prepolymer and final thermomechanical properties of polymeric materials.

EXPERIMENTAL

GPC was used to investigate the effect of the column length, the type of the sorbent and the solvent, the concentration of the solution in the loop and the chemical structure of polyelectrolyte chains upon the retention volume, V_R, and the shape of the chromatographic peaks of macro-ions.

Instruments and equipment

GPC was carried out with a ChZh-1302 liquid chromatograph (Special Design Bureau of Analytical Instruments, Academy of Sciences of the U.S.S.R., Leningrad). The chromatograph was equipped with columns of thermally stable glass and stainless steel, 1200 × 8 mm I.D., with an automatic dosimeter for six samples, a refractometric detector with a sensitivity of $n = 10^{-6}$ IR and a thermostated dropping flow-rate control which permits the measurement of V_R to within

0.2%. Two plunger pumps connected in parallel ensured uniform delivery of the eluent into the columns and the reference cell at flow-rates from 15 to 120 ml/h and at a maximum pressure of 300 p.s.i.g.

Intrinsic viscosities of polymer samples were determined with the aid of an Oswald capillary viscometer with a capillary of 0.4 mm I.D. and a mean elution time for the solvent of 80 min at 30°. The correction for kinetic energy determined for this viscometer is 1.3%.

Mobile and stationary phases

DMFA and N,N-dimethylacetamide (DMAA) used as eluents were pure grade. According to gas chromatography, their water content did not exceed 0.1%.

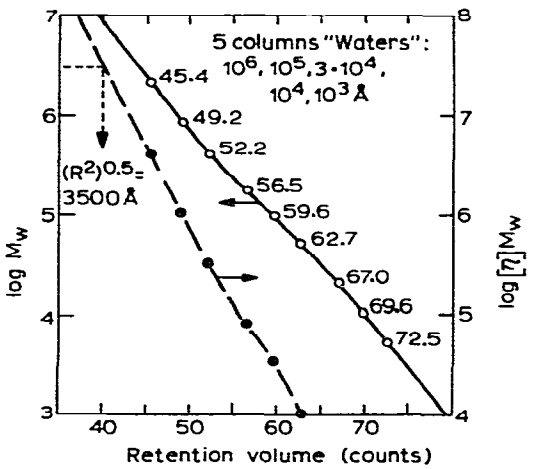
Depending on the column system, the flow-rate of the eluent through the column was 50 or 70 ml/h.

Two types of sorbent were used for the chromatographic separation:

(1) Macroporous glasses (MPG) with a mean pore diameter of 250, 1000 and 1600 Å were obtained from Prof. Zhdanov's laboratory[13] (Institute of Silicate Chemistry, Academy of Sciences of the U.S.S.R., Leningrad). MPG were fractionated with sieves and the columns were packed with glass particles of 63–70 μm by a dry method described previously[14].

(2) Styrene–divinylbenzene gels with a maximum permeability of 10^7, 10^6, 10^5, $3 \cdot 10^4$, 10^4, $3 \cdot 10^3$, 10^3 and 500 Å were packed in metal columns 1200 mm long (Waters Associates).

Figs. 1 and 2 show molecular-mass and universal calibration dependences in DMFA for narrow-disperse Waters Associates polystyrene standards obtained for both chromatographic column systems used in this experiment.



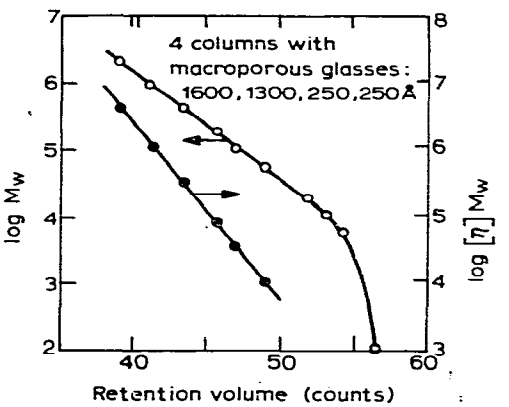


Fig. 1. Molecular mass and universal calibration dependences for five Styragel columns, 1200 × 8 mm, of 10^6, 10^5, $3 \cdot 10^4$, 10^4 and 10^3 Å porosity obtained for narrow-disperse polystyrene standards in DMFA. Elution rate, 50 ml/h; polymer concentration in the sample, 0.1 g/dl; temperature, 30°.

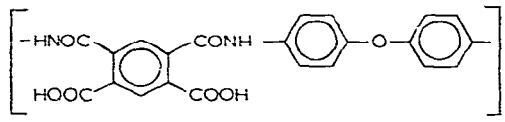
Fig. 2. Molecular mass and universal calibration dependences for four columns, 1200 × 8 mm, with macroporous glasses of 1600, 1300, 250 and 250 Å porosity obtained for narrow-disperse polystyrene standards in DMFA. Elution rate, 70 ml/h; polymer concentration in the sample, 0.1 g/dl; temperature, 30°.

Polymer samples

Our investigation was restricted to two classes of polyacids with different structures of the main chain.

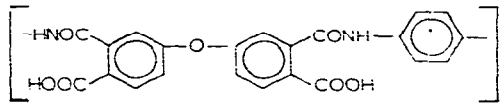
Aromatic polyacids are represented by polyamido acids (PAA) obtained by polycondensation of

(a) Pyromellitic dianhydride and 4,4-diaminodiphenyl ester: PAA PM, with the following structural formula:



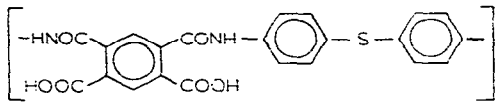
(1)

(b) Dianhydride diphenyloxide of tetracarboxylic acid and *p*-phenylenediamine: PAA DPhOPPH.



(2)

(c) Pyromellitic dianhydride and 4,4'-diaminodiphenylsulphide: PPA PMS, a sulpho analogue of PM

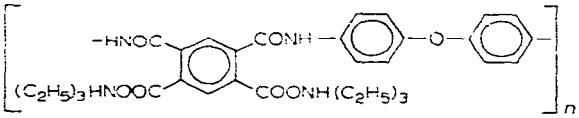


(3)

Depending on the conditions of the preparation of PAA[15] they can be obtained with intrinsic viscosities ranging between 0.3 and 3.0 dl/g. We investigated mainly polyacids with values of η between 0.3 and 0.6 dl/g. This interval corresponds to average molecular masses of 10,000–30,000 daltons[16].

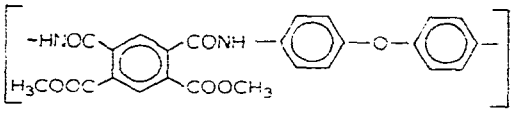
The structural formulae of these PAA show that every alternating chain sequence has two carboxylic groups capable of dissociation.

We also studied the elution behaviour of the triethylammonium salt of PAA PM



(4)

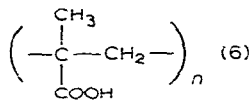
and of the methyl ester of PAA PM:



(5)

Two polymethacrylic acids of low molecular mass, PMAA-1 and PMAA-2, were chosen among aliphatic polyacids. Their viscosity-average molecular masses were

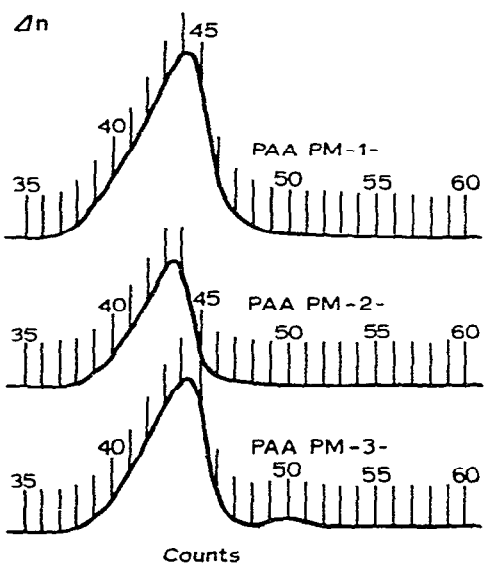
$M_\eta = 3200$ and 16,000 daltons, respectively; they have the following structural formula:


(6)

The chromatographic behaviour of PAA and MPAA was compared with that of a complete set of Waters Associates polystyrene standards, known to be electrocally neutral, and with that of a fraction of polymethyl methacrylate PMMA with a viscosity-average molecular mass of 62,000.

Gel permeation chromatography

Typical chromatograms obtained in the GPC of polyacids in DMFA are shown in Fig. 3. They are characterized by very asymmetric shapes differing from the Gaussian curve. The starts of chromatograms of polyacids with different intrinsic viscosities coincide and the maximum retention volumes do not depend on molecular masses. The chromatograms of PAA PM and DPhOPPh eluted in PMAA have similar shapes (Fig. 4).



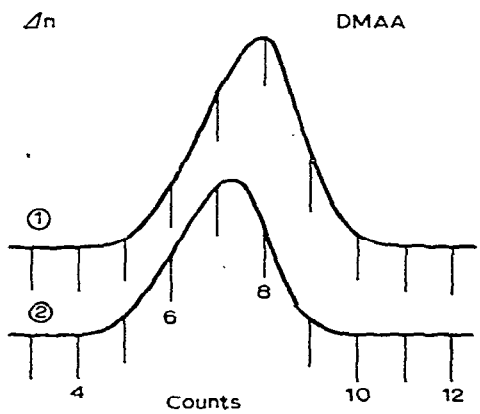


Fig. 3. Chromatograms of PAA PM: 1, 2, and 3 with intrinsic viscosities of 4.16 dl/g, 2.53 dl/g, and 0.85 dl/g, respectively. Solvent, DMFA; column system, 10^6, 10^5, $3 \cdot 10^4$, 10^4 and 10^3 Å.

Fig. 4. Chromatograms of PAA DPhOPPh (curve 1) and PAA PM (curve 2) obtained with a 300 × 8 mm column packed with MPG with the mean pore chain of 1600 Å in DMAA. Elution rate, 30 ml/h.

It was also found that the relationship between the retention time of polyacids and that of polystyrenes depends on the length of the column system. A comparison of Figs. 5 and 6 shows that for short systems, the PAA PM peaks have flatter fronts and

higher weight-average retention coefficients than for long columns packed with either MPG or Styragels of the same permeability. In the limiting case (Fig. 6) PPAs are chromatographed virtually with the exclusion limit determined by using polystyrene standards. It is characteristic that the elution behaviour of the triethylammonium salt and the methyl ester of PAA PM is the same as that of the initial PAA. It was also found that in the GPC of PAA and PMAA on Styragels, strong concentration effects are observed. Fig. 7 shows, on two scales, superposed chromatograms of PAA PM with $\eta = 0.3$ dl/g obtained at concentrations in the sample ranging between 0.003 and 2.0 g/dl. It is clear that with decreasing concentration the V_R values of the maxima on the elution curve also decrease and, as Fig. 8 shows, this dependence is not linear. The existence of breaks on the $V_R = f(C)$ curve indicates that the concentration effects in the GPC of polyelectrolytes are of a complex nature.

Similar results obtained for PMAA-1 and PMAA-2 are shown in Fig. 9. Just as for PAA PM, it is clear that with decreasing concentration the PMAA chromatographic curves tend to the lowest retention volumes. The front part of the chromatogram up to the lowest concentrations coincides with the chromatogram obtained at the maximum concentration, and only at the minimum concentration do the chromatograms become symmetrical.

In order to elucidate the nature of the processes forming the zone of polyacids, we carried cut micro-preparative fractionation of a 0.7 g/dl solution of PAA PM with $\eta = 0.3$ dl/g before and after the thermal treatment of its 0.7 g/dl solution at 60° for 5 h. Fig. 10 shows superposed chromatograms of PAA and its fractions taken from the "tail" of the chromatographic zone. Fractions introduced into the chromatograph for the second time exit in the front part of the zone rather than in its "tail" where they were collected.

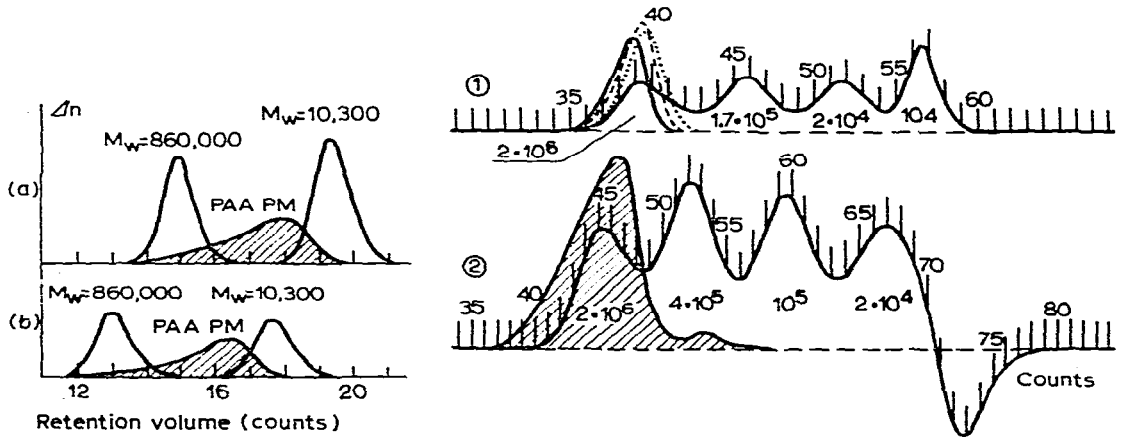


Fig. 5. Superposed chromatograms of PAA PM with $[\eta]$ — 0.41 dl/g and of narrow-disperse polystyrene standards with M_w = 860,000 and 10,300 obtained in DMFA with a two-column system (1200 × 8 mm). (a) MPG of 1600 and 250 Å. (b) Styragel of 10^5 and 10^3 Å.

Fig. 6. Superposed chromatograms (1) PAA PM (——) ,methyl ester of PAA PM (– – –) triethylammonium salt of PAA PM (....) and a mixture of "Waters" polystyrene standards with M_w = 2,145,000, 173,000, 19,850 and styrene obtained with a four-column system of 1600 and 2 × 250 Å (Fig. 2); (2) PAA PM and mixtures of "Waters" polystyrene standards of M_w = 2,145,000, 411,000, 9820 and 19,850 obtained for a five-column system packed with Styragel of 10^6, 10^5, 3 × 10^4, 10^4 and 10^3 Å porosity (Fig. 1).

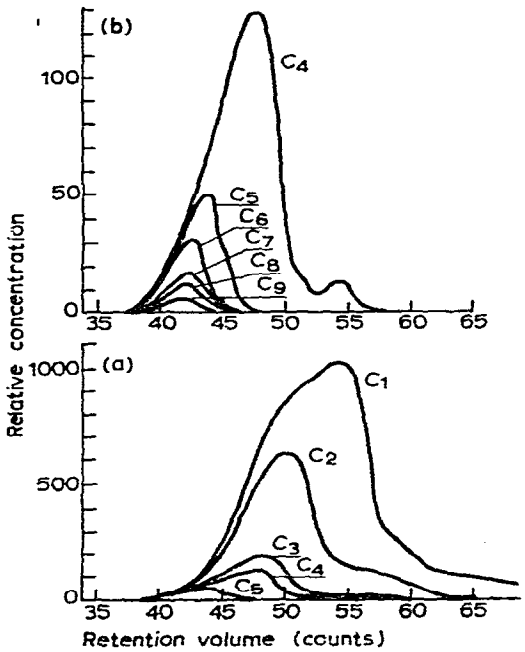


Fig. 7. Superposed chromatograms of PAA PM N32 of $[\eta] = 0.3$ dl/g in DMFA obtained with a column system of 10^6, 10^5, 3×10^4, 10^4 and 10^3 Å at sample concentrations from $C_1 = 2.0$ g/dl to $C_8 = 0.003$ g/dl. Volume of the flowmeter loop, 2 ml. Curves labelled C_4 in (a) and (b) correspond to the same concentration represented on different scales.

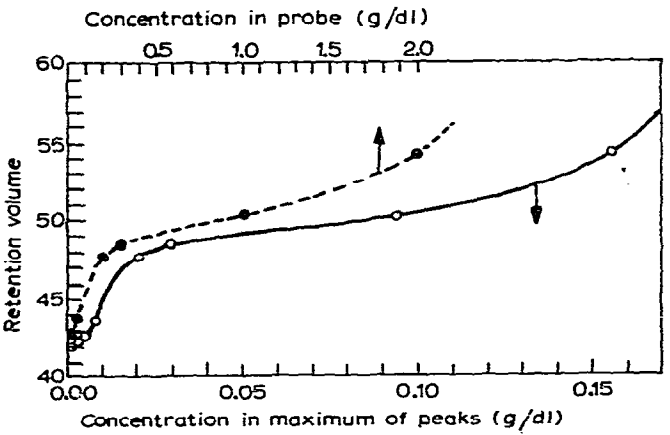


Fig. 8. Retention volume of the maximum in chromatograms of PAA PM N32 *versus* concentration in the sample (– – –) and concentrations at the peak maximum (——) obtained according to data in Fig. 7.

Viscometry

Fig. 11 shows the Mark–Kuhn–Howink dependence for polystyrene in DMFA at 30°. Numerical values of constants $k_\eta = 3.96 \cdot 10^{-4}$ and $a = 0.59$ are in good agreement with published data[17,18] and show that at this temperature DMFA is not a thermodynamically strong solvent for polystyrenes.

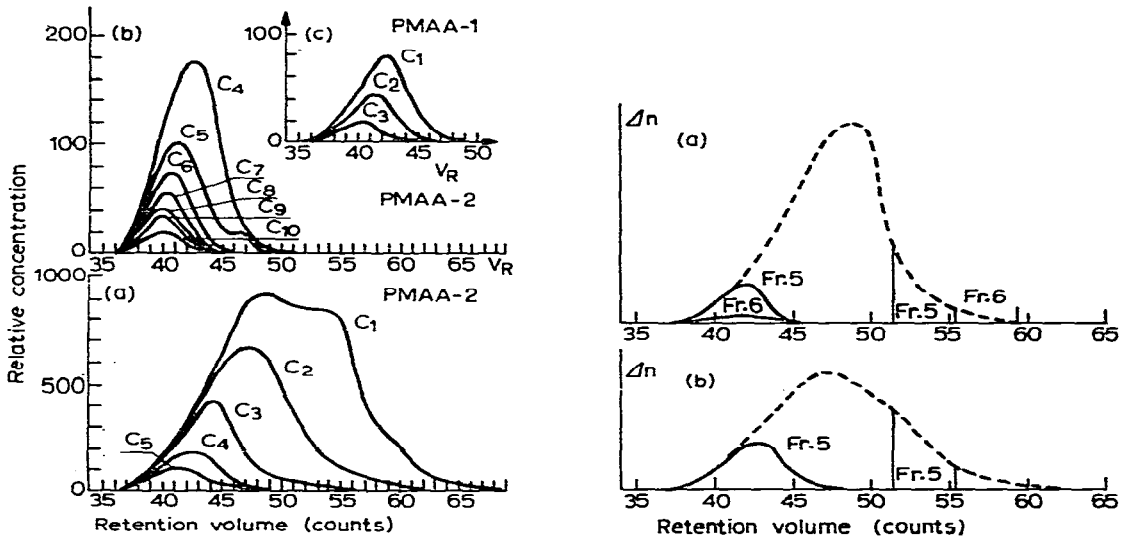


Fig. 9. Superposed chromatograms of PMAA-1 and PMAA-2 with M_n = 3200 and 1600 in DMFA obtained with the column system in Fig. 1 at concentrations for PMAA-2 (a and b) from C_1 = 2 g/dl to C_{10} = 0.015 g/dl and for PMAA-1 from C_1 = 0.08 g/dl to C_3 = 0.015 g/dl. Curves C_4 and C_5 in (a) and (b) correspond to the same PMAA-2 concentrations represented on different scales.

Fig. 10. Superposed normalized chromatograms of PAA PM N32 and of fractions taken at the tail of the zone. (a) Fractionation of the initial PAA 0.7 g/dl solution; (b) fractionation of 0.7 g/dl of PAA solution after thermal treatment at 60° for 5 h.

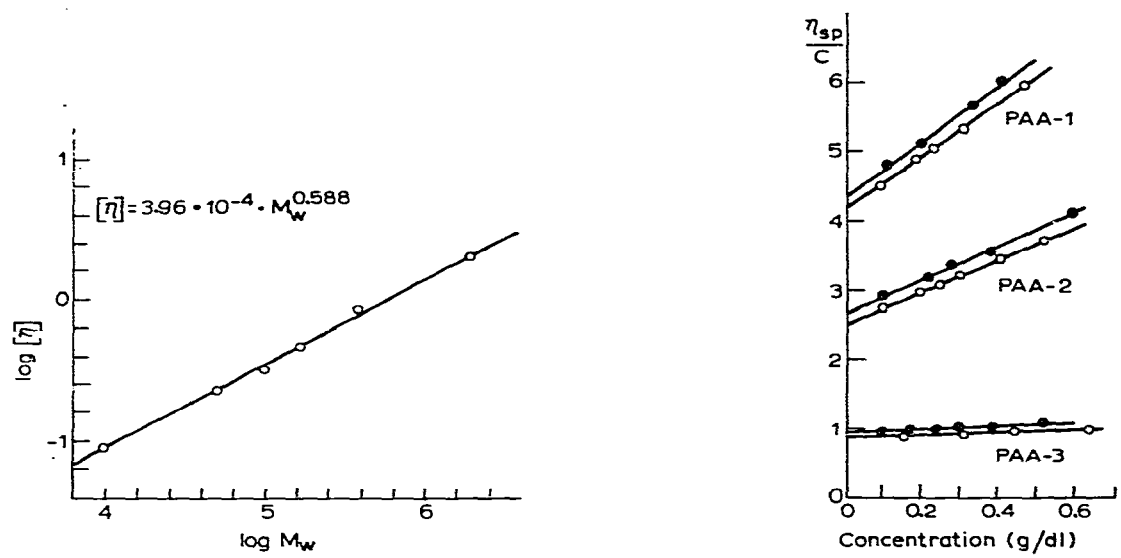


Fig. 11. Intrinsic viscosity of narrow-disperse polystyrenes *versus* their molecular mass in DMFA at 30°.

Fig. 12. Reduced viscosity, η_{sp}/C *versus* concentration for PAA PM 1, 2 and 3 obtained in pure DMFA (●) and DMFA with 0.01 mol/l of HCl (○) at 30°.

The usual indication of the polyelectrolytic nature of polymers capable of dissociation is the non-linear shape of the plot of reduced viscosity η_{sp}/C *vs.* concentration, C[19]. However, as Fig. 12 shows, over the concentration range investigated, PAA PM and PAA DPhOPPh in pure DMFA behave as neutral polymers. Moreover, the addition of small amounts of HCl leads to only a slight decrease in η (Fig. 12).

RESULTS AND DISCUSSION

The interphase distribution in GPC takes place by a mechanism in which the mean velocity of macromolecules of high molecular mass is higher than those of their low molecular weight homologues[21] and of the solvent.

Correspondingly, the distribution coefficient, K_d, cannot exceed unity in the equation

$$V_R = V_m + K_d V_s \tag{7}$$

where V_m and V_d are the volumes of the mobile and the stationary phases in the column, respectively,

This does not mean, however, that in GPC the K_d value is related only to the molecular mass of the polymer homologues and the porosity of the sorbent. It has been shown experimentally that in eluents of low thermodynamic strength[23,24] or in weakly polar eluents, K_d not only is a function of the geometrical characteristics of macromolecules and the pore shape but also depends on weak interactions of units in polymer chains with the matrix of an organic or inorganic sorbent.

The energy of these interactions can be positive (repulsion) or negative (attraction) and they can be caused by very different factors. These factors are the competition of the chain units with the eluent molecules or polar cosolvents, electrostatic interactions, entropy hindrances, etc. For GPC all these effects are shown by changes in K_d according to eqn. 7.

Intra- and inter-molecular interactions affect not only V_R but also the shape of the sorption isotherm, which finally determines the shape of chromatographic peaks. It should be noted that the correct value of K_d can be estimated from chromatographic experiments only in the linear range of the sorption isotherm.

A direct relationship is known to exist[26] between the shape of the sorption isotherm determined in static experiments and that of chromatograms obtained for the same heterophase system. Hence, the shapes of peaks in chromatograms of soluble polyelectrolytes (Fig. 3) indicate that their interphase distribution is characterized by a concave sorption isotherm[42].

It is possible to interpret this fact by taking into account the nature of macromolecules and the structure of the sorbent. A concave isotherm can be formed owing to the electrostatic exclusion observed in the chromatography of inorganic and small organic ions[27,28] on cross-linked ion exchangers or in the gel filtration of rigid globular proteins[29] on carboxylated gels. A second reason for the asymmetry of peaks is the nature of flexible-chain macro-ions: their ability to change their size when the pH or the ionic strength of solution is varied[30]. A decrease in the concentration of counter-ions when the polyelectrolyte solutions are diluted also leads to an increase in the size of macro-ions[31,32].

Because the elution behaviour of polyacids on weakly ionized MPG and non-polar Styragels is almost identical (Fig. 6), it can be concluded that the contribution of electrostatic exclusion to the chromatography of macro-ions in DMFA is slight. Moreover, as a comparison of Figs. 2 and 6 shows, in both cases PAA and PMAA are eluted virtually with the free volume of the column, and the shape of the chromatograms corresponds to a concave sorption isotherm. On the other hand, taking into account this shape of superposed chromatograms shown in Figs. 7 and 9 and their retention volumes, it is also possible to rule out the effect of adsorption on the interphase distribution. Consequently, the only possible reason for the anomalous elution behaviour of polyelectrolytes in pure DMFA and DMAA is expansion of the polyelectrolytes, which is closely related to the concentration of macro-ions in the chromatographic zone*. The influence of the concentration effects on the formation of chromatograms of polyelectrolytes is shown in Fig. 13.

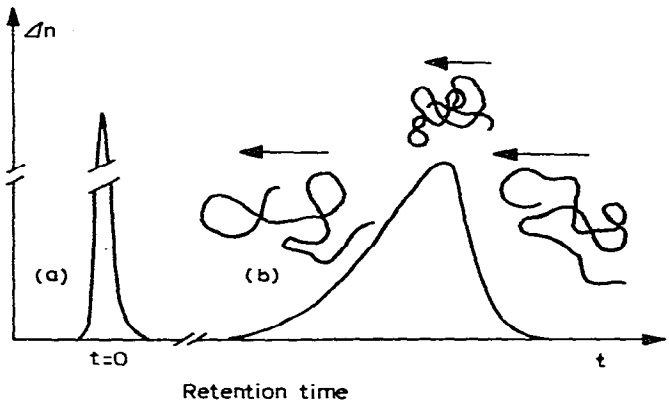


Fig. 13. Schematic representation of migration processes caused by changes in concentration and occurring in the chromatographic zone of the polyelectrolyte during its movement down the column. (a) Initial concentration profile of polyelectrolyte at the column entrance; (b) concentration profile of the polyelectrolyte in the column after a certain time *t*.

In GPC the polyelectrolyte sample is introduced into the column as a narrow zone, the concentration profile of which usually resembles that shown in Fig. 13a. Owing to the existence of the concentration gradient and of the corresponding difference in the ionic strength inside the zone, poly-ions acquire more uncoiled conformations at the edges of the zone than in its centre. As a result, in GPC the mean velocity of molecules is higher at the edges; this leads to the elongation of the front of the zone and the shortening of its rear. It should be noted that the macromolecules in the front of the zone move at a constant acceleration whereas the poly-ions in the rear undergo repeated conformational transformations.

Hence, the great difference in the elution behaviour of electrically neutral macromolecules and macro-ions in aprotic solvents is due first of all to the expansion of polyelectrolytes controlled by concentration, *i.e.* to a strong dependence of the coil size of macro-ions on their local concentration in solution.

* The question of the influence of the solvent strength on the thermodynamic compatibility of macromolecular chains with the matrix of the expanding sorbent is still unanswered[33-37]. However, the identical chromatographic behaviour of PAA on MPG makes the problem less serious.

Fig. 5 shows that the expansion of polyelectrolytes affects not only the shape of the chromatograms but also the velocity of the zone maximum.

As the zone moves down the column, the concentration in its maximum decreases and its velocity increases correspondingly. Finally, when the column length increases, the dispersion of velocities of macromolecules inside the zone should decrease and the velocity itself should increase up to a certain equilibrium value which is controlled by the porosity of the sorbent and by the maximum dimensions of poly-ion chains which they acquire at maximal dilution[35].

In our case, as will be shown below, intramolecular transformations are complicated by association; hence, this elementary interpretation can explain only qualitatively the situation observed. It should also be added that in this consideration we did not allow for the fact that the local concentration of counter-ion and, consequently, the ionic strength in the zone, decrease not only owing to the increasing concentration of poly-ions due to their chromatographic spreading and separation, but also owing to a purely chromatographic effect of separation of large poly-ions and their counter-ions. This effect is similar to the initial polyelectrolytic effect observed in ion-exchange chromatography of large ions[36], and should lead to an increasing asymmetry of peaks, which is observed exprimentally. The effect of the charge and distribution of small counter-ions around poly-ions is a general effect for the transport properties of polyelectrolyte solutions. In particular, it has a considerable influence on friction coefficients in the ultracentrifugation of macro-ions[37].

The influence of concentration effects is very great, and the final result is that the velocity of the polyelectrolyte zone and, particularly, its retention volume, are in no way related to the molecular mass or the chain length (Fig. 3). This fact is confirmed by the results of fractionation shown in Fig. 10. This figure shows that retention volumes of the PAA fractions taken from the rear part of the zone correspond to V_R of the front part and do not depend on the method of of the preparation of the PAAs. This also supports the conclusion that the elution behaviour of synthetic poly-ions which are soluble in aprotic amide solvents, and can rapidly change their conformations, is solely due to concentration effects of a polyelectrolytic nature.

A comparative analysis of elution times for chromatograms of electrically neutral polystyrene standards, PAA and PMAA at different concentrations (Figs. 2, 7 and 9) suggests that macro-ions can exist in chromatographic zones as associates and that the content of interacting polymer chains increases with decreasing concentration in the zone[43]. This comparison shows that the maximum of the PMAA-1 zone with $M_\eta = 3200$ corresponds to the retention volume of polystyrene macromolecules with a hydrodynamic radius of *ca.* 3500 Å, whereas the length of the PMAA-1 transchain cannot exceed 250 Å.

This effect is just the reverse of what is observed in the GPC of associating proteins[38], in which the association increases with concentration.

Although this phenomenon of association of synthetic polyelectrolytes with decreasing concentration (decreasing ionic strength of solution) is in apparent variance with generally accepted concepts, it is well known and has been studied in investigations of translational and rotational friction of poly-ions[32,40,44].

The following conclusions can thus be drawn. The polyelectrolyte zone in GPC in pure solvents is formed by the action of strong concentration effects, such as the expansion of polyelectrolyte association, etc., which are characterized by a concave

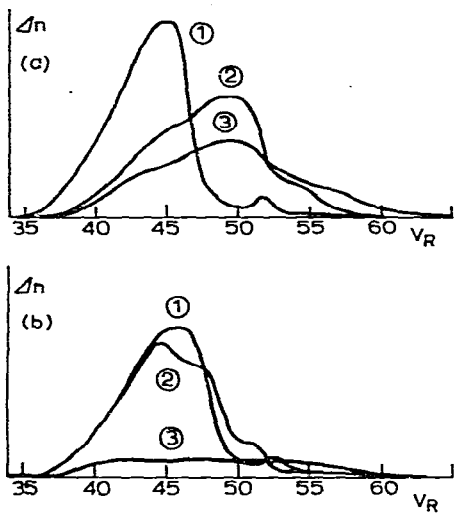


Fig. 14. Superposed chromatograms obtained for 0.2 g/dl solutions of (a) PAA PMS and (b) PAA PM (initial and after thermal treatment). (a) Curve 1, initial PAA PMS; curve 2, solution heated at 100° for 5 h. (b) Curve 1, the initial PAA PM; curve 2, a 0.2 g/dl solution stored in a refrigerator at 0° for 24 h; curve 3, solution heated at 100° for 2.5 h.

sorption isotherm. These effects eliminate the influence of the molecular mass and the molecular mass distribution of synthetic polyelectrolytes up on the shape of chromatograms and, hence, this prevents the use of GPC for determination of their molecular mass characteristics. Consequently, only when these effects are completely suppressed is it possible to use GPC for the molecular mass analysis of synthetic soluble polyelectrolytes.

Analytical possibilities of GPC of polyelectrolytes in pure solvents

Even the usual type of GPC can provide valuable information concerning the properties of polyelectrolytes.

Fig. 14 compares superposed chromatograms of PM and PMS polyamido acids exhibiting intrinsic viscosities of 0.59 and 0.62 dl/g, respectively. The figure shows that PAA PS is more thermally stable whereas PAA PM readily degrades, although on the whole it retains the main trends of the elution behaviour of a polyelectrolyte.

GPC can also serve as a convenient method for separating neutral macromolecules and poly-ions of equal molecular mass. Moreover, this separation will be caused by differences in the size of polymer coils of electrically neutral and charged macromolecules rather than by electrical exclusion characteristics of electrolytes of low molecular mass. Chromatograms of PMMA in DMFA (Fig. 15) clearly show that as the degree of saponification of the polymer increases, the peak corresponding to PMAA also increases and shifts far of front of that for PMMA.

CONCLUSIONS

GPC of synthetic polyelectrolytes is a new and unusual type of chromatography in which the interphase distribution is strongly dependent on the local concentration

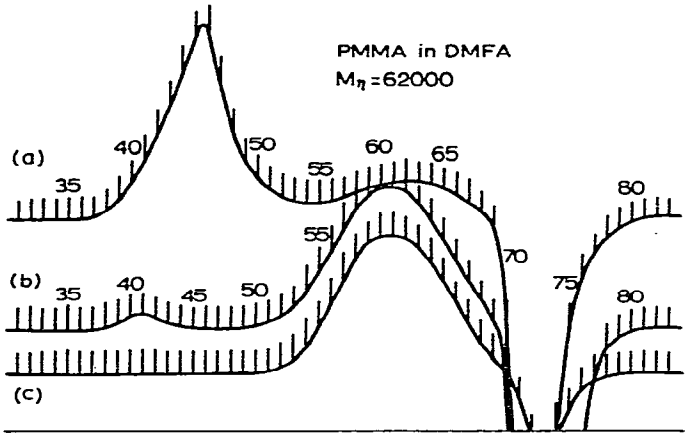


Fig. 15. Superposed chromatograms of PMAA-1 obtained for (a) a fresh 0.5 g/dl solution in DMFA, (b) the same solution stored at room temperature for 3 days, and (c) the same solution after 6 days.

of the polymer in the zone. Because the interphase distribution in GPC is determined only by the correspondence between the size of macromolecules and that of pores, this method seems very promising for solving many problems of fractionation and purification of charged and electrically neutral macromolecules (*e.g.* for separating the copolymer and the homopolymer, etc.). Moreover, very high selectivity can be attained, and the molecular mass analysis of a neutral polymer residue can be carried out simultaneously.

However, the main purpose of the GPC of polyelectrolytes, the determination of the molecular mass inhomogeneity, can be attained only if all the effects leading to the deformation and association of macromolecules in the zone are suppressed. Subsequent papers in this series will deal with the solution of this problem.

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