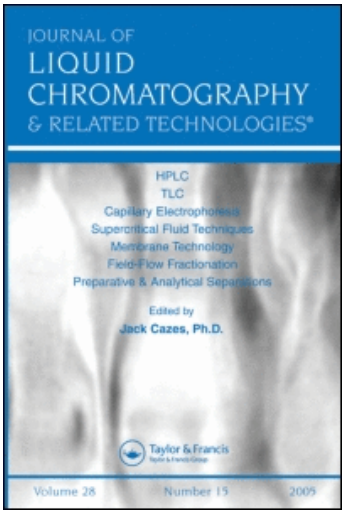

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Insight into the Chemical Heterogeneity of Cationic Poly-Electrolytes by Gradient-HPLC Using Salt Gradients

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INSIGHT INTO THE CHEMICAL HETEROGENEITY OF CATIONIC POLY-ELECTROLYTES BY GRADIENT-HPLC USING SALT GRADIENTS †

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ABSTRACT

The chromatographic behaviour of cationic poly(meth)acrylates was investigated in HPLC using salt gradients as the mobile phase. A quantitative correlation was found between retention time and number of charges per macromolecule. Information on the copolymerization behaviour of binary systems could be derived from the chromatograms. Insight into the chromatographic mechanism was gained by temperature dependent HPLC.

Thus the method is suitable for the characterisation of cationic polymethacrylates. The influence of parameters like ionic strength, pH-value, temperature, and poresize of the stationary phase corresponds widely with physico-chemical data and models of ionic macromolecules. The results offer a basis for setting up standard methods for the chromatographic characterisation of polyelectrolytes of similar structure.

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INTRODUCTION

Detailed knowledge about the correlation between molecular structure and properties of polymers is one of the main prerequisites for their preparation and technical production aiming at a tailor-made behaviour and an optimised process of synthesis. The properties of cationic copolymers e.g. depend on whether the charge distribution along the polymer chain is random or blocked. Therefore it is of major interest to get information on the molar mass and the heterogeneity of copolymers.

Up to now NMR-spectroscopy has been the method of choice for investigations on the chemical structure of copolymers [1]. In principle this method provides conclusions about the microstructure and the mean composition of polymers. However, it is not possible to discover details about the distribution of the chemical composition by NMR.

For some time past chromatographic methods like HPLC and SEC have gained increasing importance for the characterisation of polymers [2]. Gradient-HPLC of polyelectrolytes was performed by HUANG [3], who investigated copolymers of the cationic monomer diallyldimethylammoniumchloride with acrylamide with the aim of getting information on the structural heterogeneity of these copolymers of low charge density with not more than 10 per cent of charged comonomer. HUANG found after ultrasonic treatment that the polymers showed reduced retention times, a fact he attributed to a lower number of charges per molecule due to chain fragmentation. HUANG expected information about the chemical heterogeneity within single polymer chains based on the hypothesis that after fragmentation chains with a homogeneous charge distribution would give rise to a single sharp peak in the chromatogram whereas chains with a heterogeneous charge distribution would show two broad peaks, the first one relating to fragments with lower charge density and the second one covering fragments with higher charge density. HUANG, however, reported only very few results that were not able to give strong evidence for his hypothesis.

Regarding these interesting aspects it seemed worth to start a systematic investigation on the correlation between retention time and charge number of cationic polyelectrolytes. Using well characterised samples of cationic poly(meth)acrylates the chromatographic mechanism was to be investigated.

Another intention of the study was to test whether the method was suitable to get insight into the behaviour of copolymerization by characterising the chemical heterogeneity of the resulting copolymer samples. Because this group of polyelectrolytes proved easily soluble in water, aqueous solutions were used as mobile phase. Due to the

presence of ionic charges along the polyelectrolyte chaines it seemed consequent to count on the interactions of these groups with the stationary phase as the main separation mechanism [4]. Selecting the stationary phase, however, it had to be taken into account that strongly polar packings were likely to cause quasi irreversible adsorption originating from a cooperative action of multiple charges along the chain; in contrast on less suface active packings the polymers might not be retarded sufficiently.

MATERIALS

The chromatographic investigations were performed with four different types of polymers composed of the monomers trimethylammoniumethylmethacrylate-chloride (1), trimethylammoniumethylacrylate-chloride (2), and acrylamide (3). In the following the homopolymer poly-trimethylammoniumethylmethacrylate-chloride and the copolymers poly-trimethylammoniumethylacrylate-chloride-co-acrylamide and poly-trimethylammoniumethylmethacrylate-chloride-co-acrylamide are named poly-1, poly-2-co-3 and poly-1-co-3 respectively. For the experiments ten samples of poly-1 (A1 to A10), thirteen samples of poly-2-co-3 (B1 to B10 and D1 to D3) and one sample of poly-1-co-3 (E3) were used. The polymerization of samples B1 to B10 was not conducted to complete conversion, but interrupted at a small degree of conversion, G.

The molar masses were determined by size exclusion chromatography in aqueous solution using poly-1-calibrants. The maximum M_{Max} of the differential molar mass distribution W(lnM), was used for the calculation of the charge number Z; as this charge number is related to the maximum of the molar mass distribution it refers to the corresponding polymer fraction only and not to the whole sample. When correlating these Z-values with peak maximum retention times from gradient HPLC, it is assumed that it is the same polymer fraction eluting with the peak maximum in SEC and in HPLC. Furthermore, it has to be assumed that the copolymers, too, follow the calibration curve and that in HPLC the peak maximum fraction corresponds to the average composition of the sample. It is not claimed that these assumptions were close to correct, so that an error of up to about 10 percent has to be taken into account.

The charge number Z is calculated for homopolymers using eq.1 and for copolymers eq. 2, with M_1, M_2 and a_1, a_2 being the molar masses and mole fractions of the charged (1) and uncharged (2) comonomers respectively.

$$Z = \frac{M_{Max}}{M_1} \qquad (1)$$

$$Z = \frac{M_{Max}}{a_1 M_1 + a_2 M_2} a_1 \qquad (2)$$

Table 1 summarizes the SEC-results and the charge numbers for ten homopolymer samples poly-1, three samples of poly-2 and eleven copolymer samples investigated in this study.

Samples which were polymerized up to complete conversion normally show a broad distribution of their composition, especially if at the beginning of the reaction one of the monomers is consumed preferably; in any case the mean composition is identical to that of the monomers in the reaction mixture. For the high conversion binary copolymers (D1 to D3 and E3) it can be estimated from the copolymerization diagrams [5] how the composition of the resulting polymer chains changes during the course of the reaction. Samples B4 to B10, however, show a narrow distribution of their chemical composition because in this case the polymerization was interrupted at a small degree of conversion so that all polymer chains were formed under similar conditions. The composition of monomer units in the polymer, however, differs from the composition in the reaction mixture depending on the values of the copolymerization parameters. From the copolymerization parameters r_1 and r_2 the instantaneous mole fraction x_{1P} of the monomer 1 in the polymer can be calculated as a function of the mole fraction x_{1M} of the monomer 1 in the reaction mixture by using eq. 3 [6,7].

$$x_{1P} = \frac{r_1 x_{1M}^2 + x_{1M}(1-x_{1M})}{(r_1+r_2-2) x_{1M}^2 + 2(1-r_2) x_{1M} + r_2} \qquad (3)$$

For the copolymerization of monomer 2 with monomer 3, TANAKA [6] determined the copolymerization parameters to be $r_2 = 0.48$ and $r_3 = 0.64$ corresponding to a non-ideal azeotropic copolymerization. Other authors like BAADE et al. [7] determined values which

TABLE 1

Weight average molar mass M_w, peak maximum molar mass M_{Max}, charge number Z, polydispersity $U = (M_w/M_n) - 1$, degree of conversion G and mole fraction x_{3P} of acrylamide in the polymer calculated according to eq.3 for samples of poly-1 (A1-A10), poly-2 (B1-B3), poly-2-co-3 (B4-D3) and poly-1-co-3 (E3)

Sample	M_w /(10^3g/mol)	M_{Max} /(10^3g/mol)	U	G/%	Z/10^3	x_{3P}
A 1	6	5	0,9		0,02	
A 2	45	34	2,2		0,16	
A 3	44	39	1,5		0,19	
A 4	79	75	1,6		0,36	
A 5	137	93	2,0		0,45	
A 6	320	240	3,8		1,2	
A 7	765	570	3,1		2,8	
A 8	2.500	2.000	1,6		9,8	
A 9	6.350	5.400	2,0		26,0	
A10	8.300	7.600	1,5		37,0	
B 1	4.500	2.700	1,6	4,3	13,9	0,00
B 2	3.800	2.600	1,1	4,3	13,5	0,00
B 3	4.700	3.200	1,3	4,3	16,6	0,00
B 4	4.700	2.400	1,9	4,3	11,7	0,14
B 5	6.100	3.400	1,6	3,9	14,8	0,34
B 6	5.800	3.100	1,9	1,8	12,3	0,45
B 7	7.100	3.800	1,5	2,8	14,1	0,53
B 8	6.200	3.400	1,6	3,9	11,4	0,60
B 9	5.600	3.000	2,9	3,0	8,2	0,71
B10	4.200	2.200	2,0	3,0	2,9	0,89
D 1	5.000	3.400	8,1	100,0	15,3	0,30
D 2	4.300	3.000	7,9	100,0	11,2	0,50
D 3	6.100	4.100	3,1	100,0	11,4	0,70
E 3	7.700	5.800	2,1	100,0	20,8	0,50

differed only slightly from the former. In Tab. 1 the mole fractions of acrylamide x_{3P} for the B-samples are given as calculated from eq. 3.

Synthesis of Polyelectrolyte Samples

Poly-1:

An aqueous solution of 1 (c = 1.2 mol/L) is adjusted to pH 4 with dilute H_2SO_4. At a temperature of 60°C an aqueous solution of 2,2'-azobis(2-amidinopropane)dihydrochloride (c = 0.65 mol/L) is added under exclusion of oxigen, resulting in a concentration of the initiator in the reactor of $9.2*10^{-4}$ mol/L. After a reaction time of 30 min the reaction mixture is precipitated into acetone/ethanol (6/1 (v/v)) and dried at 50°C under vacuum.

Poly-2-co-3 (B1 to B10):

The monomers 2 and 3 are weighed in the desired amounts and dissolved in water, resulting in a solution with a total concentration of the monomers of 2 mol/L. At a temperature of 45°C and by excluding oxigen an aqueous solution of peroxidisulfate (c = 0.02 mol/L) is added to this solution, resulting in a concentration of the initiator of 0.005 mol/L. After a reaction time of 20 min the reaction mixture is precipitated into acetone/ethanol (6/1 (v/v)) and the precipitate dried at 50°C. The polymerization of these samples was stopped at low degree of conversion by pouring the reaction mixture quickly into the precipitation bath.

The samples poly-1-co-3, E3, and poly-2-co-3, D1 to D3, are standard products of Röhm GmbH, which are produced in technical amounts also by radical polymerization.

METHODS

All HPLC-columns contained a macroporous hydroxyethylmethacrylate-gel (HEMA) crosslinked with ethylenglycoldimethacrylate. Aqueous solutions of sodium chloride were applied as the mobile phase, the ionic strength of which steadily increased in the course of a gradient program. The pH-value of the mobile phase was kept constant at 7.4 by means of a phosphate buffer system. The gradient program described by HUANG [3] could not be used, as the salt concentration obviously was too low and not compatible with the highly charged polymers investigated in this study. Therefore new gradient programs had

to be developed which were based on the results of separations under isocratic conditions. A detailed description of the programs is given by Tab. 2. When applied to separations with the shorter column, the program P1 lead to peaks sufficiently sharp so that the maxima could be read from the chromatograms with good precision. Program P2 was used for runs with the two longer columns.

In order to achieve sufficient reproducibility of the retention times a buffered mobile phase turned out to be necessary. With unbuffered media the retention times became shorter with the number of runs; this effect was attributed to uneluted polyelectrolytes which reduced the activity of the stationary phase. Complete reconstitution of the column after each chromatographic run turned out to be indispensable for reproducible separations and was achieved by washing the column intensely with the solution of lowest salt concentration.

HPLC System

The chromatographic system consisted of a gradient HPLC-pump (L-6200), an autosampler (AS-6000), and a UV-detector (L-4000) from E.Merck (Darmstadt, F.R.G). The chromatogram s were registered by a recorder (Abimed Linear) from Linear Instruments Corp. (Irvine/California). For thermostatic control a kryostat (Lauda UKT 600) from Meßgerätewerk Lauda (Tauber, F.R.G.) and a waterbath (Julabo HC5) from Julabo Labortechnik GmbH (Seebach, F.R.G.) were applied. The detector signals were recorded digitally by the central computer of Röhm GmbH.

Separation columns were from Polymer Standard Service (Mainz, F.R.G.). The dead time/dead volume was determined by injection of an aqueous solution of sodium nitrate. Column 1 was PSS HEMA Bio 40 (dimensions: 8 x 50 mm; pore diameter 4 nm) with a dead time of 1.4 min. Columns 2 and 3 were PSW HEMA 40 and 1000 resp. (8 x 250 mm; pore diameters 4 nm and 100 nm resp.) with dead times of 9.0 and 9.3 min resp. The particle diameter of all columns was 10 µm.

Buffer-solution (ionic strength I = 0.1 mol/L; pH 7.4) :

In a 1 L measuring flask 1.18 g KH_2PO_4 p.a. (Fluka, Switzerland) and 10.85 g Na_2HPO_4 p.a. (Fluka) were dissolved and filled up to the mark with water Lichrosolv (Merck) [11].

Buffered solution of sodium chloride (I = 2.0 mol/L; pH 7.4) :

In a 1 L measuring flask 1.18 g KH_2PO_4 p.a., 10.85 g Na_2HPO_4 p.a., and 111.04 g NaCl p.a. (Merck) were dissolved and filled up to the mark with water Licrosolv.

TABLE 2

Course of ionic strength I with time for the gradient programs P1 and P2 (the delay time with respect to the detector amounted to approximately 5 min)

gradient program P1:		gradient program P2:	
time/min	I/(mol/L)	time/min	I/(mol/L)
0	0.1	0	0.1
2	0.1	20	1.1
22	1.1	21	0.1
24	0.1	50	0.1
40	0.1		

Samples for HPLC-separation:

About 100 mg of the polymer were weighed in a 20 mL measuring flask and filled up to the mark with a solution of sodium chloride (c = 0.2 mol/L) prepared with water Licrosolv (Merck). The resulting polyelectrolyt concentration was about 5 g/L.

Size Exclusion Chromatography

The apparatus consisted of an HPLC-pump from Bischoff Analysentechnik und -geräte GmbH (Leonberg, F.R.G.). Four separation columns (Ultrahydrogel) with pore sizes of 200, 100, 50, and 25 nm from Waters Chromatography Division (Eschborn, F.R.G.) were applied (dimensions: 7.8 x 300 mm). A differential refractometer from Knauer Wissenschaftliche Geräte KG (Oberursel, F.R.G.) was used, the signals of which were recorded by the central computer of Röhm GmbH. The separation columns were connected in series with decreasing pore sizes and run with an acetate buffer (acetic acid (c = 0.5 mol/L) / sodium acetate (c = 0.5 mol/L)) in water at a flowrate of 0.8 mL/min according to OTT [12]. The concentration of the polymers in the injected solution was 0.25 g/L; the injection volume was 300 µL at a temperature of 22°C.

Preparation of samples for SEC:

About 5 mg of the polymer were weighed in a 20 mL measuring flask and filled up to the mark with the above acetate buffer. The samples were kept for about 12 h and then

injected onto the column after addition of a small amount (spatula) of sodium acetate as an internal standard (dead time: 38.2 min).

The SEC system was calibrated using fifteen samples of poly-1 prepared by radical polymerization with molar mass from 6.000 to 10^7 g/mol and polydispersity $U = (M_w/M_n)-1$ varying from 1 to 5. The molar masses of these samples were determined by light scattering and ultracentrifuge measurements [13]. The calibration function was calculated by iterative optimization on the basis of the chromatogram and the M_w-value of all calibrants.

RESULTS

Peak Assignment

Fig.1 shows a typical chromatogram which was obtained with the gradient program P1 for the homopolymer sample A6. The positive baseline drift is caused by the increasing concentration of sodium chloride along the gradient when the UV-detector is set to 220 nm. Peak 1 appears with the dead volume (t_R = 1.5 min) and stems from the solvent of the sample. Peak 3 has a retention time of 16 min, is much broader and related to the polymer. Peak 2 is also found in the blank sample (deionised water); it might be suspected that with increasing salt content in the mobile phase impurities from the eluent are eluted, which before had been enriched in the stationary phase at low salt concentrations.

Fig. 2 shows a typical chromatogram for a sample of poly-2-co-3 with peak 3 corresponding to the copolymer. Peak 1 could be due to an elution of excluded polymer without or with only few charges because its retention time is shorter (1.2 min) than that of the solvent peak. As stated above, peak 2 possibly is caused by impurities from the eluent. Peaks 4 and 5 occur within the isocratic part of the chromatogram and may be attributed to polymer with low charge density, to low molar mass oligomers, or to residual monomer.

Problems with Column Overloading

Whereas in size exclusion chromatography the retention times decrease proportionally with the logarithm of the molar mass of the polymers, in gradient-HPLC of polyelectrolytes it can be expected that the retention times increase with the number of ionic charges. For

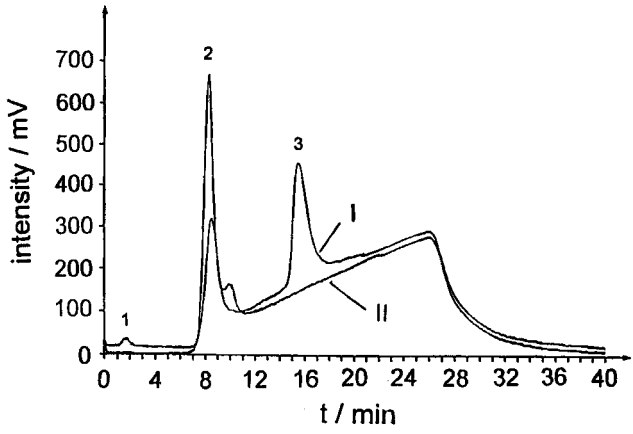

FIGURE 1 : Chromatogram for (I) the homopolymer poly-1 (sample A6; M_w = 320.000 g/mol) and for (II) a blank sample; column: PSS HEMA Bio 40; injection volume: 5 µL; analyte conc.: 5 g/L; flow rate: 1.0 mL/min; gradient P1; UV-detection: 220 nm.

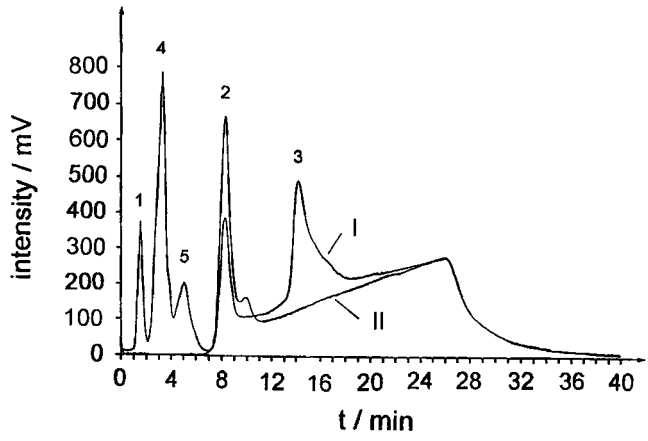

FIGURE 2 : Chromatogram for (I) the copolymer poly-2-co-3 (sample B9; M_w = 5.6 * 10^6 g/mol); and for (II) a blank sample; (for conditions see Fig.1)

homopolyelectrolytes the charge number is proportional to the molar mass, which means that under ideal conditions the chromatogram from HPLC, as an image of the charge distribution, should correlate with the distribution of the molar mass. However, such a comparison turned out to be difficult because the evaluation of the gradient-HPLC-chromatograms is complicated by the baseline drift. After subtraction of the baseline the chromatogram from HPLC (Fig.3a) of the sample A6 (poly-1) looks like a reflection of the respective distribution of the molar masses (Fig.3b) with a "tailing" for the first and a "fronting" for the latter. The asymmetric shape of the HPLC-peak may be due to an overloading of the column caused by an insufficient column capacity. Overloading of the separation column can easily occur because the large number of charges along each polymer chain will lead to an early occupation of the active sites of the gel surface even at low polymer concentrations.

The fact of increasing peak maximum retention times with decreasing analyte concentration supports the supposition of column overloading (Fig.4). The range of concentration independent retention times would be expected for concentration c below 0.2 g/L and could not be reached because of too low peak intensities. At constant analyt concentration decreasing injection volumes, too, lead to increasing retention times (Fig.5), an effect which also supports the hypothesis of column overloading.

These correlations make clear that in the following discussion it must be kept in mind that peakshapes and retention times may be effected by overloading of the column. Furthermore it has to be taken into account that multiple retention mechanisms and chromatographic effects from the polydispersity of the samples might be influencing the peakshape.

Retention Times of Homopolyelectrolytes and Copolyelectrolytes - Effect of Number and Density of Ionic Groups

Irrespective of their molar masses the homopolymer samples of poly-1 show similar retention times under isocratic conditions (at constant ionic strength). When the gradient P1 is applied, however, the retention time of peak 3 (Fig.1) increases from 13.2 min to 15.1 min with increasing molar mass (sample A2 to A10). From this a linear correlation between t_R and log M_{Max} can be derived (Fig.6): the more repetition units a polymer chain has, the longer is its retention time.

Furthermore, in Fig.6 the logarithms of the calculated charge numbers Z for the copolymers poly-2-co-3 (B-samples) from Tab.1 are plotted versus the corresponding

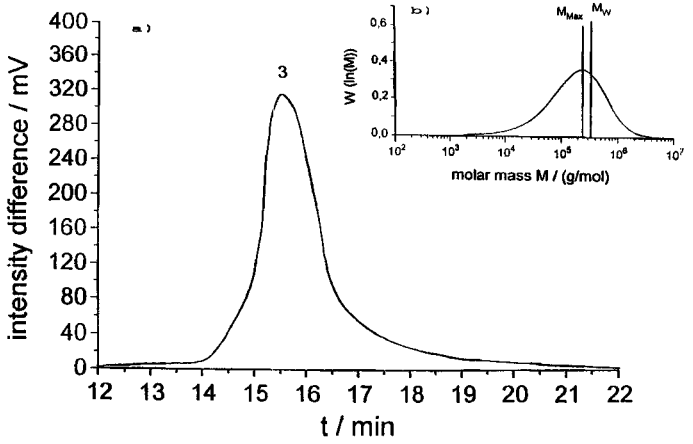

FIGURE 3 : Section-chromatogram (peak 3) of poly-1, sample A6, after subtraction of the baseline (a); (for conditions see Fig.1);
molar mass distribution (b)

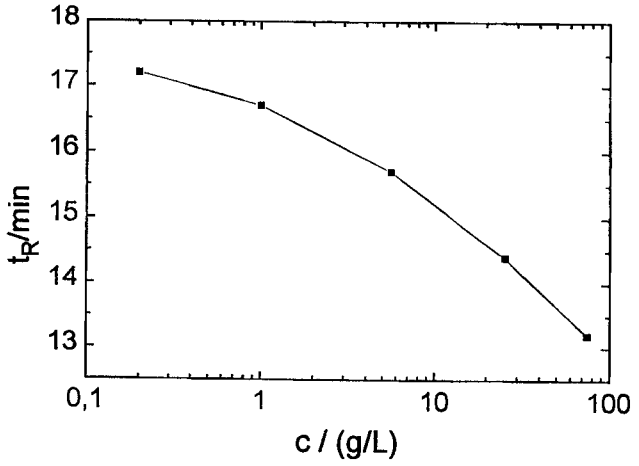

FIGURE 4 : Influence of the analyte concentration c on the retention time t_R for poly-1, sample A3 (M_w = 44.000 g/mol); injection volume: 10 µl (for other conditions see Fig.1)

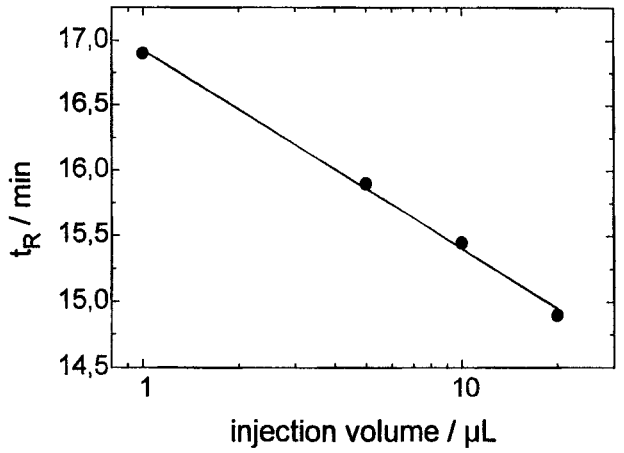

FIGURE 5 : Influence of the injection volume on the retention time t_R for poly-1, sample A2; (for conditions see Fig.1)

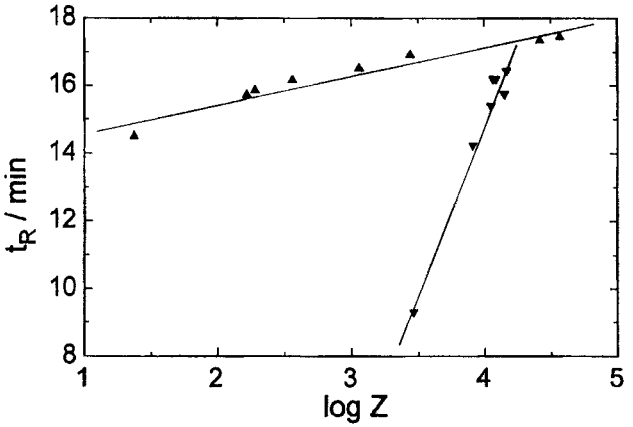

FIGURE 6 : Influence of the charge number Z on the retention time t_R for homopolymers and copolymers
(▲): poly-1; (▼): poly-2-co-3;
injection volume : 10 µL; (other conditions see Fig.1)

retention times of peak 3 (Fig.2). Due to similar charge numbers the data points for samples B4 to B7 cumulate within a small area, whereas for samples B8 to B10 the retention times show the expected dependency: t_R increases with increasing charge number. In this case the slope of the curve, however, is much steeper than that for the homopolymers. The reason for this may be that these samples of poly-2-co-3, in contrast to those of poly-1, not only have different charge numbers but also different charge densities. At a given charge number the homopolymers have longer retention times and the difference to the retention times of the copolymers is increasing with decreasing charge density of the latter.

As the M_W-values of these copolymer samples do not differ much from each other, it may be assumed that all copolymers approximately have the same chain length as far as the peak maximum fraction is concerned; it therefore can be expected that the retention times of the copolymers decrease with decreasing mole fraction x_{2P} of the charged comonomer. For low contents of the charged comonomer Fig.7 reveals a strong dependency of the retention time on the mole fraction of the comonomers; at high contents the curve flattens like a saturation function.

These results suggest that above a certain charge density the interaction of the chain with the stationary phase is at its optimum so that a further increase of the charge density cannot intensify the interactions. The following consideration may explain this: Polymer molecules with high charge density like poly-1 and the poly-2-co-3-samples B1 to B7 have many charged groups in close vicinity to each other which can interact cooperatively with the active centers of the stationary phase. Desorption of some of the groups is easily compensated for by the adsorption of adjacent groups which results in long retention times. However, excessive charges of the polyelectrolyte situated close to the active center of the stationary phase cannot intensify the binding of the analyte any more. In the case of polymer molecules with low charge density like the samples B8 to B10 (poly-2-co-3) less ionic groups can interact simultaneously with a particular binding site of the stationary phase with the result of a shorter retention time as compared to a macromolecule with the same number, but higher density of charges.

Information from the Chromatograms on the Copolymerization Behaviour

Fig.8 shows the chromatogram for sample D3 (poly-2-co-3). From the shape of peak 3 - also displayed in the insert after subtraction of the baseline - conclusions should be

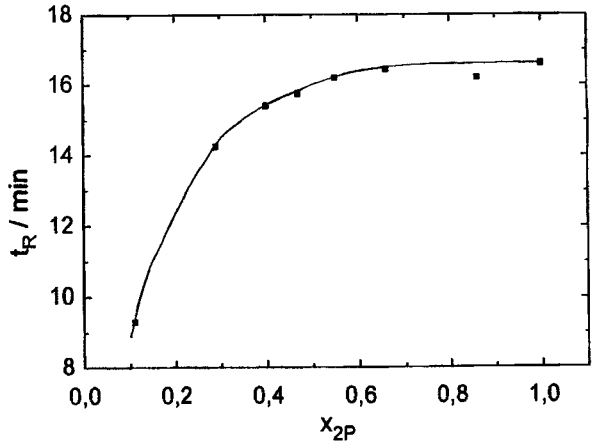

FIGURE 7 : Influence of the mole fraction x_{2P} of the charged comonomer according to Tab.2 on the retention time t_R (poly-2-co-3; B-samples); (for conditions see Fig.1)

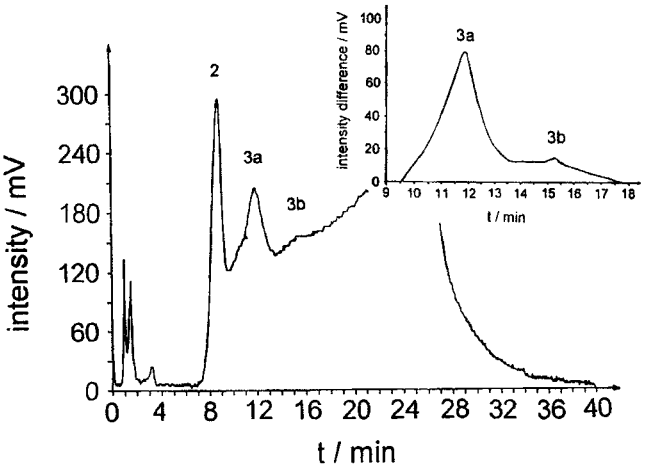

FIGURE 8 : Chromatogram of poly-2-co-3 (sample D3; x_{2P} = 0.3); insert: amplified section after substraction of the baseline (for conditions see Fig.1)

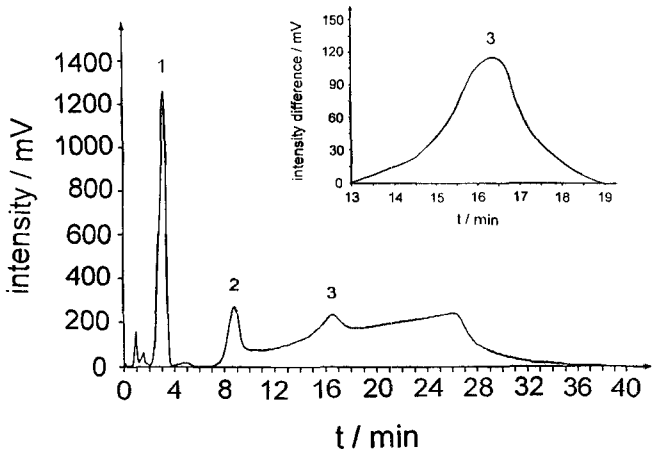

FIGURE 9 : Chromatogram of poly-1-co-3 (sample E3; x_{1P} = 0.5); insert: peak 3 after substraction of the baseline and amplification; (for conditions see Fig.1)

possible about the copolymerization behaviour of the monomers 2 and 3. In agreement with the copolymerization parameters it is expected that a reaction mixture deficient in the charged monomer 2 leads to a polymer with relatively high contents of 2 and therefore a high charge density (peak 3b). During the course of the polymerization the decreasing concentration of monomer 2 supports the reaction of the uncharged comonomer giving rise to a fraction with lower charge density (peak 3a). It is unexpected however that the alleged distribution curve exhibits a distinct bimodality; this probably is related to overloading of the separation column resulting in a strongly pronounced peak 3a.

Similarly the chromatogram for sample E3 (Fig.9) provides information on the copolymerization behaviour of the monomers 1 and 3. Again, peak 3 is shown in the insert after subtraction of the baseline and amplification and presents a distinctly flat slope despite of a possible overloading of the column. As to the copolymerization parameters (r_1 = 1.71 and r_3 = 0.25) according to TANAKA [6] it must be expected that during the polymerization of comonomers 1 and 3 the charged comonomer 1 polymerizes much faster than the uncharged comonomer 3; consequently the production of polymer chains with high charge density - corresponding to long retention times - is favoured at the beginning of the reaction. In the course of the reaction the mixture becomes more and more deficient in the charged comonomer 1 resulting in polymer chains with decreasing

number of charged groups and decreasing retention times. At the end of the reaction the charged comonomer is totally consumed and pure polyacrylamide is formed; this may be the reason for the intensive peak 1 with t_R equal to about 3 min. These results lead to the conclusion that the peakshape in the chromatogram - with some reservations due to overloading effects - contains information on the copolymerization behaviour of the respective comonomers.

Temperature Dependency of the Retention Times

The kinetics and thermodynamics of chromatographic interactions are known to be influenced by temperature [8], which also influences the solubility of the polymers in the mobile phase. For the investigation of the influence of temperature on the separation of the polymers the column was placed in a water bath at seven different (8, 15, 22, 29, 36, 43, and 50°C) but constant temperatures. Whereas the retention times for polymers with low charge density (samples B9 and B10) did not vary significantly with temperature, its influence proved more and more pronounced with increasing charge density of the polymers (samples B8 and A10): retention times increased with increasing temperature (Fig.10). Obviously it is the charge density which controls the elution behaviour at different temperatures. This supports the assumption that the charged groups of the polymer molecules cause interactions between the polyelectrolyte molecules and the stationary phase. Within the temperature interval from 22 to 29°C the slope of the resulting curve is remarkably steeper compared to the other temperature intervals which suggests a change in the separation mechanism.

Influence of Column Length and Pore Size on the Chromatographic Separation

As to the homopolymer samples poly-1 application of the gradient program P2 results in a linear correlation between retention time and log Z both for short (50 mm) and long (250 mm) columns. However, on the longer column the net retention time starts at lower values (9.2 min) and increases more rapidly with the charge number (up to 12.8 min). Consequently, the resolution R_S calculated for the samples A2 and A9 (poly-1) is better for the long column (R_S = 0.82) than for the short column (R_S = 0.55). This result is in disagreement with investigations on the chromatographic separation of proteins by SNYDER et al.

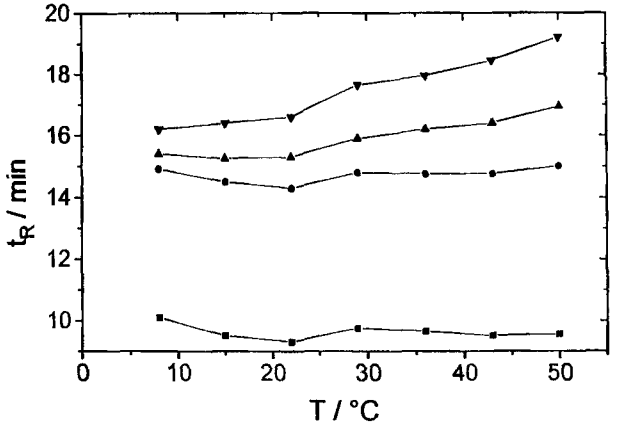

FIGURE 10 : Influence of temperature T on the retention time t_R for poly-1 and poly-2-co-3; samples (▼) A10;(▲) B8; (●) B9; (■) B10; (for conditions see Fig.1)

[9], who found the retention time influenced by the salt concentration of the mobile phase, but not by the column length. These differences in retention time and resolution observed for columns of different lengths in this study may be caused by a slightly different column packing material and they underline that - in contrast to proteins - polyelectrolytes interact intensely with the surface of the stationary phase.

For the experiments described so far column packings with a pore size of 4 nm were used in order to ensure the same separation conditions for all polymers by excluding the analytes from the pore volume. On packing materials with a pore size of 100 nm, however, the shorter chain molecules of A1 and A2 (poly-1; M_W = 6.000 and 45.000 g/mol resp.) had longer retention times than sample A10 (M_W = 8.300.000 g/mol) despite of their much lower charge number. This indicates that short chained polymer molecules can also interact within the pores of the stationary phase. The longer the polymer chains are the better they are excluded from the pores. Thus, for the high molar mass samples A6, A8, and A10 a linear correlation between retention time and log Z is found for these large pore materials as well as for the small pore materials discussed above.

From these results the conclusion is drawn that interactions of the ionic groups of the polymers with the surface of the stationary phase may occur in the pores as well as in the outer grain spheres. Therefore, it is recommended that for comparative investigations the pore size of the packing material has to be chosen in a way that either all sample constituents can get into the pores or all are excluded from the pores.

DISCUSSION

Concluding, it can be stated that moderately polar HPLC-separation columns of the type PSS HEMA are suitable for the characterization of cationic poly(meth)acrylates. The ionexchange capacity of this stationary phase is much lower than that of typical ionexchange materials of commercial standard; this helps to avoid irreversible sorption. The interactions of the polyelectrolytes with the stationary phase and their retention times depend on the ionic strength of the mobile phase. Therefore it turned out to be advantageous to vary the ionic strength by application of a gradient.

The retention time of the polymers depends on the number of charges per molecule and on the charge density along the polymer chain. A linear correlation between the retention time and the logarithm of the charge number was found.

The dependency of the retention time on the charge density of the polyelectrolytes can be explained by a qualitative model where in the case of macromolecules with high charge density the active centers of the stationary phase are attacked by many charges simultaneously; this makes the desorption of the macromolecule more difficult than in the case of molecules with lower charge density. Satisfactory reproducibility of the retention times was achieved by applying buffered media as mobile phases in order to prevent retention times from increasing with increasing pH. It may well be that the stationary phases contain anionic residues from initiator fragments which interact with the analyte molecules. Such anionic groups underlie an acid-base-equilibrium so that ionization is enhanced at higher pH-values. As a consequence of the presumably low consumption of initiator during the production process a very small number of active centers on the stationary phase would be expected - this could be the reason for the observed effects of column overloading.

Different separation mechanisms may be dominant in the chromatography of polyelectrolytes with moderately polar stationary phases. The total activation energy E_a of the interaction process can be estimated from the variation of the capacity factor k' with temperature by plotting the ln k'-values versus 1/T (eq.4) [10]. The order of magnitude of E_a allows conclusions about the predominant mechanism.

$$\ln k' = \ln k_0 - \frac{E_a}{RT} \qquad (4)$$

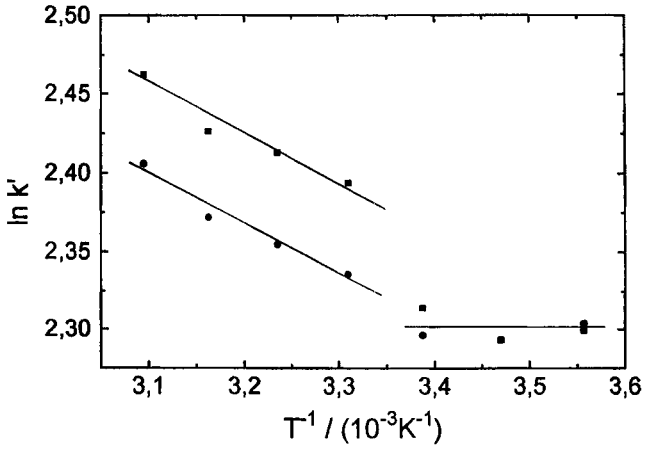

FIGURE 11 : Arrhenius plot for ln k' versus 1/T;
(■) poly-1 (sample A6); (●) poly-2-co-3 (sample B6)

As an example Fig.11 shows an Arrhenius plot for a homopolymer and a copolymer sample. Roughly, three areas can be distinguished: i) between 8 and 22°C k' is almost constant and happens to be identical for copolymer and homopolymer, ii) between 22 and 29°C the value of k' strongly increases, especially for the homopolymer sample, iii) within the temperature interval from 29 to 50°C k' steadily increases and a regression line can be drawn from which the activation energy is calculated equal to 1.8 kJ/mol and 2.1 kJ/mol for samples A6 (poly-1) and B6 (poly-2-co-3) respectively. From the fact that the activation energies for both samples were determined to be equal within the limits of error it can be concluded that in both cases the same chromatographic mechanism is predominant. The energy values lie in the lower range of what is expected for an adsorption mechanism. Although the properties of polyelectrolytes are known to be very complex it seems not inconsiderate to state that adsorption interactions play an important role in gradient chromatography of polyelectrolytes. However, the temperature dependency (Fig.11) is not as simple as to exclude the existence of other mechanisms and it seems very likely, indeed, that the predominant mechanism changes within the temperature interval investigated.

The chromatogram obtained by gradient-HPLC can be taken as an image of the charge distribution of the polymer given by the distribution of molar mass and chemical

composition. Moreover, the chromatograms contain information about the copolymerization behaviour of the different pairs of monomers. As far as the copolymerization parameters for the respective comonomers were known, qualitative agreement was found between experimental and expected results. Unfortunately, it cannot be ruled out that the chromatograms were influenced by overloading effects; from this point of view it would be appropriate to increase the relative detector sensitivity by applying salt solutions as mobile phases which are more transparent, whereby it would be possible to further decrease the absolute amounts injected. Even more information would be accessible by cross fractionation via coupling of SEC and HPLC so that the single SEC-fractions of a polyelectrolyte sample could be separated subsequently according to charge numbers by gradient-HPLC.

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