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Characterization of synthetic polyelectrolytes by capillary electrophoresis

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Abstract

Capillary electrophoresis in entangled polymer solutions was applied to determine the molecular mass and polydispersity of polyelectrolytes. The separation selectivities of different polyethylene glycols as buffer additive can be correlated to their average molecular mass. A universal curve correlating the selectivity and the molecular mass could be obtained by using the instrinsic viscosity of the polyethylene glycol. The separation of poly(2-vinylpyridine) standards was compared to the separation of poly(4-vinylpyridine) standards. An indirect detection system was developed to characterize the cationic polyelectrolyte polydiallyldimethyl ammonium chloride. Various polymers with oppositely charged groups (polycarboxybetaines) were investigated with respect to structure dependence, pH dependence and molecular mass dependence of inter- and intramolecular association.

Keywords: Polyelectrolytes; Buffer composition; Selectivity; Molecular mass; Polyvinylpyridines; Polybetaines; Polymers

1. Introduction

In polymer characterization, the determination of average molecular masses and their distribution are one of the most important criteria. With neutral polymers, one of the fastest and easiest techniques is size-exclusion chromatography (SEC) besides ultracentrifugation and light-scattering techniques. The latter is often used in combination with SEC. The characterization of polyelectrolytes in SEC is problematic because these polymers are mainly soluble in aqueous media and may exhibit adsorption and exclusion effects with the stationary phase. In addition, aggregation effects of the analytes can interfere with the molecular mass characterization.

Capillary electrophoresis (CE) on the other hand has found wide applications in the analysis of biopolymers, like DNA, proteins and carbohydrates. The intrinsic advantages of this technique are the principal use of aqueous buffers, the diminishing of adsorption effects on the capillary wall and the suppression of the electroosmotic flow (EOF) by special surface modification techniques. Therefore, CE is an alternative for the separation of synthetic polymers [1]. Due to the almost identical mass-to-charge ratios of linear polyelectrolytes in free solution, the mobility of different molecular masses of the same polyelectrolyte is very similar. To improve molecular mass separation in principal, sieving polymeric matrices have to be used. In DNA separation, linear polyacrylamide solutions are applied besides other water-soluble polymers, like polyethylene glycols, dextrans and cellulose derivatives.

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Only a few papers have been published about the characterization of synthetic polyelectrolytes with CE. Capillary gel electrophoresis (CGE) of polystyrene sulfonates [2–4] and polyvinylpyridines [5,6] were performed. Besides the characterization of polyelectrolytes in entangled polymer solution, the migration behavior of polyelectrolytes and polyelectrolyte copolymers in free solution can give additional information about counterion effects [7] and composition of copolymers such as 2-acrylamido-2-methylpropanesulfonate (AMPS) [8,9]. Very little is known about the migration behavior of amphoteric polymers with positively and negatively charged groups [10].

Two important parameters have to be considered when selecting the appropriate separation matrix for CGE. The first issue is the viscosity of the buffer solution, because a high viscosity of the buffer increases the rinsing time of the capillary. Additional bubble formation may cause problems. The buffer viscosity containing various sieving polymers in varying concentrations can easily be measured directly in the CE instrument [11].

The concentration of the sieving polymer in the buffer should exceed a certain concentration. In has been found in the separation of small DNA fragments, that so-called entangled sieving polymer solutions give superior separations over dilute ones [12]. This entanglement threshold (c^*, concentration of polymer in solution, where the entanglement of independent molecular chains begins) depends on the type of polymer and its average molecular mass. It can be determined by viscosity measurements directly with the CE instrument or can be calculated approximately with Eq. (1) [13]:

$$c^* \cong 1.5/[\eta] \tag{1}$$

in which $[\eta]$ is the intrinsic viscosity of the polymer. The intrinsic viscosity can also be estimated by the empirical expression (Mark–Houwink–Sakurada equation) [13]:

$$[\eta] \cong KM^a \tag{2}$$

K and a are characteristic constants with a given polymer–solvent system.

In reality, synthetic polymers have always a molecular mass distribution. Thus, the entanglement of the different chain lengths starts at different concentrations and c^* describes more or less a concentration interval than a certain point. To make sure the entanglement is complete, the concentration used should be beyond c^*. A concentration twice as high as c^* seems to be appropriate in praxis. Comparing with SEC, it would be interesting to determine the average ''mesh size'' of the transient entangled polymer network. This so called ''blob size'' can be estimated using the Mark–Houwink parameters with the following expression [14]:

$$\xi_b \cong 1.43K^{-1/3a}c^{-(a+1)/3a}(1.5^{1+1/a}/2\pi N_A)^{1/3} \tag{3}$$

where ξ_b is the blob size and N_A is Avogadro's number. Once the entanglement threshold is surpassed, the blob size should be independent from the chain length of the polymer. A conclusion would be, that, if the migration behavior of polyelectrolytes in capillary electrophoresis would only depend on this ''blob size'', any molecular size of a given polymer additive would lead to the same mass selectivity as long as the concentration is beyond c^*.

When plotting the logarithm of the relative mobility of a given polyelectrolyte versus the logarithm of its chain length (or molecular mass), a calibration curve as depicted in Fig. 1 can be obtained.

When the radius of gyration of the analyte is smaller than the ''blob size'' of the polymer network, the Ogston [15,16] model can be used to describe the migration behavior. It mainly depends on the accessibility of the ''pores'' between the entangled polymer fibers for the analytes. Since this regime has only a low mass selectivity, it is not important in

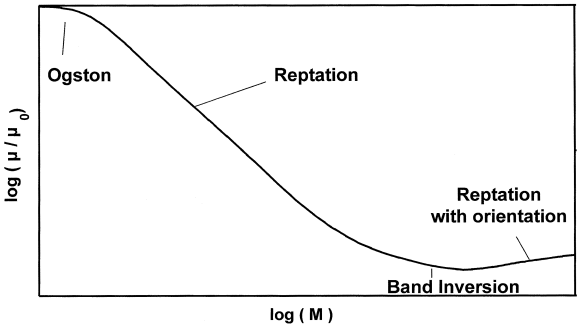

Fig. 1. Schematic calibration curve obtained for the separation of polyelectrolytes in entangled polymer solutions.

molecular mass determination and will not be discussed further in this paper.

The regime with the highest mass selectivity can be described by the so-called reptation model [14]. The radius of gyration of the polymer to be separated is larger than the blob size of the sieving matrix. In order to be able to migrate through the matrix, the molecule has to change its shape from a coil to a stretched conformation. The molecule migrates reptile-like through the entangled polymer network. Usually, a limit is given in mass selectivity with high-molecular-mass analytes due to the increasing orientation of the matrix parallel to the electric field. This phenomenon has been called ''band inversion''. Any analyte with a higher molecular mass will migrate irregularly and cannot be characterized anymore in the specific system. A simplified mathematical description is given in Eq. (4) [17] to describe the migration behavior of the analytes in this regime:

$$\mu \approx K_{\text{rep}} \cdot \left(\frac{1}{N_{\text{p}}} + bE^2 \right) \tag{4}$$

where K_{rep} is a constant, N_{p} the solute's molecular size, E the field strength and b is a function of the mesh size of the polymer network as well as of the charge and persistence length of the migrating analyte.

It becomes evident from this equation, that the mobility is only inversely proportional to the analyte chain length when the second term is negligible. This holds, however, only when relatively low field strengths ($<$200 V/cm) for CE are used.

Contrary to SEC, no universal calibration curve for the capillary electrophoresis of polyelectrolytes is known so far. Therefore, well characterized polyelectrolyte standards with low polydispersity are required to correlate the relative mobilities to a certain molecular mass. For our use, calibration curves will be practically used by relating the relative mobility:

$$\mu_{\text{r}} = \frac{\mu_{\text{S}}}{\mu_{\text{M}}} \tag{5}$$

to the molecular mass average in a double logarithmic plot; μ_{S} represents the mobility of the polymer sample and μ_{M} the mobility of a low-molecular-mass marker.

The molecular mass averages can be calculated in a histogram as in SEC. It is essential to analyze whether the detector signal is related to molar or mass concentration. The signal of end group labeled compounds is related to their molar concentration. The signal gained with polyelectrolytes with repeating chromophore units or, with indirect detection, repeating charges is related to the mass concentration of the analyte.

The number-average molecular mass can be calculated by the known equation [18]:

$$M_n = \frac{\sum w_i}{\sum N_i} = \frac{\sum N_i M_i}{\sum N_i} \tag{6}$$

while w_i is the mass of i molecules with the molecular mass M_i and N_i is the number of ith molecule with the molecular mass M_i. The weight-average molecular mass can be determined by [18]:

$$M_{\text{w}} = \frac{\sum w_i M_i}{\sum w_i} = \frac{\sum N_i M_i^2}{\sum N_i M_i} \tag{7}$$

The ratio of M_{w} and M_n [18]:

$$d = \frac{M_{\text{w}}}{M_n} \tag{8}$$

is a measure for the polydispersity of the molecular mass distribution in a polymer sample.

Sometimes, with polymer standards, one can find the molecular mass M_{p} which describes the molecular mass at the apex of the polymer peak and should be used preferably for recording calibration curves in CE.

2. Experimental

2.1. Chemicals

Phosphates, phosphoric acid, 4-aminopyridine, sodium chloride and polyvinyl alcohol (PVA) (M_{r} 49 000) were obtained from Fluka (Buchs, Switzerland); polyethylene glycol and hexadimethrine bromide were purchased from Sigma–Aldrich (Deisenhofen, Germany).

2.2. Polymer samples

Poly(2-vinylpyridine) standards were purchased from Polymer Standard Service (Mainz, Germany). Dextran standards were obtained from Pharmacosmos (Viby Sjælland, Denmark).

The syntheses of cationic polyelectrolytes (Fig. 2, structures b [19], c [19], d [20]) and polycarboxybetaines (Fig. 2 structures e [21], f [16], g [16], h [22]) were performed as described elsewhere.

Cationic polyelectrolytes and polyelectrolyte samples were dissolved in 0.1 mol/l HCl, containing 150 ppm 4-aminopyridine standard or, with indirect detection, 200 ppm sodium chloride. The sample concentration varied from 250 to 500 mg/l.

2.3. Preparation of the buffer solutions

Phosphate buffers at pH 2.0 and 2.5 were prepared by adjusting a 50 mmol/l sodium dihydrogenphosphate solution with 50 mmol/l phosphoric acid. The phosphate buffer at pH 6.0 was obtained the same way with 50 mmol/l sodium dihydrogenphosphate and 50 mmol/l disodium hydrogenphosphate buffer. Polyethylene glycol and polyvinyl sulfate additives were dissolved under permanent stirring with a magnetic stirrer for 3–5 h in the respective buffer stock solutions. The hexadimethrine bromide rinsing buffer was obtained by dissolving 0.4% (w/w) hexadimethrine bromide in the phosphate buffer stock solution as used in the separation buffers. PVA rinsing buffer was obtained by dissolving 1% (w/w) PVA (M_r 49 000) in the respective phosphate buffer stock solution at 95°C in a water bath.

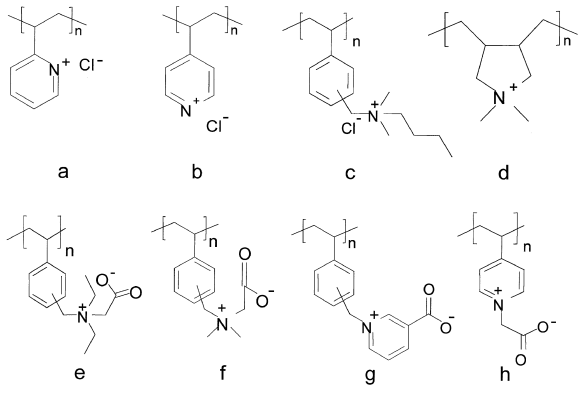

Fig. 2. Structures of the studied cationic polyelectrolytes (a–d) and polycarbobetains (e–h).

2.4. Instrumentation

CE measurements with UV detection were performed in a PACE/MDQ capillary electrophoresis instrument (Beckman Instruments, Fullerton, CA, USA) and recorded with the respective PACE/MDQ software.

2.5. Capillary material

Fused-silica capillaries of 75 μm I.D. were purchased from Polymicro (Phoenix, AZ, USA) and modified with a PVA coating according to Ref. [23]. The capillaries were used for all polyelectrolyte and polycarboxybetaine separations. The detection window was made by peeling off the polyimide coating with a razor blade.

2.6. Capillary rinsing

For cationic polyelectrolyte separations, the polyvinyl alcohol-coated capillaries were rinsed at 20 p.s.i. for 1–3 min with 0.4% (w/w) hexadimethrine bromide rinsing buffer (1 p.s.i. = 6894.76 Pa). For polycarboxybetaine separations, a new PVA capillary was treated with 1% (w/w) PVA rinsing buffer for 2 min. at 30 p.s.i. In both cases, the separation was then prepared by flushing the capillary for 2 min at 20 p.s.i. with the separation buffer.

3. Results and discussion

3.1. Quantification of the mass selectivity of a given buffer system

A variety of water-soluble sieving polymers can be used as buffer additives in CE. Therefore it is important to quantify the mass selectivity to be able to select the best polymer matrix. Ways to quantify the selectivity per base pair in DNA separation have been described by Dolnik and Gurske [24]. For synthetic polymers, this approach is not directly applicable because of their polydispersity. Therefore, we tried to quantify the mass selectivity by compar-

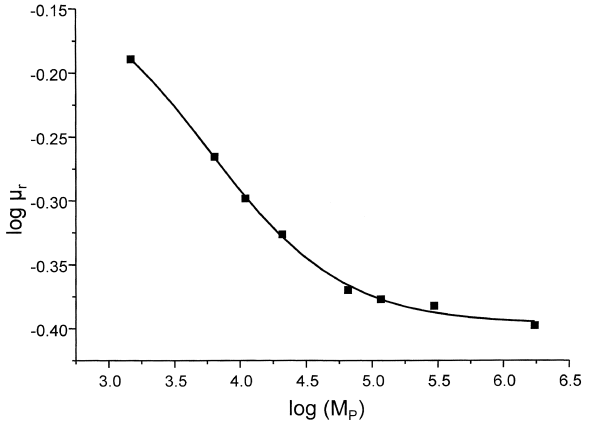

Fig. 3. Calibration curve for poly(2-vinylpyridine) standards. Conditions: buffer: 50 mmol/l phosphate, 5% (w/w) PEG $20 \cdot 10^3$ g/mol, pH 2.5; capillary: PVA 60 cm (effective length 50 cm); $U = 20$ kV; injection: 15 s, 3.45 kPa; detection: UV at 214 nm; marker 4-aminopyridine.

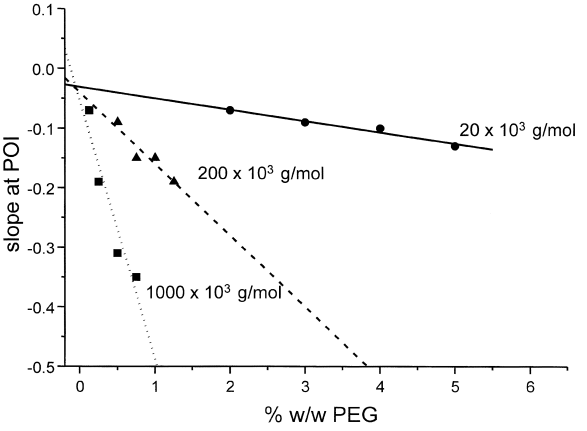

Fig. 4. Sieving performance of polyethylene glycol as buffer additive. Analyte: poly(2-vinylpyridine) (conditions as in Fig. 3). The maximum slope at the point of inflection (POI) is plotted versus polymer additive concentration.

ing the slopes of the calibration curves in the reptation region. A typical calibration curve is shown in Fig. 3 for poly(2-vinylpyridine) standards. The conditions for the separation are identical to those previously described [5]. The standards used are summarized in Table 1.

The sigmoidal shaped curve has the greatest slope at the point of inflection (POI). This slope at the point of inflection can be plotted versus the concentration of the sieving polymer additive. The resulting plot is linear. The greater the value of intercept with the *y*-axis and the greater the slope of this graph, the better is the mass selectivity of the sieving polymer. Fig. 4 shows this sieving performance plot achieved by using polyethylene glycols (PEGs) as buffer additives in different concentrations and with different molecular mass averages. The mass averages of the sieving polymer differ about one magnitude. The highest separation performance is obtained with the PEG of the highest average molecular mass. This leads to the assumption, that not only the mesh size of the sieving buffer additive is responsible for the separation effect. Obviously, other molecular mass dependent parameters may be responsible for the separation performance of this buffer additive.

Table 1
Molecular mass averages and polydispersity of the poly(2-vinylpyridine) standards investigated

Sample	M_p (g/mol) SEC	M_w (g/mol) SEC	M_n (g/mol) SEC	Polydispersity SEC
a1	1480	1340	1500	1.12
a2	6400	6100	6500	1.07
a3	11 000	10 700	11 100	1.04
a4	20 900	19 900	20 500	1.03
a5	65 700	62 800	64 300	1.02
a6	117 000	112 000	115 000	1.03
a7	297 000	257 000	287 000	1.11
a8	1 730 000	920 000	1 260 000	1.37

The situation becomes clearer when plotting the ratio of the point of inflection of the calibration curve and the intrinsic viscosity $[\eta]$ versus the polymer additive concentration (Fig. 5). The intrinsic viscosity for PEG was calculated with Eq. (2) with the Mark–Houwink parameters $K = 1.25 \cdot 10^{-2}$ ml/g and $a = 0.78$ at 25°C in aqueous solutions [13]. It can be seen that all points are correlated in a linear graph. We can therefore assume, that the sieving performance is directly proportional to the intrinsic viscosity of a polymer solution.

Considering the selection of a sieving matrix, it is obvious that the highest molecular mass of a given additive should be used to achieve the highest separation efficiency. However, the increasing viscosity with increasing molecular mass may cause problems, like extended rinsing times, light scattering effects in the detection system and bubble

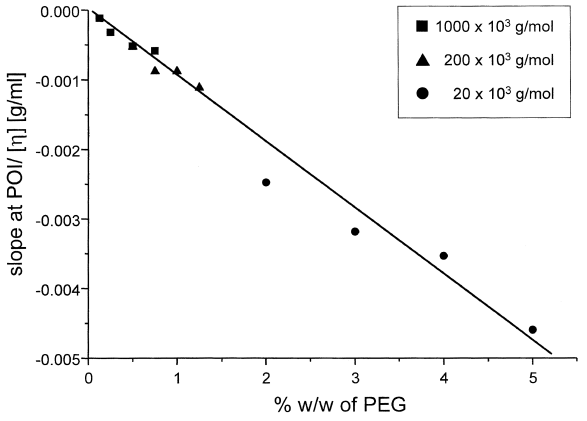

Fig. 5. Universal sieving performance plot by using the intrinsic viscosity of polyethylene glycols. For details see text.

formation. In our experience, polyethylene glycol with a molecular mass average of 200 000 has the most practical properties, i.e., good mass discrimination and tolerable viscosity.

3.2. Comparison of mass selective separation of the cationic polyelectrolytes poly(2- and poly(4-vinylpyridine)

Poly(2-vinylpyridine) (p-2VP) and poly(4-vinylpyridine) (p-4VP) can be protonized in acidic buffers and separated in CE as cationic polyelectrolytes. Although the chemical structure is very similar, the physical and chemical properties are quite different. The synthesis of standards with low polydispersity and their molecular mass determination with SEC is more problematic for p-4VP than for p-2VP.

Therefore, it is of practical interest whether the calibration curves of p-2VP standards can be also used to characterize p-4VP.

Fig. 6a shows the size-selective separation of poly(4-vinylpyridine) standards in the identical system as used for p-2VP. The samples have been characterized by SEC in dimethyl formamide using polystyrene standards. The characteristic data for the standards are summarized in Table 2. The samples were obtained by controlled radical polymerization [21]. This polymerization method is limited to be applied on the synthesis of lower-molecular-mass standards. The sample with the highest molecular

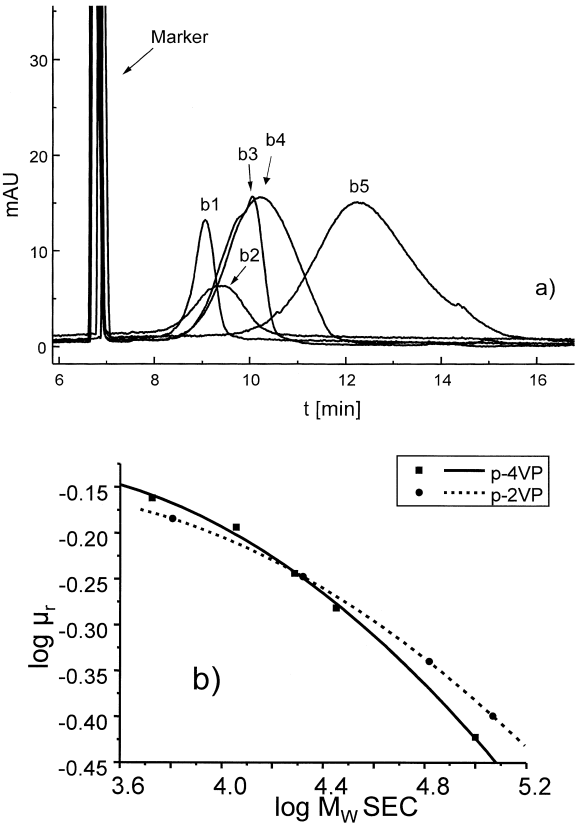

Fig. 6. (a) Overlay of the single injections of different poly(4-vinylpyridine) samples. Conditions: buffer: 50 mmol/l phosphate, 0.75% (w/w) PEG $200 \cdot 10^3$ g/mol, pH 2.5; capillary: PVA 60 cm (effective length 50 cm); $U = 20$ kV; injection: 15 s, 3.45 kPa; detection: UV at 214 nm; marker 4-aminopyridine. (b) Comparison of the calibration curves of poly(2-vinylpyridine) (p-2VP) and poly(4-vinylpyridine) (p-4VP). Conditions as in (a) with 1% (w/w) PEG $2000 \cdot 10^3$ g/mol additive.

Table 2
Molecular mass averages and polydispersity of the poly(4-vinylpyridine) samples used[a]

Sample	M_n (g/mol)	M_w (g/mol)	Polydispersity
b1	5320	7280	1.37
b2	11 420	15 600	1.37
b3	19 370	27 700	1.43
b4	28 350	42 500	1.5
b5*	100 000	–	–

[a] M_n of the highest molecular mass compound (marked with *) was calculated from reaction conditions.

mass has been synthesized by free radical polymerization and has therefore a much greater polydispersity. No signs of adsorption could be notified, the separation was finished in less than 20 min in this range of molecular mass. Unlike in SEC, the peak width of a polymer peak cannot be directly related to its polydispersity since the signal generated in the detector depends on the velocity of the sample zone and hence on the electrophoretic mobility of the compound.

Fig. 6b compares the calibration curves of p-2VP with p-4VP. It can be seen, that the calibration curves move from the Ogston in the reptation regime with those low-molecular-mass standards. The curves do not correlate. When moving to higher or lower polymer additive concentrations, the intercept of both calibration curves does not occur at the same molecular mass. It is shifted to higher molecular masses with increasing concentration of the polymer additive. Nevertheless, a good mass selectivity for the p-4VP polyelectrolytes can also be achieved. The reptation slope is even higher than with p-2VP. Unfortunately, the standards of the latter cannot be used for the determination of average molecular masses of the p-4VP samples. An explanation could be the different ternary structure of the molecule in solution and entropic energy differences during the molecule stretching in the reptation regime.

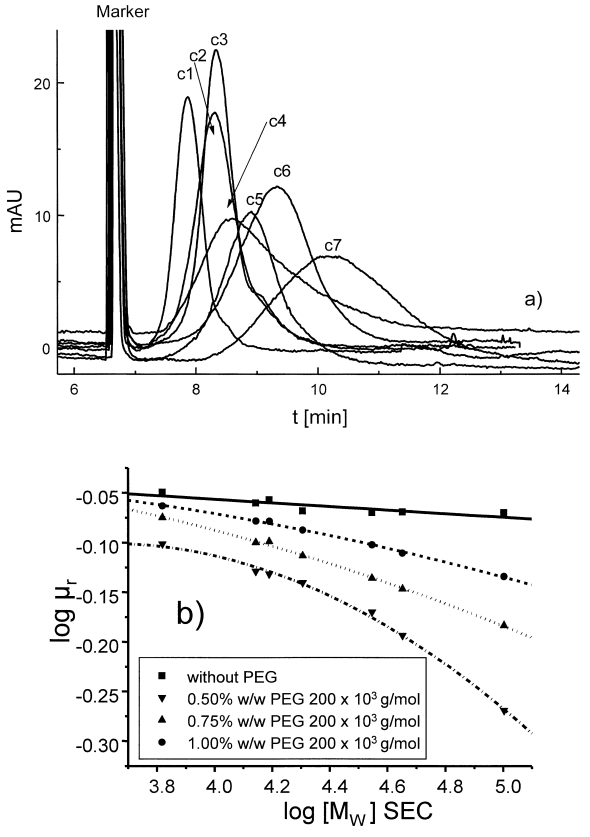

Fig. 7. (a) Overlay of the single injections of polyelectrolyte c samples. Conditions: buffer: 50 mmol/l phosphate, 0.75% (w/w) PEG $200 \cdot 10^3$ g/mol, pH 2.5; capillary: PVA 60 cm (effective length 50 cm); $U = 20$ kV; injection: 15 s, 3.45 kPa; detection: UV at 214 nm. (b) Calibration curves for polyelectrolyte c for various polyethelene glycol (PEG) concentrations as buffer additive. Other conditions as in (a).

3.3. Characterization of cationic polyelectrolytes with direct detection

As already mentioned, the characterization of some polyelectrolytes is problematic in SEC. In some cases a precursor of the polymer is characterized before cationic modification. If the degree of substitution measured, for example, by titration or nuclear magnetic resonance (NMR), one can calculate the molecular mass of the modified compound. Although this method gives an estimation of the molecular mass average, it cannot provide any information about changes in the polydispersity during the modification.

The polyelectrolyte c (Fig. 2 c) was obtained by modification of the polystyrenes. The molecular mass distribution has been determined by SEC. The cationic poylyelectrolytes have been separated by CE as shown in Fig. 7a. The corresponding calibration curves obtained with different concentrations of the polyethylene glycol additive can be found in Fig. 7b. It can be seen that the low-molecular-mass samples are more in the Ogston regime of the calibration curve.

The calculated molecular mass averages and polydispersity of the modified polyelectrolyte from the precursor are compared to the data obtained with CE in Table 3. The molecular mass averages were calculated from the pherograms as usual. It can be seen that a high correlation of the molecular mass averages and the polydispersity between CE and SEC is obtained for the medium sized samples. In order to be able to determine the polydispersity of

Table 3
Molecular mass averages and polydispersity estimated from the precursor for polyelectrolyte c and comparison with the capillary electrophoresis results

Sample	M_n calculated from precursor (g/mol)	Polydispersity of precursor (g/mol)	M_w CE (g/mol)	M_n CE (g/mol)	Polydispersity CE (g/mol)
c1	6586	1.17	9439	7668	1.23
c2	13 906	1.26	24 516	16 893	1.45
c3	15 470	1.20	25 584	17 423	1.47
c4	20 208	1.93	68 469	35 600	1.92
c5	35 182	1.33	50 625	34 893	1.45
c6	44 841	1.44	63 035	45 607	1.38
c7	100 636	1.84	152 900	105 470	1.45

the highest- and lowest-molecular-mass sample, the calibration curve had to be extrapolated. Therefore, the polydispersity of compound c7 is remarkably lower than the calculated value from the precursor.

3.4. Characterization of cationic polyelectrolytes with indirect detection

So far, polyelectrolytes with UV active chromophores have been separated. Indirect detection methods have found wide applications in capillary zone electrophoresis (CZE). To diminish electrodispersion, however, the mobilities of the analytes and the UV active ions of the background electrolyte have to match closely. Otherwise, asymmetric peaks of the polymer standards are observed. The influence of electrodispersion is demonstrated schematically in Fig. 8. Only if the mobility of the background electrolyte is equal to the mobility of the analyte, a symmetric peak can be observed. A polymer can be considered as an overlap of several monodisperse peaks. In this case, the sieving effect is greater than the electrodispersion and normal gaussian polymer peaks can be expected. If the mobility of the analyte differs strongly from the mobility of the background electrolyte, electrodispersion rules over the sieving effect and causes asymmetric peaks. In this case, a different background electrolyte should be chosen since strongly asymmetric peaks do not represent the molecular mass distribution of the polymer.

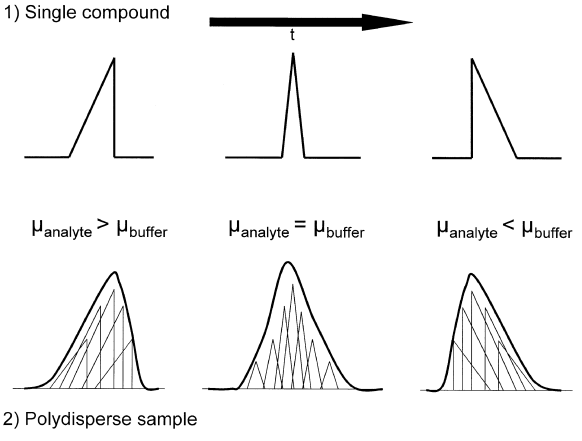

Fig. 8. Effect of electrodispersion on peak shape in the capillary electrophoresis of polyelectrolytes. For details see text.

Polydiallyldimethylammonium chloride (p-DADMAC) is a well investigated polyelectrolyte with low UV absorption [20,25]. Fig. 9 shows an overlay of the separation of p-DADMAC samples with different molecular mass (Table 4) with pyridine as background electrolyte. In this system, the sodium cation could be used as a migration time marker. The mobility of the background electrolyte was adjusted to the polyelectrolyte in free buffer solution without polymer additive. However, the sieving effect induces a high mobility difference of the background electrolyte with high-molecular-mass analytes and causes high electrodispersion and therefore a high peak asymmetry. Also, the peak shape of the lowest molecular mass seems to be tailing. This is not due to a mismatch with the mobility of the background electrolyte but can be referred to the increasing interaction with the sieving matrix with increasing molecular mass of the analyte.

Of course, the calibration curve corresponds to those achieved in direct detection systems, as shown in Fig. 1. Band inversion occurs at a molecular mass of 250 000, while the maximum molecular mass

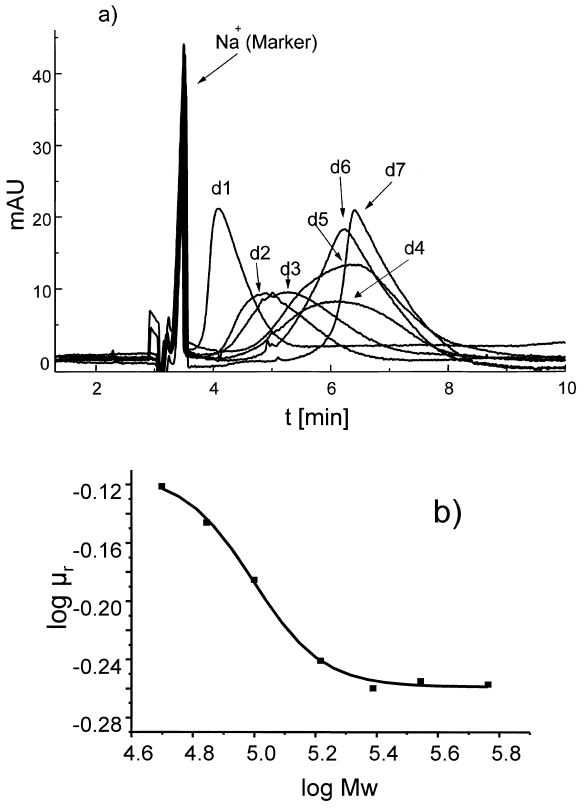

Fig. 9. Indirect detection of polyelectrolytes. (a) Overlay of the single injections of poly(diallyldimethylammonium) chloride (p-DADMAC). Conditions: buffer: 5 mmol/l pyridine, 1.0% (w/w) PEG $200 \cdot 10^3$ g/mol, pH 2.5; capillary: PVA 30 cm (effective length 20 cm); $U = 7$ kV; injection: 20 s, 3.45 kPa; detection: UV indirect at 214 nm; marker: sodium chloride. (b) Calibration curves for polyelectrolyte with 1% (w/w) polyethelene glycol (PEG) concentrations as buffer additive. Conditions as in (a).

selectivity is found around 100 000. With respect to the given separation limits, the molecular mass averages could only be calculated for the three samples with the lowest molecular mass. The molecular mass distribution of the larger polyelectrolyte samples exceeds the band inversion limit. As can be seen in Table 4, a very good correlation could be observed.

3.5. Separation of zwitterionic polyelectrolytes – polycarboxybetaines

Polybetaines are polyelectrolytes containing oppositely charged ionic groups at each monomer unit. A similarity in the structure to polypeptides or proteins is obvious although the compounds investigated (Fig. 2e–h) carry permanent cationic groups due to their quaternation. Another difference to proteins is, that the heterogeneity of the structures due to the polydispersity of the polymer is much higher than that of proteins. The similarity to proteins leads to the prediction of the following phenomena: pH dependence of the overall charge of the molecule, mobility differences due to the primary structure, and the formation of a specific ternary structure due to intra- and intermolecular association.

Fig. 10 shows the determination of the electrophoretic mobility of the carboxybetaines e–h in free solution under identical acidic conditions. The analytes have been selected to exhibit average molecular masses to diminish the influence of differences in chain length of the polymer on electrophoretic

Table 4
Molecular mass averages and polydispersity of p-DADMAC samples calculated from SEC and capillary electrophoresis for p-DADMAC samples

Sample	M_w (g/mol) SEC	M_n (g/mol) SEC	Polydispersity SEC	M_w (g/mol) CE	M_n (g/mol) CE	Polydispersity CE
d1	56 000	40 000	1.40	56 968	38 360	1.48
d2	74 000	52 000	1.42	75 717	52 763	1.43
d3	106 000	81 000	1.31	93 568	72 054	1.29
d4	187 000	138 000	1.36			
d5	245 000	180 000	1.36			
d6	336 000	232 000	1.45			
d7	580 000	420 000	1.38			

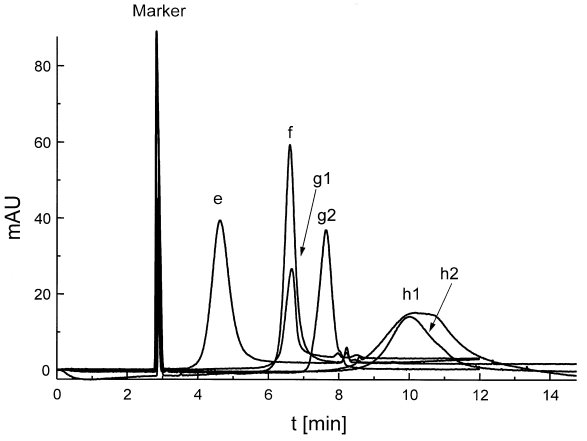

Fig. 10. Analysis of carbobetaines. Overlay of single injections of the carbobetaines e–h in free solution. Conditions: buffer: 50 mmol/l phosphate, 50 mmol/l acetic acid, pH 2.0; capillary: PVA 30 cm (effective length 10 cm); $U=5$ kV; injection: 5 s, 2.07 kPa; detection: UV at 214 nm; marker: 4-aminopyridine.

mobility. The characteristic data of the carbobetaines used are summarized in Table 5.

At the low pH of 2, the dissociation of the carboxylic function is suppressed and the analytes migrate as cationic compounds to the cathode. Comparison of the peakwidth of the betaines with the peakwidth of the monomolecular standard 4-aminopyridine shows, that a mobility distribution broadens the betaine peaks. Furthermore, the mobility of these betaine structures at this low pH is still rather low compared to other cationic polyelectrolytes. For this reason, the samples were injected at the short end of the capillary. The electrophoretic mobility of the betaines at their peak maxima are summarized in Table 6.

The mobility differences of analyte e and f as well

Table 5
Molecular mass averages and polydispersity of polycarbobetaine structures e–h investigated

Sample	M_w (g/mol)	M_n (g/mol)	Polydispersity
e	15 700	22 200	1.41
f	46 800	38 500	1.22
g1	51 100	27 000	1.89
g2	53 300	90 700	1.70
h1	20 000	13 300	1.50
h2	32 600	23 600	1.38
h3	46 100	32 900	1.40
h4	66 000	44 000	1.50

Table 6
Electrophoretic mobilities taken from the peak maxima for polycarbobetaines from Fig. 10

Sample	μ ($cm^2\ V^{-1}\ s^{-1}$)	RSD(μ) (%)
e	$1.7\cdot10^{-3}$	0.29
f	$1.2\cdot10^{-3}$	0.17
g1	$1.2\cdot10^{-3}$	0.62
g2	$1.1\cdot10^{-3}$	0.60
h1	$7.8\cdot10^{-4}$	1.69
h2	$8.0\cdot10^{-4}$	0.09

as the differences of g and h can be explained by looking at their primary structures. The cationic group of betaine e is shielded by ethyl substituents which are bulkier than the methyl groups of analyte f. Thus, the association of the carboxylic group with the quaternary amine is lower. It is therefore easier to protonate the carboxylic functions in compound e. The overall cationic charge of the molecule is higher and the mobility greater than for compound f. The association of the oppositely charged groups in compound g is probably hindered due to the low flexibility of the aromatic ring compared to the more flexible C–C bonds between the oppositely charged functions in structure h.

It can also be seen with analytes g and h, that different molecular mass averages of the betaines lead to different electrophoretic mobilities. Fig. 11 shows the electropherograms of four different sam-

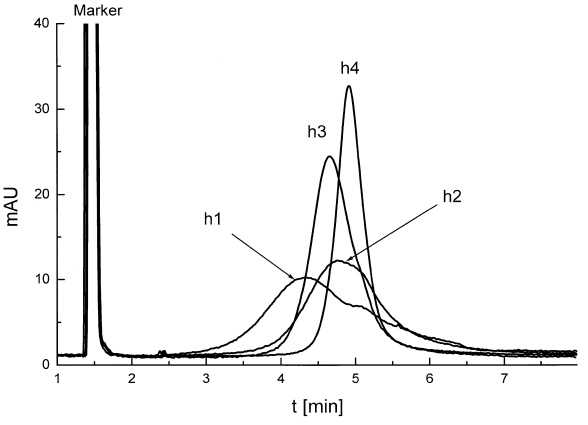

Fig. 11. Analysis of carbobetaines. Overlay of single injections of polycarbobetaine samples h1–h4. Conditions: buffer: 50 mmol/l phosphate, 50 mmol/l acetic acid, pH 2.0; capillary: PVA 30 cm (effective length 10 cm); $U=10$ kV; injection: 5 s, 2.07 kPa; detection: UV at 214 nm.

ples with increasing molecular mass averages in PVA capillaries with strong EOF suppression. As no sieving matrix is used, the increasing migration time is caused by decreasing mobility with increasing molecular mass. Also, a decrease in peakwidth can be observed. We assume a size dependent inter- and intramolecular association mechanism with changes in the ternary structure to be responsible for this behavior.

To study, whether intra- or intermolecular association dominates for the respective betaine structure, we mixed two samples of different molecular mass averages. If the intramolecular association dominates, the resulting electropherogram of the mixture should be the overlap of the two single injections. When the electropherogram of the mixture results in a new narrow peak with a mobility in-between the peak maxima of the single injections, the dominating process should be intermolecular association. Fig. 12 shows, that the dominating association mechanism for compound h is of intramolecular nature whereas intermolecular association is dominating in aqueous solution with compound g as shown in Fig. 13.

This intramolecular association is pH dependent as expected. With increasing pH of the buffer solution and increasing dissociation of the carboxylic group, the mobility of the betaine decreases drastically as shown in Ref. [10].

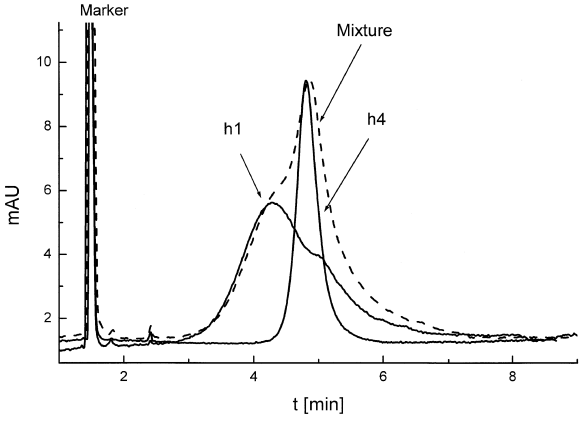

Fig. 12. Intramolecular association of carbobetaines. Overlay of single injections of compounds h1 and h4 and the mixture of both. Conditions: buffer: 50 mmol/l phosphate, 50 mmol/l acetic acid, pH 2.0; capillary: PVA 30 cm (effective length 10 cm); $U = 10$ kV; injection: 5 s, 2.07 kPa; detection: UV at 214 nm.

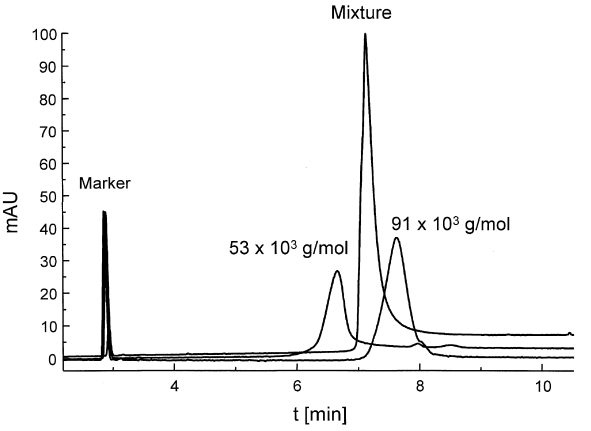

Fig. 13. Intermolecular association of carbobetaines. Overlay of single injections of compounds g1 and g2 and the mixture of both. Conditions: buffer: 50 mmol/l phosphate, 50 mmol/l acetic acid, pH 2.0; capillary: PVA 30 cm (effective length 10 cm); $U = 10$ kV; injection: 5 s, 2.07 kPa; detection: UV at 214 nm.

4. Conclusions

CE is suitable for the analysis of synthetic polyelectrolytes. Fast repeatable separations with low adsorption effects help to decrease separation time as well as solvent and sample volumes. An universal calibration system for the separation efficiency due to the polymer additive is likely to be developed whereas a universal calibration with polymer standards appears more complicated. Various applications have shown, that the determination of molecular mass averages and molecular mass distribution is possible with CE. Polyelectrolytes without highly UV absorbing chromophores can be investigated by using indirect detection systems. Furthermore, CE can help to investigate inter- and intramolecular association effects with more complex polymers, such as polycarboxybetaines. Further aspects will be investigations on quantifying the properties of polymer additives and the improvement of indirect detection systems.

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