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Journal of Chromatography A, 802 (1998) 149–157

JOURNAL OF CHROMATOGRAPHY A

Separations of anionic and cationic synthetic polyelectrolytes by capillary gel electrophoresis

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Abstract

The separation of anionic [poly(styrene-4-sulfonates)] and cationic [poly(2-vinylpyridines)] synthetic polyelectrolytes in CE is described. Dextrans have been used as sieving media, because they combine excellent UV translucence and good water solubility. The poly(styrene-4-sulfonates) as strong polyelectrolytes can be separated under alkaline as well as acidic conditions. The separation under acidic conditions is possible for low and high molecular masses even in uncoated fused-silica capillaries. Systems without electroosmotic flow have to be used in alkaline medium. For the cationic polymers surface modification of the capillary is obligatory to avoid wall adsorption of the solutes. All analytes exhibit similar migration properties to DNA in sieving media. The mass-selectivity range can be adjusted by the chain length (molecular mass) of the dextran. The resolution of the standards can be optimized via dextran concentration in the buffer. In contrast to CE separations of DNA and small molecules increasing band broadening here means better monitoring of solute polydispersity. © 1998 Elsevier Science B.V.

Keywords: Buffer composition; Polyelectrolytes; Poly(styrene sulfonate); Poly(vinyl pyridine); Polymers

1. Introduction

Capillary electrophoresis (CE) has demonstrated its enormous separation potential for the separation of biopolymers [1] for several years. Proteins can be separated according to three different mechanisms. The easiest possibility is the separation according to differences in their native mobility by capillary zone electrophoresis (CZE). The development of stable capillary coatings [2,3] led to very efficient and reproducible separations. Additional information on these proteins can be gathered by separations according to their isoelectric points in capillary isoelectric focusing (CIEF) [4–6] and in mass-discriminating systems like capillary gel electrophoresis (CGE) and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) [7,8].

DNA fragments show constant mass-to-charge ratios and can therefore only be separated according to their molecular size in polymer sieving systems or in gels. Most efficient separations have been achieved in crosslinked poly(acrylamide) gels. However, these gels are not replaceable and the buffer cannot be exchanged. Furthermore Joule heating can cause bubble formation, thus the capillary lifetime is very limited.

Similar efficiencies can also be achieved in so-called linear poly(acrylamide) gels. The remaining problems are a relatively high UV-cutoff around 250 nm and the toxicity of the starting material. Mass-selective separations are preferentially achieved in so-called entangled polymer solutions based on

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PII S0021-9673(97)01077-7

poly(ethylene oxide) or cellulose derivatives differing in concentration and molecular mass range. The optimum polymer concentration varies widely and even in ultra-dilute polymer solutions mass selective separations have been achieved [9,10].

In all cases a nonlinear dependence of relative polymer mobility on its molecular mass can be found. Several theories on the migration behavior of DNA have been developed, depending on the matrix and molecular mass range to be investigated [11]. A common method of depicting the dependence of the migration velocity on the molecular size is the double logarithmic plot given in Fig. 1. The resulting sigmoidal curve shape is caused by different migration mechanisms occurring for polymers of different molecular size and shape. These mechanisms can be explained by the theories of Ogston [12], the Reptation theory and others [13–16]. These theories have been verified by fluorescence microscopy [17].

So far, these systems have seldom been adapted to the separation of synthetic polyelectrolytes although they are difficult to characterize by aqueous size exclusion chromatography due to ionic interactions between the stationary phase material and the solutes, which may lead to poor accuracy, reproducibility, and precision of the mass determination. Those effects can be suppressed by using buffers of higher ionic strength, but then other problems caused by adsorption and salting-out effects are noticeable. On the other hand, CGE has revealed an enormous separation potential for biopolymers, e.g. proteins and DNA.

Poly(styrene sulfonates) have been analyzed by CE with hydroxyethylcellulose as sieving medium and suppressed EOF by applying coated capillaries [18]. The use of coated capillaries is not necessary when working under acidic conditions at pH 2.5 with polystyrene sulfonate (PSS), because analyte–wall interactions and the EOF are negligible. After field reversal (injection at the cathodic end) the PSS can be detected at the anodic end of the capillary. On the other hand, cationic polyelectrolytes tend to interact with capillary surfaces, consequently coated capillaries are required here.

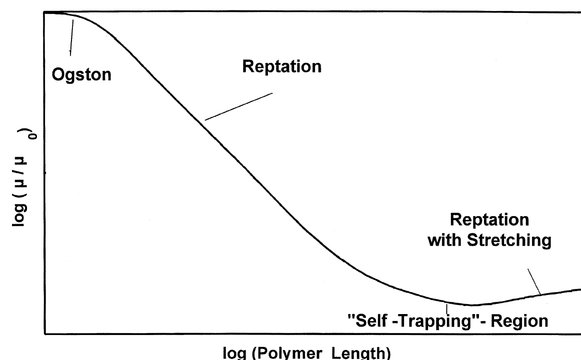

Fig. 1. Schematic presentation of polymer mobility in sieving matrices. M_r=molecular mass.

Because of the poor solubility of cellulose derivatives and their slow dissolution rate in cold water we decided to study other sieving media for their applicability in the characterization of anionic as well as cationic polyelectrolytes. Dextrans proved to be very advantageous. Besides their good water solubility [e.g. more than 15% w/w of dextran T70 (M_r 70 000) can be dissolved quickly in cold water] they show excellent UV translucence at wavelengths >200 nm which is crucial for the study of nonaromatic polyelectrolytes. The viscosities of dextran solutions are relatively low, thus simplifying the buffer exchange.

2. Materials and methods

2.1. Instrumentation

A Beckman P/ACE System 2050 (Beckman Instruments, Munich, Germany) automated CE instrument with SYSTEM GOLD instrument control and data evaluation software was used for analysis. The detection wavelength was 254 nm. The temperature of the analysis was set at 25°C.

2.2. Materials

All buffer substances except phosphoric acid were purchased from Fluka (Buchs, Switzerland). Phosphoric acid was purchased from Merck (Darmstadt, Germany). The dextrans T10, T70, T2000 and ficoll 70 were purchased from Pharmacia (Uppsala, Sweden). The e-CAP 200 for SDS-gel electrophoresis was purchased from Beckman Instruments.

2.3. Capillaries

Most separations were performed in 75 μm I.D.×375 μm O.D. capillaries with 57 cm (50 cm effective length). The fused-silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ, USA). The covalently bonded poly(vinyl alcohol) (PVA) coated capillaries were prepared according to the literature [3].

Some separations were performed with a Beckman e-Cap capillary with 27 cm (20 cm effective) length×100 μm I.D.

2.4. Electrophoresis

A 50 m*M* phosphate buffer (pH 2.5) was used for the separations under acidic conditions. The buffer was prepared by mixing 50 m*M* stock solutions of phosphoric acid and sodium dihydrogen phosphate. The 60 m*M* AMPD (2-amino-2-methyl-1,3-propanediol) buffers were prepared by dissolving the correct amount of AMPD and adjusting pH with 1 *M* hydrochloric acid or 1 *M* phosphoric acid. To achieve a sieving effect various amounts (1–10%, w/w) of dextrans differing in molecular mass were added to the background buffer. The electric field strength was kept at 300 V/cm unless reported otherwise. Standards of known molecular mass were used. To standardize for migration time fluctuations, monomolecular cationic (4-aminopyridine) and anionic (naphthalene-1,5-disulfonic acid) solutes were used as internal standards. All samples and buffer substances were dissolved in purified water (Milli-Q, Millipore, Eschborn, Germany).

The samples were introduced hydrodynamically at 0.5 p.s.i. (35 mbar) for 5–30 s, depending on the buffer viscosity. The capillary was rinsed for 3–5 min with running buffer, prior to analysis. Before the first use, the fused-silica capillaries were pretreated by rinsing with 1 *M* NaOH, water and running buffer for 15 min each. The PVA coated capillaries were rinsed with methanol instead of sodium hydroxide.

2.5. Samples

The poly(styrene-4-sulfonates) were purchased from Pressure Chemical (Pittsburgh, PA, USA). The native poly(2-vinylpyridines) were purchased from Polymer Standards (Mainz, Germany). The modified poly(2-vinylpyridines) were kindly provided by Professor Manfred Schmitt (Institute of Physical Chemistry, Johannes Gutenberg Universitaet, Mainz, Germany).

3. Results and discussion

3.1. Separation of anionic polyelectrolytes

PSSs are linear anionic polymers, bearing a constant mass-to-charge ratio, comparable to DNA. As they are strong polyelectrolytes, they can be analyzed both under alkaline as well as acidic conditions. They can be separated in a counter- or coosmotic-mode as well as in coated capillaries with suppressed EOF.

3.1.1. Separations under alkaline conditions

In the pH range of 8–9 the electrophoretic mobilities of the PSS are slightly higher than that of the oppositely directed EOF. When working under CZE conditions in counterosmotic mode all the PSS can be detected at the anodic end of the capillary. The addition of the sieving polymer hardly affects the EOF but leads to hindered migration of the polymer molecules. The electrophoretic velocity of the various polymers is reduced significantly for the samples with a molecular mass higher than a certain threshold, while for smaller molecules a minor influence is noticed. The polarity has to be switched to cathodic end detection, because the EOF velocity is higher now than the intrinsic sample mobilities. For the smallest polymer molecules, (M_r<5000) which interact only very weakly with the sieving matrix, the electrophoretic mobility is still higher than the EOF mobility, so they cannot be detected simultaneously with the higher mass polymers.

However, using coated capillaries with suppressed EOF, all PSSs can be detected at the anodic end simultaneously. A separation of standards with the Beckman e-Cap 200 system for SDS-proteins is shown in Fig. 2. As can be seen a good separation over three magnitudes of molecular mass can be achieved easily. The signals for the single standards are certainly not as narrow as those observed for DNA fragments due to the polydispersity of the

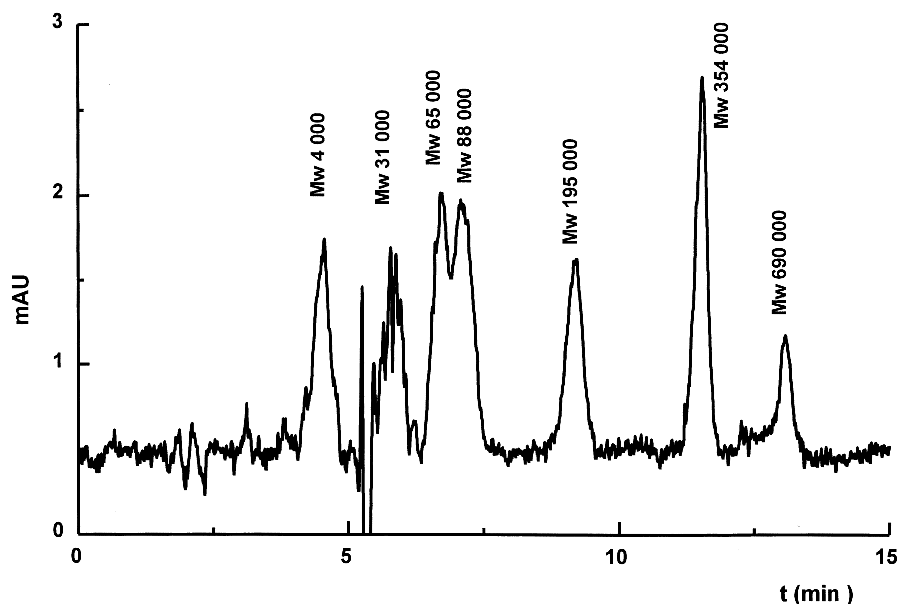

Fig. 2. Separation of PSSs under alkaline conditions. Capillary: Beckman e-Cap 27 cm (20 cm effective length)×100 μm I.D.; buffer: 60 m*M* AMPD-cacodylic acid, 10% (w/v) dextran T 2000, 0.1% SDS; Injection: 5 kV, 5 s; Detection: 254 nm.

investigated synthetic polymers, considering mass and/or charge distribution. This demonstrates on the other hand the advantage of the system for the determination of sample polydispersity.

3.1.2. Separations under acidic conditions

When working under acidic conditions uncoated capillaries can be used which simplifies the instrumentation. PSSs as strong electrolytes are already widely dissociated at a pH of 2.5. At this pH the EOF is negligible. It is not to be expected that wall interactions are likely, consequently it is possible to work with bare fused-silica capillaries. Separations were carried out in 50 m*M* phosphate buffers containing different amounts of dextrans of different average molecular masses. Fig. 3 shows the resulting electropherograms for separations under identical conditions except the variation of the dextran molecular mass. A separation over a range of three magnitudes of molecular mass could be achieved in both cases, but the resolution for the slower migrating high-molecular-mass polymers increases dramatically when the dextran molecular mass has been increased to $2\cdot10^6$ (Fig. 3b).

The relative migration of the polymer shows the same sigmoidal curves observed in the separation of DNA fragments. As can be seen in Fig. 4, the higher the molecular mass of the sieving polymer, the stronger is the dependence of the relative migration of the standards on their size. Polymer sieving in solutions of linear polymers occurs due to interactions between the polymer chains and the analytes. Very effective DNA separations can be achieved when the polymer concentration exceeds the entanglement threshold, when a physical network is formed [11]. This threshold depends on the sieving polymer structure and chain length and is about 0.5% w/w for dextran T2000 (M_r=2 000 000). With the mid-sized dextran of a molecular mass of 70 000 this threshold is not reached yet with the dextran concentration applied in our separations [11]. Nevertheless separations could be achieved for PSSs. DNA separations are also possible at polymer concentrations far below the entanglement threshold.

In Fig. 5 the influence of the sieving polymer concentration on PSS migration is shown. Increasing the polymer concentration leads to an improvement of resolution for a given molecular mass range. The reason for this improvement is the increasing density

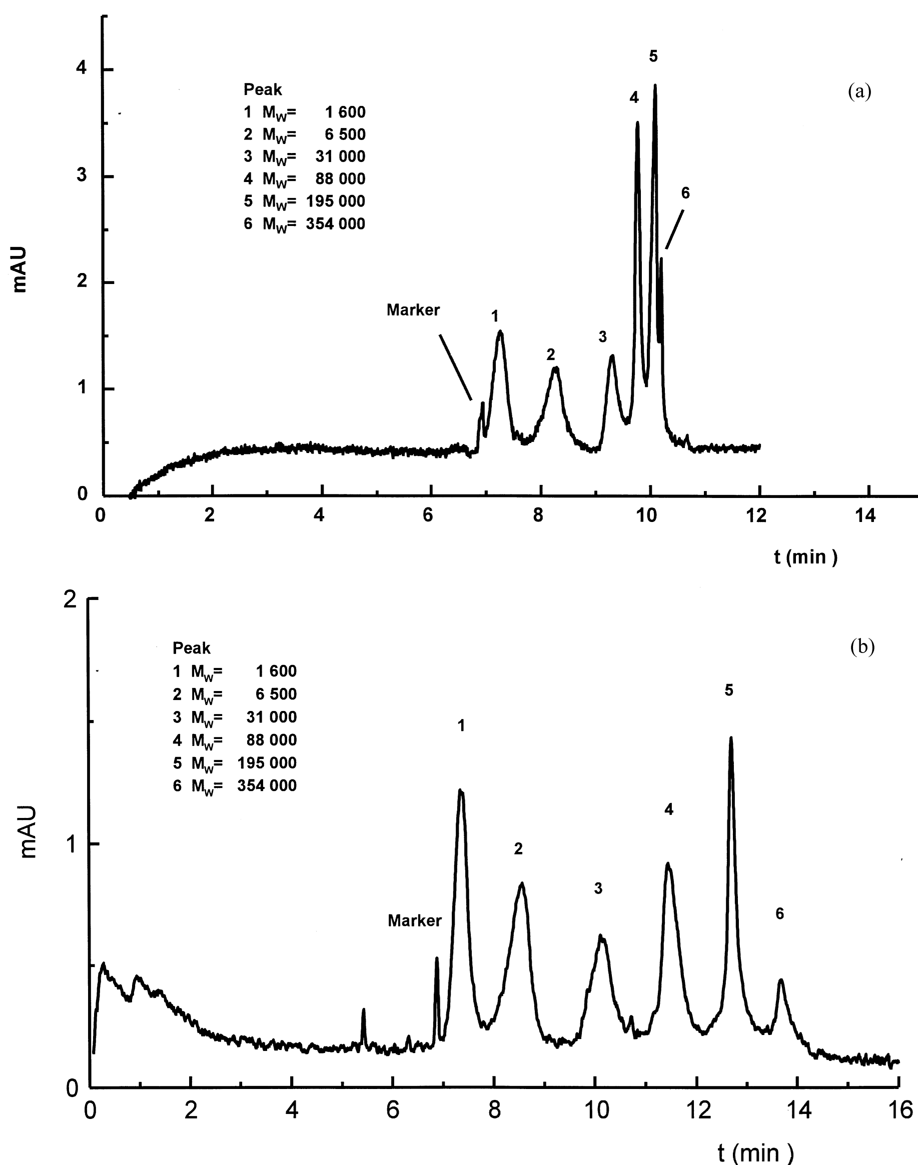

Fig. 3. (a) Separation of PSSs in dextran T 70 (3%). Conditions: capillary: fused-silica 57 cm (50 cm effective length)×75 μm I.D.; buffer: 50 m*M* phosphate (pH 2.5)+3% dextran T 70; U=−17.1 kV; injection: 15 s (0.5 p.s.i.; 1 p.s.i.=6894.76 Pa); detection: UV, 254 nm; marker: naphthalene-1,5-disulfonate. (b) Separation of PSSs in dextran T 2000 (3%). Conditions: buffer: 50 m*M* phosphate (pH 2.5)+3% dextran T 2000; capillary and other conditions as in (a).

of polymer chains per unit buffer volume which are hindering the migration of the analytes.

3.2. Separation of cationic polyelectrolytes

Under acidic conditions poly(2-vinylpyridines) are protonated and behave like cationic polyelectrolytes. The phosphate buffer system described above can be used for the characterization of those polycations successfully, as demonstrated in Fig. 6, but as these analytes interact very intensively with the fused-silica surface, coated capillaries have to be used here

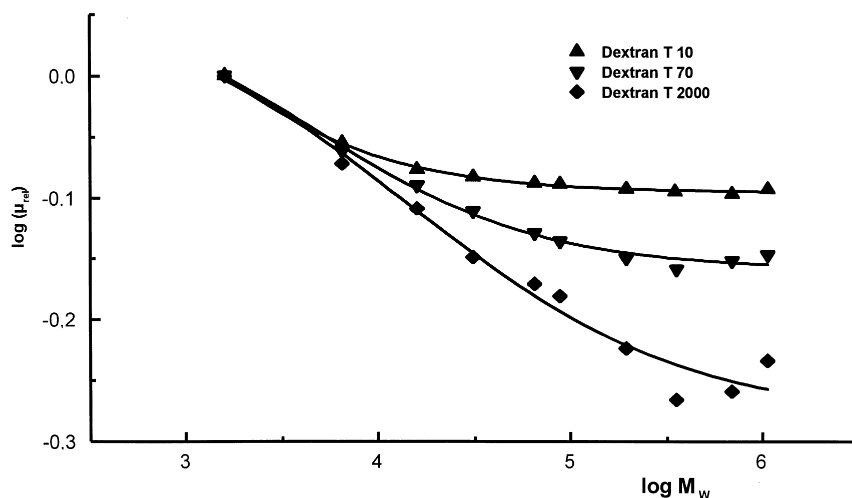

Fig. 4. Dependence of the separation of PSSs on dextran molecular mass. Conditions: capillary: fused-silica 57 cm (50 cm effective length)×75 μm I.D.; buffer: 50 m*M* phosphate (pH 2.5)+3% dextran; $U=-17.1$ kV; injection: 5 s (0.5 p.s.i.); detection: UV, 254 nm; marker: naphthalene-1,5-disulfonate.

and the polarity has to be switched back to cathodic end detection.

A separation over four magnitudes of molecular mass could be achieved, starting from 1480 to 1 700 000. The PVPs exhibit the same migration behavior as the PSSs in the sieving matrix and the

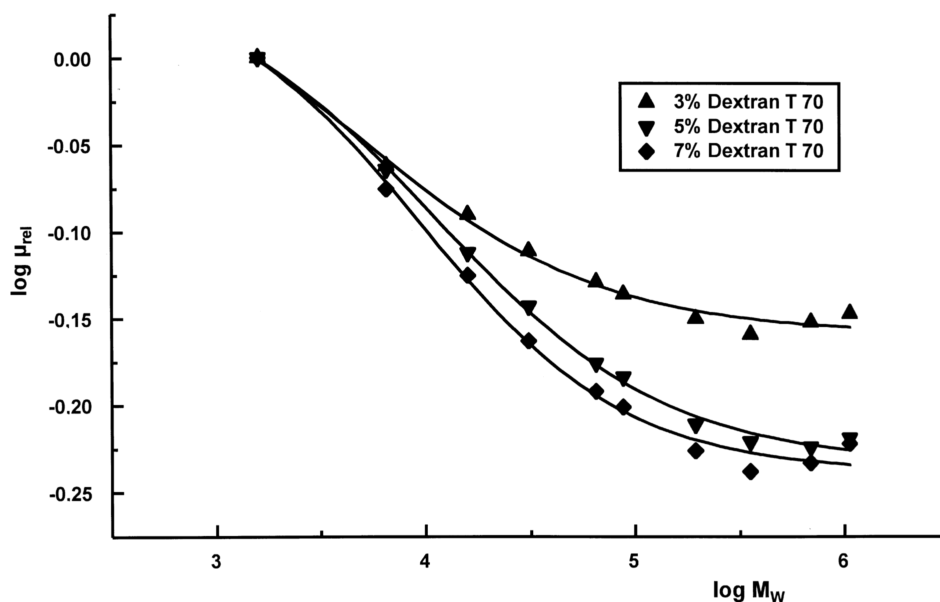

Fig. 5. Dependence of the separation of PSSs on dextran concentration. Conditions: capillary: fused-silica 57 cm (50 cm effective length)×75 μm I.D.; buffer: 50 m*M* phosphate (pH 2.5)+X% dextran T70; $U=-17.1$ kV; injection: hydrodynamic 0.5 p.s.i.; detection: UV, 254 nm;

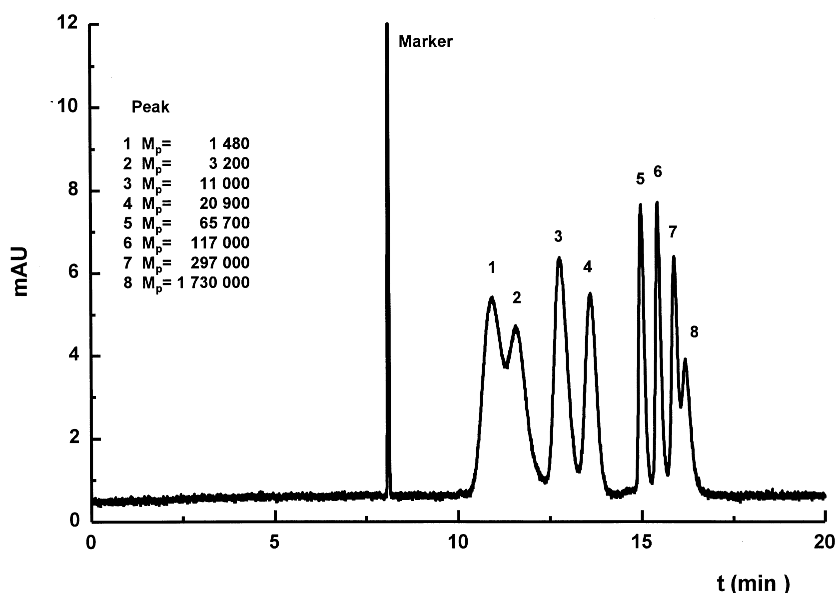

Fig. 6. Separation of PVP standards in dextran T 70 (5%). Conditions: capillary: PVA/VTCS 57 cm (50 cm effective length)×75 μm I.D.; buffer: 50 m*M* phosphate (pH 2.5)+5% dextran T70: E=300 V/cm; Injection:10 s (0.5 p.s.i.); Detection: UV, 254 nm; marker 4-aminopyridine.

same dependence of the separation on sieving polymer chain length and concentration can be recognized (Fig. 7).

In Fig. 8 the separation of differently alkylated PVPs is shown. These solutes have been derived from the same polymer by alkylation with ethyl- and benzyl-bromide. This demonstrates the possibility of separating synthetic polymers, not only on the basis of differences in backbone chainlength, but also according to side chain modifications in entangled polymer solutions.

4. Conclusions

A separation technique for both the separation of anionic and cationic synthetic polyelectrolytes has been developed. The mass selectivity ranges from M_r 1000 to 1 000 000. It can be adjusted by the proper selection of the sieving polymer chain length and concentration. Dextrans proved to be an optimal sieving medium for the separation of synthetic polyelectrolytes combining excellent water solubility at room temperature with good UV translucence. While anionic polyelectrolytes can be separated in bare fused-silica capillaries the use of coated capillaries is obligatory for the separation of cationic polymers to avoid wall adsorption of the samples. The problems occurring in gel permeation chromatography are of no importance in the CGE method. The method can be adapted to other polymer samples easily.

The capability of the system is not limited to linear polymers with different chain length. To avoid system breakdown the electric field strength should be kept at about 300 V/cm. Higher field strengths do not lead to increased resolution as Joule heating and the tendency to irregular migration increase with higher field strengths.

Acknowledgements

We appreciate the financial support of this work by the Deutsche Forschungsgemeinschaft (DFG) in Bonn-Bad Godesberg.

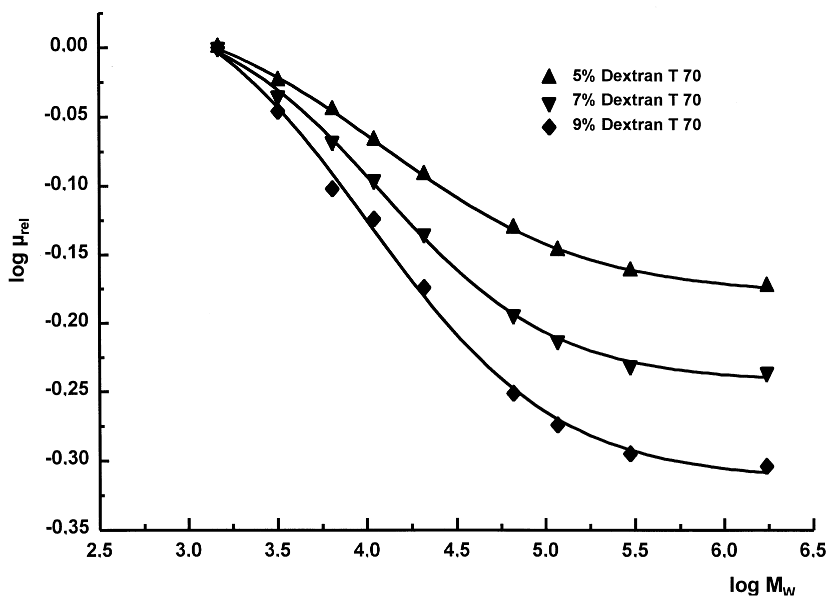

Fig. 7. Dependence of the separation of PVPs on dextran concentration. Conditions: capillary: VTCS/PVA 57 cm (50 cm effective length)×75 μm I.D.; buffer: 50 m*M* phosphate (pH 2.5)+X% dextran T70; samples: poly(2-vinylpyridines) U=17.1 kV; Injection: hydrodynamic 0.5 p.s.i.; Detection: UV, 254 nm.

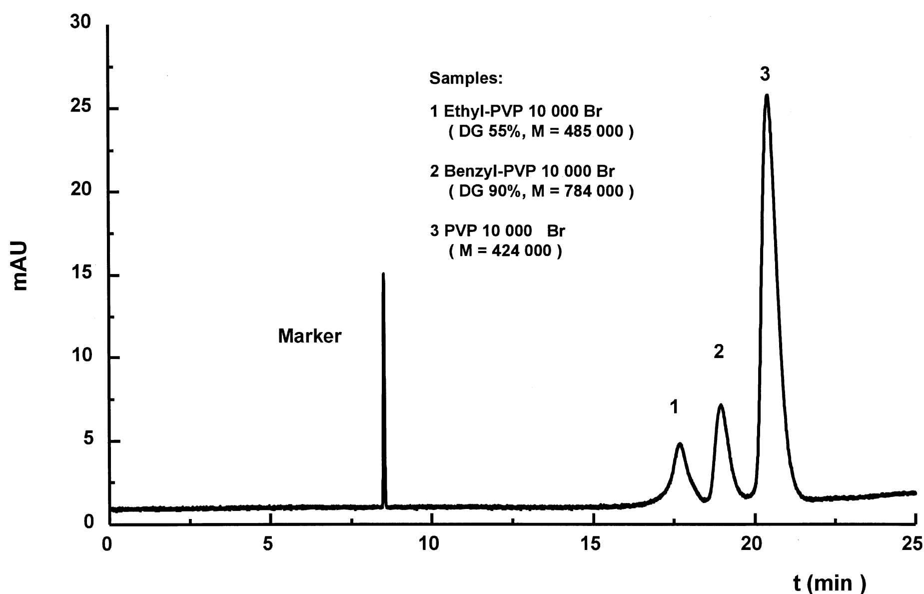

Fig. 8. Separation of PVP-derivatives of identical contour length. Conditions: capillary: VTCS/PVA 57 cm (50 cm effective length)×75 μm I.D.; buffer: 50 m*M* phosphate (pH 2.5)+7% dextran T70; E=300 V/cm; Injection: 20 s (0.5 p.s.i.); Detection: UV, 254 nm; marker 4-aminopyridine.

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