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Mixed polymer networks in the direct analysis of pharmaceuticals in urine by capillary electrophoresis

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Abstract

Two-component polymer mixtures of polyethylene oxide–polydextran have been investigated as unique separation media for capillary electrophoresis. The effects of concentration of the individual polymers and their mixtures on the electroosmotic velocity and electrophoretic mobility of small pharmaceutical compounds were investigated. The molecular masses of polymers, buffer concentrations and percentages of organic solvents and cyclodextrins were varied to explore their effects on the separation process.

The plate height against field strength curves were also generated for a better understanding of the kinetic processes involved. The two-component polymer mixtures were found as stable and selective media for the analysis of an anti-ulcer drug famotidine directly in untreated urine.

1. Introduction

A direct analysis of low-level pharmaceuticals and their metabolites in physiological fluids, such as plasma or urine, presents an important and methodologically challenging problem. To ascertain specificity of such determinations, extensive sample purification is often necessary, but this happens only at the expense of potential sample losses and methodological complexity. Another unfortunate attribute of the extensive removal of a biological matrix is frequently the removal of structurally related metabolites from the parent drug [1]. Yet, biological matrices are complex, containing numerous compounds that may directly or indirectly interfere with the analytes of interest. Urine, for example, contains a large number of organic acids, amines, bile acids, porphyrins, etc., in addition to various electrolytes.

Due to the recent popularity of electromigration techniques in biomedical analysis, high-performance capillary electrophoresis (HPCE) has been increasingly employed in analyzing endogenous urinary components [2,3] and excreted pharmaceuticals [4–6], either directly or after sample fractionation. The most popular HPCE separation mode seems to be micellar electrokinetic capillary electrochromatography (MECC), which utilizes the solubilizing properties of detergents for a desirable removal of interfering compounds from the "analytical window" for the analytes of interest. Still, this approach is not generally applicable and effective in various types of analytical problems. During the search for a more general approach to the direct HPCE analysis of pharmaceuticals in urine, we started to investigate various poly-

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mer matrices dissolved in typical buffer media and came across the area of mixed polymer systems. Such systems appear to exhibit unusual properties that could potentially lead to substantial improvements in the HPCE analysis of low-level pharmaceuticals. The present report describes the initial observations of the related phenomena.

Single-component polymer networks, also referred to as "entangled polymer matrices", have recently become popular in the HPCE separations of biopolymers such as oligonucleotides [7,8], proteins [9,10] and polysaccharides [11]. The sample component separation is believed to take place through sieving in the size-dependent fashion inside the "dynamic pores" of the physically entangled polymeric network. Such polymer solutions typically represent UV-transparent and relatively non-viscous media, which can be easily replaced in the separation capillary after each analytical run. Linear polyacrylamide, polydextran and modified cellulose media have been thus far confined to the biopolymer applications, although small quantities of polymer additives to the HPCE buffers can also aid in suppressing electroosmosis at the capillary wall [12,13] in all applications of interest.

The present study is primarily concerned with two-component polymer matrices which are somewhat analogous to the systems pioneered by Albertsson [14,15] for fractionating macromolecules and biological particles at a preparative scale. In this type of work, aqueous solutions of the relatively low-molecular-mass polydextrans and polyethylene glycols are typically used. At certain ratios and temperatures, such systems may become immiscible, generating distinct interface layers and partitioning media. Under a different set of conditions, the polymers form a homogeneous mixture. The multiple-phase systems have been characterized thermodynamically [15]. To our knowledge, two-component polymer matrices have not previously been applied in CE.

We demonstrate here that a two-component polymer matrix can offer a desirable medium in terms of the effective analysis of small drug molecules, sampled from unfractionated urine samples. Using a two-phase system of polyethylene oxide–polydextran (PEO–PD), a direct analysis of an anti-ulcer drug famotidine is shown at a therapeutically relevant level. Further modification of the system's selectivity has been possible through the use of appropriate additives such as organic solvents and cyclodextrins.

2. Experimental

2.1. Instrumentation

Laboratory-built CE equipment was used in all experiments. The system consisted of a Jasco (Tokyo, Japan) Uvidec 100-IV UV absorbance detector set at 220 nm. The high-voltage power supply was from Spellman High Voltage Electronics (Plainview, NY, USA). Voltages of 20–22 kV (negative or positive ground) were used. Uncoated capillaries (50 and 75 μm I.D., 200 μm O.D., 64 cm long with 48 cm effective length) were obtained from Polymicro Technologies (Phoenix, AZ, USA). Sample introduction was performed hydrodynamically by creating a 20-cm height difference between the buffer reservoir and the sample levels.

2.2. Chemicals

6-Aminocaproic acid, adipic acid, PDs (average M_r of 18 300, 39 000, 73 000, 267 000, 515 000 and 525 000) and heptakis(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD) were received from Sigma (St. Louis, MO, USA). PEOs (average M_r of 100 000 and 300 000) and tetrahydrofuran were products of Aldrich (Milwaukee, WI, USA). Methanol and 2-propanol (Photrox grade) were purchased from J.T. Baker (Phillipsburg, NJ, USA). Acetonitrile (HPLC grade) was from Fisher Scientific (Fair Lawn, NJ, USA).

2.3. Pharmaceuticals

The test mixtures contained several different pharmaceutical compounds (see Fig. 1). All their pK_a values were close to neutral; cimetidine,

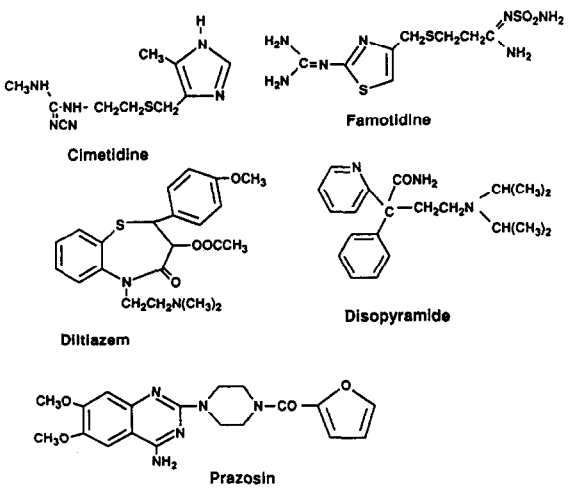

Fig. 1. Chemical structures of the model compounds.

famotidine, disopyramide and prazosin are listed in an increasing hydrophobicity order. In the demonstration of chiral separations, racemic pharmaceuticals nitrendipine, pindolol and disopyramide were used. All test compounds were a gift from Orion-Farmos Pharmaceuticals (Espoo, Finland).

2.4. Urine sample preparation

Thawed urine was briefly centrifuged with a Fisher Scientific microcentrifuge. After spiking with drug solutions, 100 μl of urine were diluted with 200 μl of buffer (32 m*M* 6-aminocaproic acid/18 m*M* adipic acid, pH 4.5).

3. Results and discussion

The entangled polymer networks, which are now used extensively in HPCE of large biopolymers, are either linear or branched polymers that are dissolved in aqueous buffer solutions. A certain critical concentration of these polymers is viewed as essential for forming the "dynamic pores" [16] for the biomolecular solutes. These polymer solutions have typically low or medium viscosity (<6 cP). Other than controlling the electroosmosis through the viscosity effect or modifying the capillary wall, such polymer solutions have no foreseen benefits for the separations of small molecules.

The study presented here has utilized polymer binary mixtures in buffer solutions: PEOs and PDs, at different concentrations, were employed. PEO is a linear, neutral polymer with a dihedral helical structure [17], while PDs are branched polysaccharides that are more hydrophilic than PEOs. The main linkage between glucopyranose residues is (1-6)-α-D-glucan, but to a variable extent, other linkages may occur, with the (1-3)-α-D-branched species being dominant [18]. Both polymers are typically polydisperse, consisting of an array of polymer fragments with different molecular masses.

We shall start discussion of our results with the separation of model pharmaceuticals in different polymer solutions (Fig. 2). When 5% PD solution was used as the buffered matrix, the four components of the test mixture (cimetidine, famotidine, diltiazem and prazosin) separated only marginally from each other (Fig. 2A), under the conditions of a strong electroosmotic flow. (Without a polymer addition, practically no separation could be achieved, with all components emerging in the same zone.) When PEO alone was used in the buffer solution at 0.02% concentration, a substantial improvement in resolution was achieved, while electroosmosis was slowed down considerably (Fig. 2B). When both polymers were present (Fig. 2C), very substantial improvements in selectivity and resolution were realized, resulting in the additional separation of two drug degradation products (designated as F′ and D′). Electroosmosis was negligible ($<0.4\cdot10^{-5}$ cm^2 V^{-1} s^{-1}) under these conditions. This resolution improvement cannot be readily explained by the additive effects of PEO and PD, or the change in electroosmosis alone, but rather points to formation of a qualitatively different separation matrix. Consequently, we proceeded to study variations of this experiment: electromigration under different polymer concentrations and molecular masses, buffer concentration, organic additives, and band-broadening phenomena under different experimental conditions.

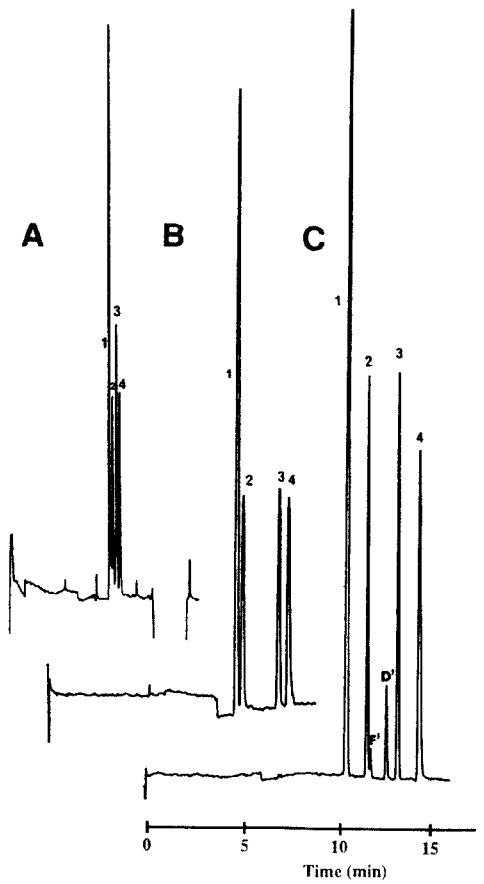

Fig. 2. Effect of polymeric additives in the same buffer [32 m*M* 6-aminocaproic acid/18 m*M* adipic acid, pH 4.5/5% (v/v) methanol]. (A) 5% (w/w) Dextran, M_r 18 300; (B) 0.02% (w/w) PEO, M_r 300 000; (C) 5% (w/w) dextran (M_r 18 300) and 0.02% (w/w) PEO (M_r 300 000). Capillary: 64 cm (48 cm effective length) × 75 μm I.D.. Run voltage, 22 kV; UV detection at 220 nm, 0.01 AUFS. Analytes: 1 = cimetidine; 2 = famotidine; F' = degradation product of famotidine; 3 = diltiazem; D' = degradation product of diltiazem; 4 = prazosin.

3.1. Migration in single-polymer matrices

When comparing the effects of polymer addition in one-phase systems on electroosmosis, both PEO and PD decreased the electroosmotic effects on untreated fused silica, but did so to a different degree. In addition, the polymers with different molecular masses differed in this capacity (results not shown). PDs of different molecular masses (18 300, 73 000 and 267 000) exhibited a different effect on electroosmosis. In the buffer of 32 m*M* 6-aminocaproic/18 m*M* adipic acid (pH 4.5), the smallest PD needed a 15% concentration to reduce substantially electroosmosis, while for the two larger PDs at 10% concentrations, electroosmosis between $15 \cdot 10^{-5}$ and $30 \cdot 10^{-5}$ $cm^2\ V^{-1}\ s^{-1}$ was still visible. In contrast, already 0.08% (w/w) PEO (M_r 100 000) suppressed electroosmosis as effectively as 5% PD with M_r 18 300. Once again, the polymer size did make a difference with PEO in this capacity: M_r 100 000 was more effective in decreasing electroosmosis than M_r 300 000. Using M_r 100 000 PEO, suppression of electroosmosis was practically complete at 0.20% polymer addition (results not shown). During the initial electroosmotic measurements using the same polymers at different concentrations, only successive measurements were made (to avoid long equilibration times), interrupted by washing the capillary with distilled water for 60 min before the measurement series with a new buffer. Between different types of polymer buffers, the capillaries were flushed overnight with aqueous 0.1 *M* sodium hydroxide. Success of rinsing was verified through the test solution using the buffer without polymer additives. Equilibration times for a new polymer solution were permitted for at least 1 h under the conditions of applied voltage. Consecutive injections of the test sample verified the system's stability.

A greater effectiveness of smaller polymers in decreasing the negative charge on the capillary surface is in agreement with the results of Hommel et al. [19] who studied PEO adsorption dynamics on a silica surface: the process favors kinetically smaller molecules, and displacement of the surface-adsorbed polymers by high-M_r chains moving into the diffuse layer can be quite slow. Adsorption isotherms, which parallel the blockage of surface charge, are expected to be quite dependent on the polymer polydispersity.

When the used buffer contained a single polymer in different concentrations, electrophoretic mobilities differed substantially. In Fig. 3 (A vs. B), the electrophoretic mobilities for cimetidine and prazosin (the most hydrophilic

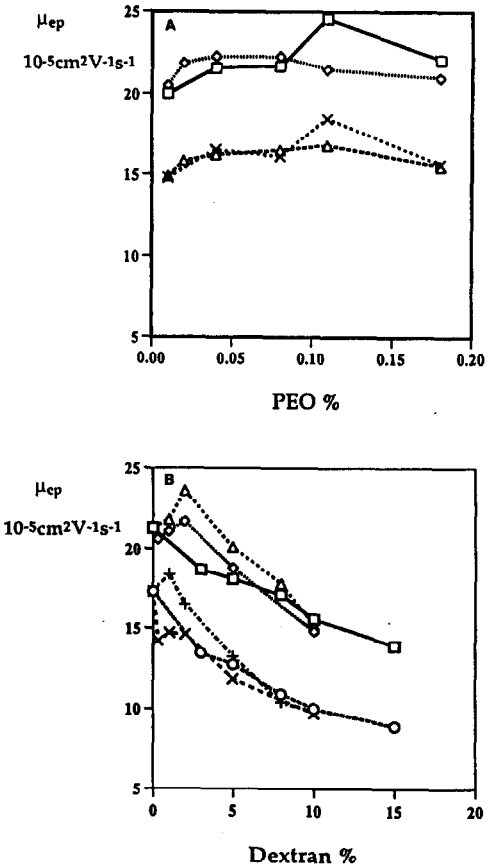

Fig. 3. (A) Effective mobilities (μ_{ep}) of cimetidine and prazosin as a function of PEO percentage (w/w) for the polymers of M_r 100 000 (□ = cimetidine, × = prazosin) and M_r 300 000 (◇ = cimetidine, △ = prazosin) in the buffers containing 32 m*M* 6-aminocaproic acid/18 m*M* adipic acid, pH 4.5. Other analytical conditions as in Fig. 2. (B) Effective mobilities of cimetidine and prazosin as a function of PD percentage (w/w) for the polymers of M_r 18 300 (□ = cimetidine, ○ = prazosin), M_r 73 000 (◇ = cimetidine, × = prazosin) and M_r 267 000 (△ = cimetidine, + = prazosin) in the buffers containing 32 m*M* 6-aminocaproic acid/18 m*M* adipic acid, pH 4.5. Other analytical conditions as in Fig. 2.

and hydrophobic solute, respectively, from our test mixture) are plotted against the polymer concentrations. The most pronounced decrease in mobilities was observed with PDs with increasing polymer concentration. However, for all studied dextrans, mobilities were roughly identical at 10% (w/w), suggesting primarily the effect of increased viscosity in control of migration. Increasing concentrations of PEO (up to 0.20%) had very little effect on mobilities of both analytes.

3.2. Two-component polymer solutions

Phase-separated, aqueous polymer mixtures are known to be "soft" and non-denaturing media used in the selective extractions of biopolymers. The larger the molecular mass difference between the two polymers, the lower polymer concentrations are necessary for the phase separation to occur. Besides this molecular mass effect, temperature and solution additives may influence the phase separation conditions. Interface between the polymer phases features a low surface tension and may accommodate emulsion droplets. The mixtures such as PD–PEO or PD–polyethylene glycol, at certain transition concentration, qualify well [15] for such two-phase partitioning systems. While the solutions of polymers used to fill the electrode reservoirs in our experiments do appear as visibly homogeneous, the phase separation under electric field, inside the separation capillary, cannot be ruled out, and, in fact, is quite likely. The thermodynamic basis for polymer de-mixing is entropy-gaining, when the large polymers interact with their own kind rather than with other polymer solutes in the mixture [15].

When the PD and PEO polymers were first added to the same electrophoretic buffer, an increased migration window was experienced between the most hydrophilic (cimetidine) and hydrophobic (prazosin) solute for an increasing PD concentration (see Fig. 4). This selectivity gain appears quite obvious for such small, weakly basic solutes of approximately the same molecular mass, each carrying one positive charge.

For untreated fused-silica capillary wall, dynamic conditions at the surface require consideration. While both PD and PEO can interact with the hydrated silica structure, PEO exhibits considerably greater affinity (hydrogen bonding) to the capillary wall. However, the surface binding is known to be a dynamic process allowing a polymer layer rearrangement and slow replacement [19]. Wall treatment with PEO (M_r

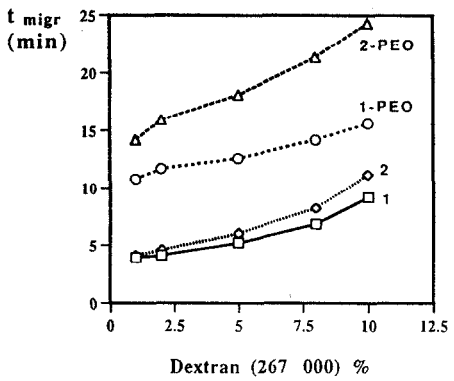

Fig. 4. Migration window between the migration times (t_{migr}) of cimetidine (1-PEO) and prazosin (2-PEO) vs. dextran M_r 267 000 percentage (%, w/w). Buffer: PEO 300 000 0.02% (w/w)/32 m*M* 6-aminocaproic acid/18 m*M* adipic acid, pH 4.5. Other analytical conditions as in Fig. 2. For comparison, cimetidine (1) and prazosin (2) migration times are shown in the buffer containing no PEO.

10 000–86 000) has been reported in HPCE [20] to aid separation of synthetic diamine oligomers. After untreated silica surface is exposed to a relatively weak solution of PEO, an adsorption plateau will be reached: Parnas et al. [21] reported a plateau value of 0.3 mg/m^2 (for M_r 400 000 PEO) on non-porous, untreated silica. The equilibrium situation was thus established already with 0.025% (w/w) solution in their experiment. In all our experiments, the amounts corresponding to PEO concentration were larger than 1.8 mg/m^2, assuming a complete monolayer coverage of the silica surface. After a short stabilization time, a PEO adsorption plateau should be reached in our system in the presence of a dextran polymer as well. The application of electric field probably assists the equilibrium process through organizing polymer orientation, as we have observed that the solute migration stability was achieved faster by applying electric field after a brief capillary rinse period between the runs, as compared to a rinse-only situation.

3.3. Solvation effects

The effects of buffer (6-aminocaproic acid/adipic acid) concentration or migration times of the test solutes were studied in the system of 5% (w/w) 18 300 PD–0.02% (w/w) 300 000 PEO. Within the range of 5–35 m*M* 6-aminocaproic acid concentration, migration times for all pharmaceuticals increased slightly. Repeatability of migration time measurements was most favorable at roughly 15 m*M* concentration (0.6% R.S.D., $n = 5$). The observed slight increases (around 3–6%) of migration times at higher concentrations may be due to a viscosity increase.

Acetonitrile, 2-propanol, tetrahydrofuran and methanol were tested as the buffer additives, in concentrations ranging from 0.5–5% (v/v), in the system of 3% PD (M_r 267 000)–0.05% PEO (M_r 300 000), at pH 4.95. The solvent addition generally improved the peak symmetry. Both the electroosmotic flow and solutes' electrophoretic mobilities were affected through addition of solvent modifiers (Figs. 5 and 6).

Except for methanol, all solvents increased electroosmosis, with 2-propanol and acetonitrile being most potent (Fig. 5). Interestingly, the behavior of our mixed-polymer system is very different from the phenomena observed in MECC, where organic solvents are known [22] to change the migration of small organic mole-

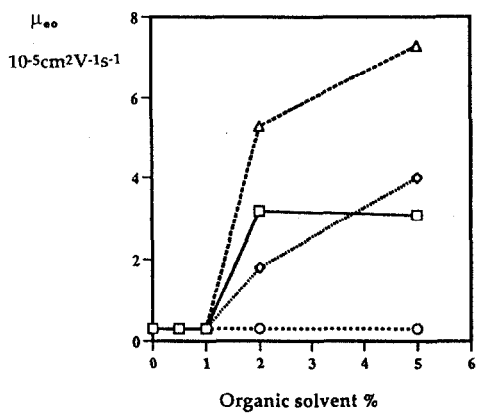

Fig. 5. Effect of organic solvents on electroosmotic flow (μ_{eo}). Solvent additions (%, v/v) in the buffer containing 37 m*M* 6-aminocaproic acid/6 m*M* adipic acid (pH 4.95)/3% (w/w) dextran 267 000/0.05% (w/w) PEO 300 000. Other analytical conditions as in Fig. 2. Solvents: □ = tetrahydrofuran; ◇ = acetonitrile; ○ = methanol; △ = 2-propanol.

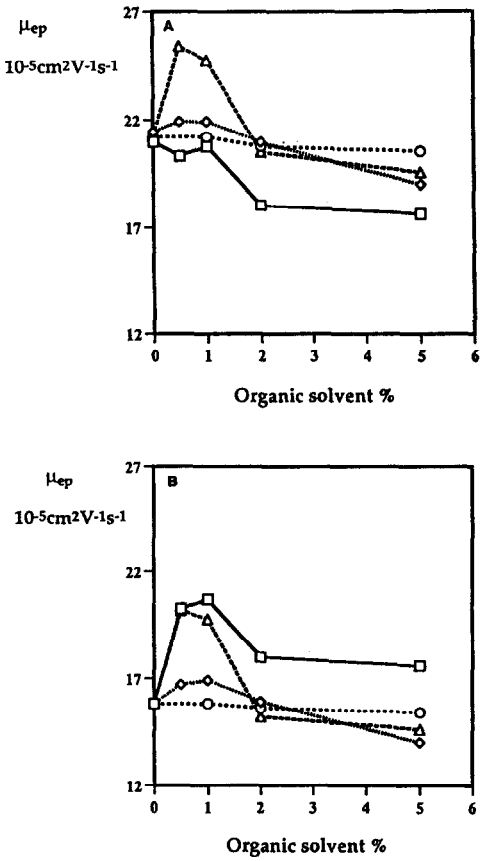

Fig. 6. Effect of organic solvents on mobilities of (A) cimetidine and (B) prazosin. Analytical conditions as in Fig. 5. Solvents: □ = tetrahydrofuran; ◇ = acetonitrile; ○ = methanol; △ = 2-propanol (△).

cules dramatically while the electroosmotic flow remains relatively stable.

Various solvation phenomena that may influence polymer swelling and displacement at the wall can be quite complex to begin with. Additionally, in the two-phase polymer extraction systems, various organic solvents have been reported [23] to lower dramatically the phase-separation transition concentrations of polymer components, with acetonitrile reportedly being most effective.

In the experiments shown here (Fig. 6), the organic modifiers may change the probability ratio [19] of the physically adsorbed polymers and the polymers moving freely in the buffer solution. Acetonitrile and 2-propanol appear most effective in solvating the adsorbed polymer layers. Additionally, they may modify the effective charge of the test solutes.

3.4. Kinetic considerations

What is the prevalent separation mechanism for these solutes in our mixed-polymer system? Does it resemble capillary zone electrophoresis in gel media, or is it more like electrokinetic chromatography? These questions can potentially be answered through measuring the band-broadening effects as a function of the separation velocity (applied voltage) [24]. Attempts have been made here to clarify these phenomena through generating a series of plate height vs. voltage curves for (a) different solutes; (b) two different column diameters (50 and 75 μm I.D.); and (c) different polymer media. Examples of such kinetic plots are shown in Figs. 7–9.

In both 50- and 75-μm capillaries with mixtures of PEO and PD, the plate heights gradually decreased, with voltages up to 500 V/cm, for cimetidine and famotidine (Figs. 7 and 8). However, for the more hydrophobic prazosin, a strong tendency toward increasing the plate height was observed at about 400 V/cm (22 kV

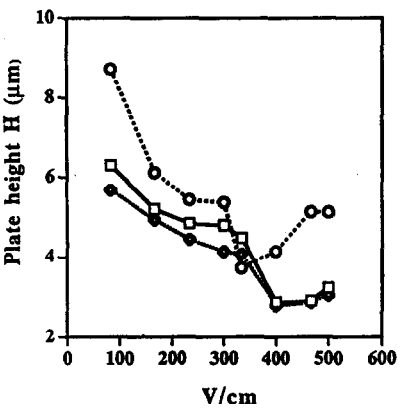

Fig. 7. Plate heights for cimetidine (□), famotidine (◇) and prazosin (○) measured against the electric field strength with a 60 cm × 50 μm I.D. capillary. Buffer system: 32 m*M* 6-aminocaproic acid/18 m*M* adipic acid, (pH 4.5)/5% (w/w) dextran 525 000/0.05% (w/w) PEO 300 000.

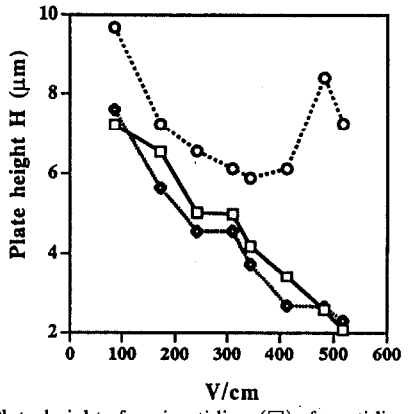

Fig. 8. Plate heights for cimetidine (□), famotidine (◇) and prazosin (○) measured against the electric field strength with a 60 cm × 75 μm I.D. capillary. Buffer system as in Fig. 7.

power-supply setting). Under the optimum voltage conditions, the separation efficiencies increased with the molecular mass of PDs approaching roughly 500 000 theoretical plates/m. At a constant percentage (5%, w/w) of PDs, the higher polymers obviously formed a more rigid network minimizing the band dispersion. For cimetidine and famotidine in the mixed-polymer media, the observed curves are consistent with the efficiencies for small molecules measured in rigid gels [24], and correspond generally to the zone electrophoresis mode of separation. The

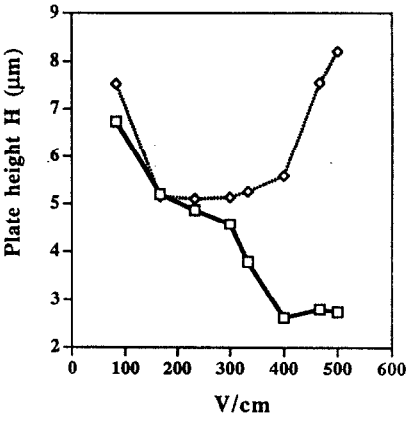

Fig. 9. Plate heights for famotidine in 60 cm (44 cm effective length) × 50 μm I.D. capillary against electric field strength. Run buffer as in Fig. 2; additives: ◇ = 5% (w/w) dextran 267 000 and □ = 5% (w/w) dextran 267 000/0.05% (w/w) PEO 300 000.

measured current–voltage characteristics for all media concerned (results not shown) were also in agreement with the expected thermal effects [24]. The observed band-broadening for prazosin can tentatively be explained by an interaction with the PEO polymer residing at the capillary wall (electrochromatographic separation principle).

When the plate height values were measured for famotidine in the buffer containing M_r 267 000 PD (Fig. 9), a rapid loss of efficiency in the absence of PEO became apparent. Simultaneously, a significant tailing of the famotidine peak seemed to indicate an undesirable interaction of the solute molecules with the bare silica wall. This observation verifies the role of PEO as a dynamic coating agent in the used mixed-polymer system. The apparent diffusion coefficient for cimetidine in the mixed-polymer system was estimated from the slope of its H vs. $1/E$ curve (H = plate height, E = field strength) to be $1.4 \cdot 10^{-7}$ cm^2 s^{-1}.

3.5. Preliminary analytical applications of the mixed-polymer systems

As suggested already in Fig. 2, the mixed-polymer media can form analytically useful separation systems of their own. It became of further interest to investigate whether such media are also compatible with cyclodextrins, now the commonly used buffer additives for enhanced separation selectivities. Selected separations of enantiomers in the mixed-polymer systems containing a cyclodextrin additive are shown in Fig. 10. These results are roughly comparable to our earlier investigations [25] in the ordinary aqueous buffers.

One of the model solutes used in this study has been famotidine. Famotidine, a relatively new and effective anti-ulcer drug [26], is of considerable pharmacological interest. The reported analytical methods for measuring famotidine in urine have been based on HPLC [27,28], necessitating sample preconcentration through solvent or solid-phase extraction. It became worth investigating whether the mixed-polymer media are

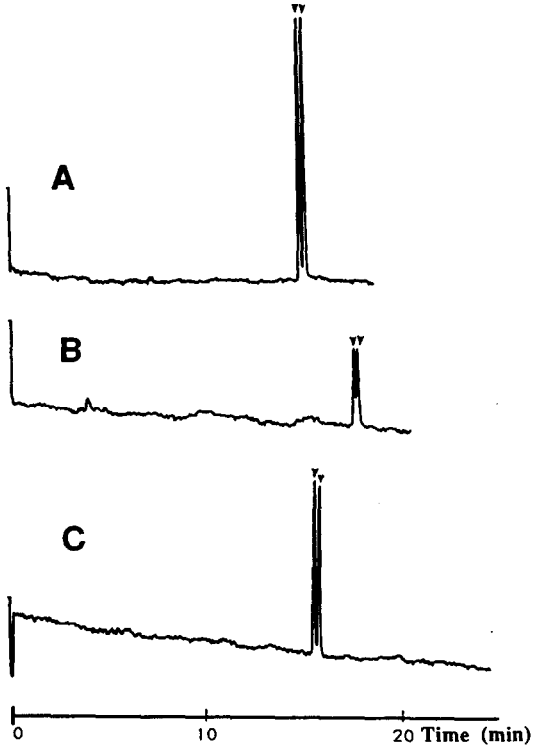

Fig. 10. Separation of the enantiomers (indicated by arrows) from their racemic mixtures: (A) pindolol; (B) disopyramide; (C) nitrendipine. Run buffer: 14 m*M* 6-aminocaproic acid/8 m*M* adipic acid (pH 4.5)/ 3% (w/w) dextran 39 000/0.05% (w/w) PEO 300 000/7.5 m*M* DM-β-CD/0.6% methanol. Other analytical conditions as in Fig. 2. Sensitivity 0.01 AUFS.

applicable to improvements in the analysis of urinary famotidine by HPCE.

After a brief optimization study of various additives, a medium containing 4% PD–0.04% PEO, with a cyclodextrin and methanol used as further additives (effective in adding selectivity to resolving famotidine from the urinary endogenous compounds), was selected as optimal for the urinary analysis. (Cyclodextrins were also reported to stabilize famotidine against degradation in acidic solutions [29].) Varying further the molecular mass of PDs (at a constant 4% concentration), the important analytical parameters, such as the precision of migration time, peak height reproducibility, separation efficiency, and linearity, were also evaluated. The results and shown in Table 1. Using the peak height ratios of famotidine and disopyramide, the coefficients of correlation for the linear regression calibration plots in the famotidine concentration of 0.5–30 μg/ml ($n = 7$) were 0.993–0.996. Generally, the use of larger PD polymers appears to result in improved reproducibility, separation efficiency and quantitation. Whereas the exact analysis of these effects would require additional studies, a more tightly packed polymeric network seems to have a generally favorable effect in stabilizing the system for analytical purposes.

With an increasing size of the PD molecules

Table 1
Precision, stability and efficiency of the famotidine analysis in urine with polydextran additives

Dextran M_r	Precision of injection, R.S.D. (%, $n = 5$)[a]	Precision of migration, R.S.D. (%, $n = 5$)[b]	Number of theoretical plates/m)[c]
18 000	6.6	2.2	180 000
39 000	2.7	1.2	210 000
73 000	2.3	0.8	220 000
515 000	1.7	0.9	550 000

[a] Famotidine and disopyramide peak-height ratios used in calculations.
[b] Migration time for famotidine.
[c] Measured for famotidine peak.

used in the analytically desirable system, an increased medium viscosity tends to decrease the sample uptake during the hydrodynamic injection. Fig. 11 shows the electropherograms obtained after sampling the urine specimens (containing famotidine at 10 μg/ml) for 2-, 5- and 9-min time intervals, using a 515 000 PD buffer. In spite of the long sampling times, the solute zones remained sharp, and migration times became shorter. The peak height increases for both famotidine and disopyramide remained linear for all injection times, with the correlation coefficient of 0.999. Under similar circumstances in a strictly aqueous buffer medium (without PD), already after 2-min injection the zones became very diffuse and the hint of enantioseparation for disopyramide was completely lost.

The serendipitously occurring on-column preconcentration of our analytes is presumably due to a large concentration of urinary salts and the negatively charged porphyrins which upon being introduced onto capillary simultaneously with the measured solutes behave as a "stacking material". After the voltage is turned on, the

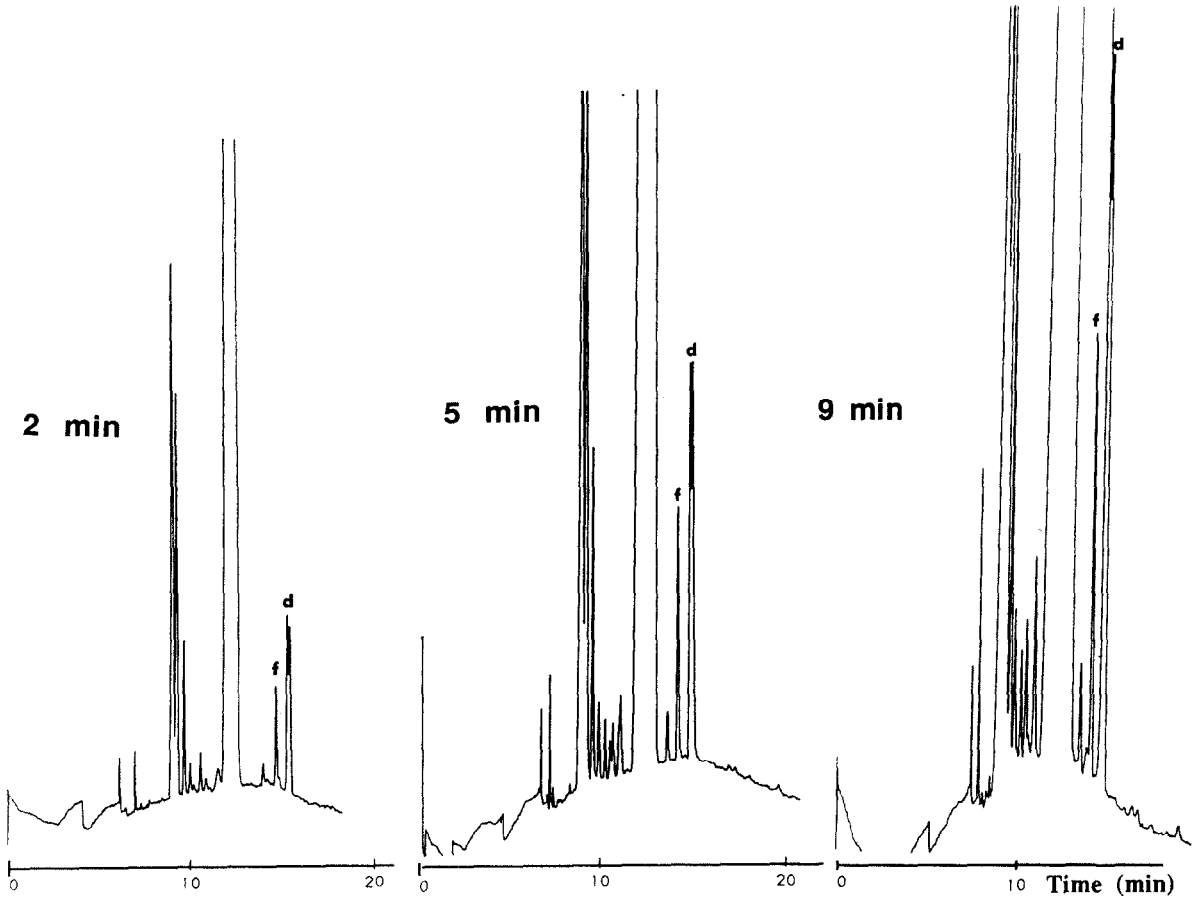

Fig. 11. Electropherograms of a directly sampled urine after hydrodynamic injection for 2, 5 and 9 min (height differential, $\Delta h = 20$ cm). Run buffer (pH 4.5) additives: 4% (w/w) dextran 515 000/0.04% (w/w) PEO 300 000/2% (v/v) methanol/7.5 m*M* DM-β-CD. Peaks: f = famotidine, 10 μg/ml; d = disopyramide, 20 μg/ml (internal standard). Other analytical conditions as in Fig. 2. Sensitivity 0.01 AUFS.

negatively charged species begin migrating in the opposite direction from the analytes, which form the sharp zones on positively charged species [30,31]. When a large plug of water was introduced, the local polymer equilibrium was disturbed without any stacking effect and the analyte zones became broader. When the injection time was kept to less than 60 s, identical migration times and peak heights were obtained for the same famotidine concentrations in urine or water. Fig. 12 shows comparison of a blank urine electropherogram (A) together with the runs of a famotidine-spiked urine (10 μg/ml) (B), and aqueous sample of the same concentration (C), after injection into a 73 000 PD–300 000 PEO medium for 45 s.

4. Conclusions

Two-component systems comprising PEO–PD solutions were found effective in separating small pharmaceuticals with high efficiency and separation selectivity. Additionally, such systems

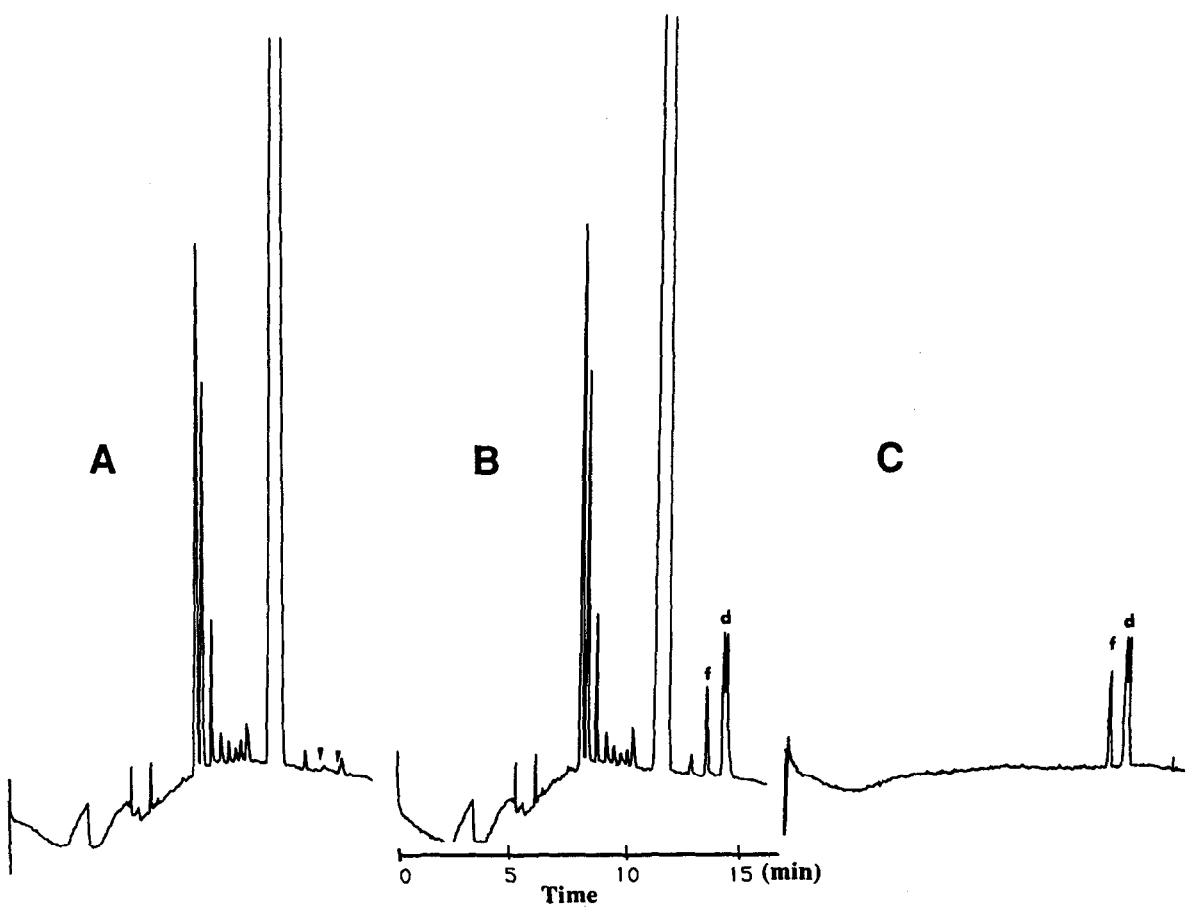

Fig. 12. Electropherograms of (A) directly injected blank urine, (B) urine sample with famotidine, 10 μg/ml [peaks: f = famotidine; d = disopyramide (internal standard)], (C) aqueous sample containing the compounds at the same concentrations as in B. Buffer (pH 4.5) additives: 4% (w/w) dextran 73 000/0.04% (w/w) PEO 300 000/2% (v/v) methanol/7.5 m*M* DM-β-CD. Hydrodynamic injection for 45 s (Δh = 20 cm). Other analytical conditions as in Fig. 2. Sensitivity 0.01 AUFS.

appear desirable in the direct analysis of complex samples, such as human urine. The analytical properties of these mixed-polymer separation media can further be modified through addition of organic solvents or cyclodextrins. The mixed-polymer systems described in this study have been briefly characterized in terms of polymer component concentration, solvent effects and kinetic behavior. The potential of mixed-polymer systems in the analyses of complex biological and environmental samples warrants further investigations.

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References

[1] R.D. McDowall, *J. Chromatogr.*, 492 (1989) 3.
[2] R. Weinberger, E. Sapp and S. Moring, *J. Chromatogr.*, 516 (1990) 271.
[3] M. Miyake, A. Shibukawa and T. Nakagawa, *J. High. Resolut. Chromatogr.*, 14 (1991) 181.
[4] S. Li, K. Fried, I.W. Wainer and D.K. Lloyd, *Chromatographia*, 35 (1993) 216.
[5] C.-X. Zhang, Z.-P. Sun, D.-K. Ling, J.-S. Zheng, J. Guo and X.-Y. Li, *J. Chromatogr.*, 612 (1993) 287.
[6] P. Lukkari, H. Siren, M. Pantsar and M.-L. Riekkola, *J. Chromatogr.*, 632 (1993) 143.
[7] M. Zhu, D.L. Hansen, S. Burd and F. Cannon, *J. Chromatogr.*, 480 (1989) 311.
[8] P.D. Grossman and D.S. Soane, *Biopolymers*, 31 (1991) 1221.
[9] K. Ganzler, K.S. Greve, A.S. Cohen, B.L. Karger, A. Guttman and N.C. Cooke, *Anal. Chem.*, 64 (1992) 2665.
[10] A. Guttman, J. Horvarth and N.C. Cooke, *Anal. Chem.*, 65 (1993) 199.
[11] J. Sudor and M.V. Novotny, *Proc. Natl. Acad. Sci. U.S.A.*, 90 (1993) 9451.
[12] J. Snopek, H. Soini, M.V. Novotny, E. Smolkova-Keulemansova and I. Jelinek, *J. Chromatogr.*, 559 (1991) 215.
[13] D. Belder and G. Schomburg, *J. High Resolut. Chromatogr.*, 15 (1992) 686.
[14] P.-Å. Albertsson, *Nature*, 177 (1956) 771.
[15] P.-Å. Albertsson, *Partition of Cell Particles and Macromolecules*, Wiley, New York, 3rd ed., 1986.
[16] A.G. Ogston, *Trans. Faraday Soc.*, 54 (1958) 1754.
[17] P.G. Bruce and C.A. Vincent, *J. Chem. Soc., Faraday Trans.*, 89 (1993) 3187.
[18] M. Yalpani, *Polysaccharides*, Elsevier, Amsterdam, 1988.
[19] H. Hommel, A.P. Legrand, H. Ben Ouada, H. Bouchriha, H. Balard and E. Papirer, *Polymer*, 33 (1992) 181.
[20] J. Bullock, *J. Chromatogr.*, 645 (1993) 169.
[21] R.S. Parnas, M. Chaimberg, V. Taepaisitphongse and Y. Cohen, *J. Colloid Interface Sci.*, 129 (1989) 441.
[22] W. Thormann, S. Lienhard and P. Wernly, *J. Chromatogr.*, 636 (1993) 137.
[23] G. Johansson and M. Joelsson, *J. Chromatogr.*, 464 (1989) 49.
[24] J. Liu, V. Dolnik, Y.-Z. Hsieh and M.V. Novotny, *Anal. Chem.*, 64 (1992) 1328.
[25] H. Soini, M.-L. Riekkola and M.V. Novotny, *J. Chromatogr.*, 608 (1992) 265.
[26] T. Takagi, M. Takeda and H. Maeno, *Arch. Int. Pharmacodyn.*, 256 (1982) 49.
[27] W.C. Vincek, M.L. Constanzer, G.A. Hessey, II and W.F. Bayne, *J. Chromatogr.*, 338 (1985) 438.
[28] Y. Imai and S. Kobayashi, *Biomed. Chromatogr.*, 6 (1992) 222.
[29] M.S. Islam and M.M. Narurkar, *Drug Devel. Ind. Pharm.*, 17 (1991) 1229.
[30] D.S. Burgi and R.-L. Chien, *J. Microcol. Sep.*, 3 (1991) 199.
[31] P. Gebauer, W. Thormann and P. Boček, *J. Chromatogr.*, 608 (1992) 47.