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Separation of Diastereomers by Capillary Zone Electrophoresis with Polymer Additives: Effect of Polymer Type and Chain Length

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Diastereomeric derivatives of enantiomers are separated by capillary zone electrophoresis in nonchiral separation systems in the presence of linear polymers. These polymers significantly influence the mobilities of the analytes as well as the stereoselectivity of the system. Three types of linear polymers, poly(vinylpyrrolidone), poly(ethylene glycol), and poly(acrylamide), are investigated to determine their influence on the stereoselective separation of diastereomeric derivatives of α-amino acids obtained by reaction with optically pure (+)-*O,O'*-dibenzoyl-L-tartaric anhydride. Differences are found in the strength of the polymer effect and the effected migration order. Polymer chain length had no impact on stereoselectivity.

In previous papers,[1-3] the electrophoretic separation of diastereomeric derivatives of racemic amino acids has been reported, where the diastereomers were obtained by reaction with (+)-*O,O'*-dibenzoyl-L-tartaric anhydride (DBT anhydride). It was shown that in free solution using no further additives, many of the investigated compounds are resolved at appropriate pH conditions. It has been found that the presence of linear poly(vinylpyrrolidone) in the electrolyte solution significantly increases stereoselectivity and allows one to separate a larger number of diastereomeric analytes.[1,2] This increased stereoselectivity is supposed to be based on intermolecular interactions between the analytes and the polymeric pseudophase. In aqueous electrolyte solutions, it can be assumed that the pseudophase acts predominantly on the basis of free energy contributions responsible for "hydrophobic" behavior, as well as on the basis of dipole and $\pi-\pi$ interactions between appropriate structural moieties in analyte and polymer. Interactions between aromatic and π-electron-rich structural groups seem to be of special significance.[4]

In this paper, three different types of polymers are compared with respect to this observed effect on stereoselectivity: poly(vinylpyrrolidone) (PVP), poly(ethylene glycol) (PEG), and poly(acrylamide) (PAA). As stereorecognition might be affected by the conformation of the polymer, the parameter of chain length is investigated, too, using PVP and PEG with three different chain lengths. Test analytes were racemic α-amino and α-hydroxy acids converted to diastereomeric derivatives by reaction with DBT anhydride.

EXPERIMENTAL SECTION

Apparatus. The experiments were carried out using a laboratory-made apparatus as described in refs 1 and 5. The dimensions of the fused silica capillary used (Polymicro Technologies, Phoenix, AZ) were 56 cm × 100 μm i.d., with 39 cm length to the detector (UV absorption at 233 nm). A constant voltage of 12 kV was applied to the capillary during electrophoresis in the anionic mode. The capillary was coated to suppress the electroosmotic flow; it was kept at ambient temperature (24–25 °C) without thermostating. Sampling was done by the hydrodynamic method (5 s at a height of 10 cm).

For measurements of dynamic viscosities at three different temperatures, an automated microviscosimeter (AMV 200, A. Paar, Graz, Austria) was used.

Chemicals. Optically pure (optical purity >99.5%) and racemic α-amino acids, as well as buffering electrolytes and coating reagents, were purchased from Aldrich (Steinheim, Germany) in the purest obtainable quality. (+)-*O,O'*-diacetyl-L-tartaric anhydride (optical purity >99.5%) was obtained from Aldrich, and (+)-*O,O'*-dibenzoyl-L-tartaric anhydride was synthesized as described in refs 1 and 5, ending with an optical purity >99.5%.

PVP-15, PVP-25, and PVP-90, as well as PEG-200, PEG-20 000 and PEG 100 000, were obtained from Serva (Heidelberg, Germany). PAA was polymerized according to ref 6, with the given concentration of acrylamide.

Procedure. The derivatization of the racemic or optically pure analytes with DBT anhydride was performed as in refs 7 and 8; the poly(acrylamide)-type coating was made according to the procedure given in ref 6.

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(1) Schützner, W.; Caponecchi, G.; Fanali, S.; Rizzi, A.; Kenndler, E. Electrophoresis **1994**, *15*, 769.
(2) Schützner, W.; Fanali, S.; Rizzi, A.; Kenndler, E. *J. Chromatogr.* **1993**, *639*, 375.
(3) Schützner, W.; Fanali, S.; Rizzi, A.; Kenndler, E. *J. Chromatogr.*, in press.
(4) Blatny, P.; Fischer, H.-C.; Rizzi, A.; Kenndler, E. *J. Chromatogr.*, in press.
(5) Fanali, S.; Ossicini, L.; Foret, F.; Boček, P. *J. Microcolumn Sep.* **1989**, *1*, 190.
(6) Kilar, F.; Hjerten, S. *Electrophoresis* **1989**, *10*, 23.
(7) Zetzsche, F.; Hubacher, M. *Helv. Chim. Acta* **1926**, *9*, 291.
(8) Lindner, W.; Leitner, C.; Uray, G. *J. Chromatogr.* **1984**, *316*, 605.

0003-2700/95/0367-3866$9.00/0

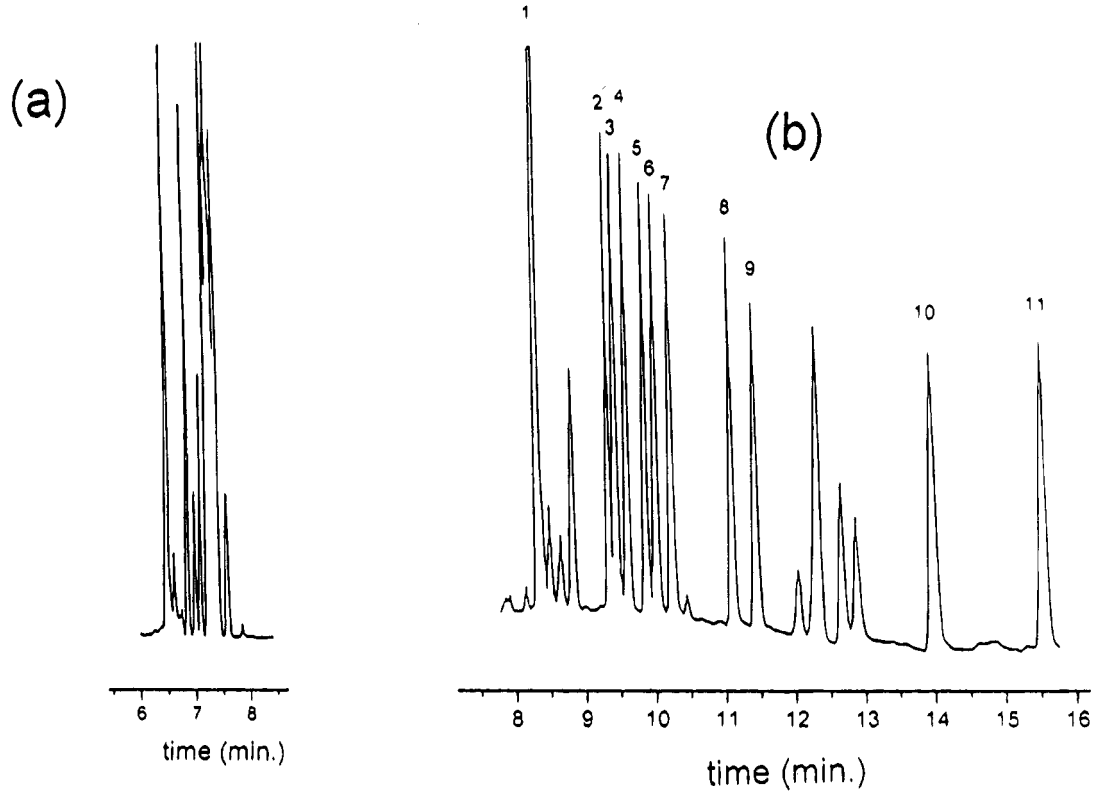

Figure 1. Electropherograms of a mixture of DBT-derivatized racemic amino acids without (a) and with PVP (b) added as pseudophase. (a) Sample: DBT-derivatized racemic Ser, Thr, Gln, Met, Leu, Phe, Phegly, and Trp. No polymer in the BGE. (b) Sample: DBT-derivatized D-Ser (2), L-Ser (3), D-Gln (4), L-Gln (5), D-Leu (6), L-Leu (7), L-Phe (8), D-Phe (9), L-Trp (10) and D-Trp (11). Peak 1 originates from DBT-acid. Additional peaks are byproducts of reaction. Electrophoretic conditions: coated fused silica capillary, dimensions 56 cm × 100 μm i.d., 39 cm length to the detector; voltage, 12 kV; ambient temperature; UV absorption at 233 nm. Composition of the BGE: aqueous buffer solution, 30 mM sodium phosphate, pH 5.8; 2.5% (w/v) PVP.

The BGE was composed of 30 mmol/L sodium dihydrogen phosphate, adjusted with NaOH to pH 5.8. The polymers were added to the BGE solution prior to pH adjustment in a concentration range from 0.5% to 3% (w/v) (PVP and PAA) and 5% (w/v) (PEG).

RESULTS AND DISCUSSION

Chemical Structure of Polymer and Analyte. The retardation of the analytes induced by interaction with the polymer network generates selectivity with respect to the chemical nature of the analytes, in particular the chemical structure of the amino acid side chains and their configuration.

Side-Chain-Related Selectivity. The retardation of the single DBT-derivatized amino acids by interaction with the different polymeric pseudophases is illustrated by the decrease in their effective mobilities given in Table 1. With PVP, aromatic amino acids are seen to be slightly more affected than aliphatic ones, and the hydrophilic groups in serine, threonine, and glutamine diminish the effect of PVP on mobility. The impact of PEG and PAA is generally of the same type, but weaker compared to PVP, and the side-chain-specific selectivity does not distinguish as clearly between aliphatic and aromatic moieties. The spreading of mobility values by interaction with the polymer is strongest with PVP. The thus-achieved broadening of the mobility window of a set of analytes allows us to enhance the number of separable components, as shown in Figure 1. Ten analytes are easily resolved in the presence of PVP which can hardly be separated in absence of the polymer.

Stereoselectivity. The impact of three different types of polymers on the stereoselectivity coefficients is shown in Figure 2 as a function of the polymer concentration. Stereoselectivity coefficients are calculated as the ratios of the effective mobilities, μ_D/μ_L, where D and L indicate the diastereomer carrying the D and L amino (or hydroxy) acid, respectively. The pH was adjusted to 5.8, where fairly complete dissociation of the carboxylic groups of the analytes can be assumed and where selectivity effects resulting from polymer-induced pK_a shift can widely be excluded. The results obtained with PVP have been discussed in a previous paper[2] and are repeated here to allow a direct comparison of polymer-type related effects. PVP (Figure 2a) affects the stereoselectivity coefficients of aliphatic and aromatic amino acid DBT

Table 1. Dependence of the Effective Mobilities of the DBT L-Analytes[a] on Type and Concentration of Polymer[b]

		polymer type						
		PVP[c]			PEG[c]		PAA[c]	
	no polymer	0.5	2.0	3.0	2.0	5.0	1.5	3.0
PheGly	39.5	36.2	27.1	25.9			35.3	33.8
Val	39.0	29.1	28.7	27.4				
Leu	38.8	32.4	28.0	27.5	31.5	24.8	35.1	30.6
Met	37.9	34.0	29.2	30.5				
Gln	37.4	34.0	32.0	30.0				
Phe	36.5	30.5	26.1	26.3	32.1	25.9	34.5	30.0
Trp	38.4	26.4	21.7		29.5	22.8	34.5	27.9
Ser	40.2	32.7	31.6	31.1	33.2	26.2	37.4	32.7
Thr	39.3	34.0	29.4	31.8	33.9	25.4	36.0	31.0
mandelic acid	36.0		25.6	24.3	31.6	24.0	35.7	32.2

[a] $\mu_i^L \times 10^5$ cm^2 V^{-1} s^{-1}. [b] BGE as specified in the Experimental Section. [c] Polymer concentrations in % w/v.

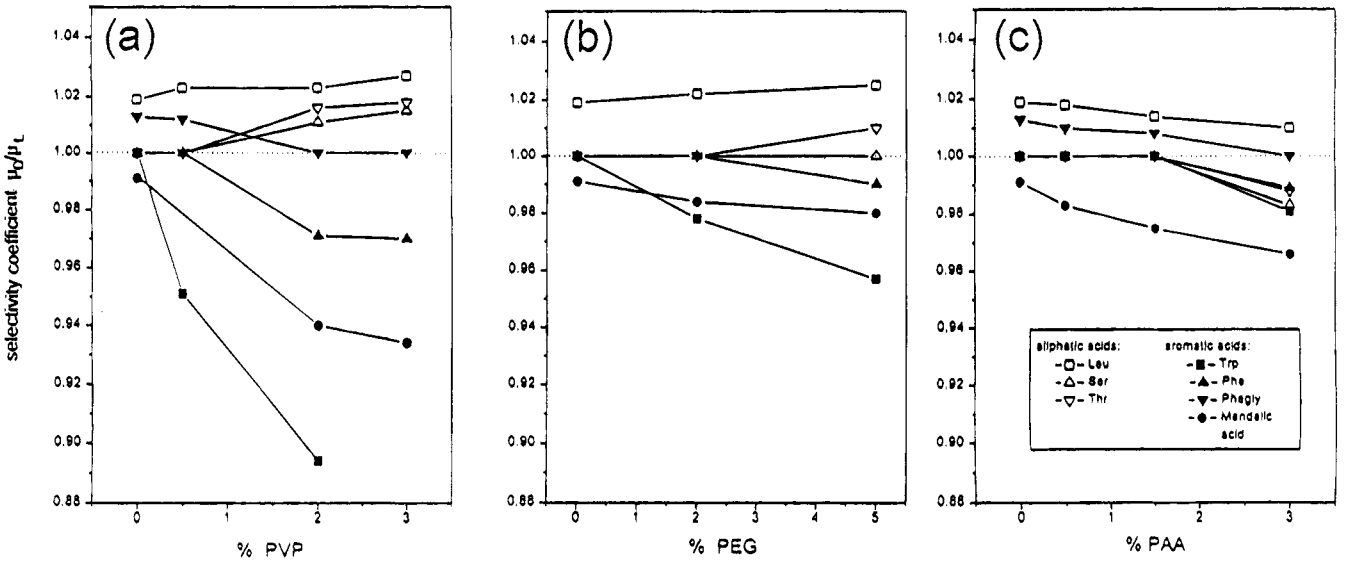

Figure 2. Stereoselectivity coefficients of various DBT-derivatized aliphatic and aromatic α-amino acids and mandelic acid as a funciton of the concentration (% w/v) of polymer in the BGE: (a) PVP-15, (b) PEG-20 000, and (c) PAA. Electrophoretic conditions as specified in the Experimental Section; pH 5.8.

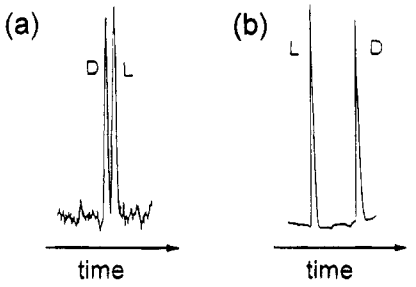

Figure 3. Electropherograms of (a) DAT-derivatized DL-Trp and (b) DBT-DL-Trp in the presence of PVP in the electrolyte solution. Composition of the BGE: aqueous buffer solution, 30 mM sodium phosphate, pH 5.8; (a) 6% and (b) 2% (w/v) PVP. Migration times: (a) 12.93 and 13.14 min; (b) 13.96 and 15.62 min. All other experimental conditions as in Figure 1.

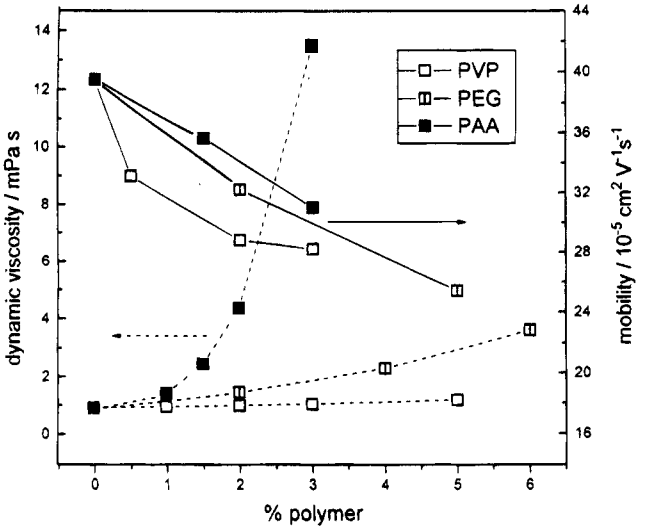

Figure 4. Dynamic viscosity of aqueous polymer solutions and effective mobilities of DBT-L-leucine as a function of polymer type and concentration. Polymers: PVP-15, PEG-20 000, and PAA in aqueous solution; temperature, 25 °C. Electrophoretic conditions as specified in the Experimental Section. Solid lines, effective mobility; broken lines, dynamic viscosity.

derivatives in opposite direction. The D-analytes of the aromatic acids are more strongly retained by the polymer in all instances. The alteration of stereoselectivity is found to be considerably stronger for most of the aromatic acids than for aliphatic ones. With PEG (Figure 2b), essentially the same pattern is observed as with PVP, i.e., the stereoselectivity coefficients of aliphatic and aromatic amino acid derivatives are affected in opposite direction, although the strength of this effect is significantly less, even at a polymer concentration of 5% (w/v). With PAA (Figure 2c), however, stronger retardation of the D-analytes was found for aliphatic as well as aromatic amino (and hydroxy) acids. The strength of the polymer's effect on selectivity coefficients is comparable to that of PEG, i.e., less than PVP. Due to the differing stereospecifity of the polymer's effect, different migration order was found for racemic DBT-threonine depending on the type of polymer added to the BGE.

Separations carried out employing differently substituted derivatizing agents showed that the chemical structure of the *O,O'*-substituents attached to the L-tartaric acid decisively influences the separation factors and even the migration order of the diastereomers. The isomers of DL-tryptophan derivatized by di-*O,O'*-acetyl-L-tartaric anhydride (DAT derivative) showed the opposite migration order from those derivatized by di-*O,O'*-benzoyl-L-tartaric anhydride (DBT derivative), as documented in Figure 3. The magnitude of the separation factors was very different in these cases.

Viscosity of Polymer Solution and Retention Effect. Data for the dynamic viscosities of the BGE solution were measured at three different temperatures covering polymer concentrations of up to 3% (w/v) (for PAA), 5% (for PVP-15), and 6% (for PEG-20 000) in water. The viscosity data at 25 °C are shown in Figure 4, together with the mobility data of DBT-L-leucine. The increase in viscosity is small when PVP is added, considerably stronger for PEG, and drastic when PAA is added. The concave viscosity versus polymer concentration curves exhibit the steepest slope at higher polymer concentrations. Unlike these curves, the graphs displaying the decrease in the analytes' mobilites caused by the presence of the polymer are either approximately linear (PAA) or concave (PVP and PEG), with a stronger decrease at lower polymer concentrations. PVP, exhibiting the smallest increase

Table 2. Dependence of Stereoselectivity Coefficients[a] on the Polymer Chain Lengths at Different Polymer Concentrations[b]

(a) PVP

	concn of PVP, % (w/v)					
		0.5			2.0	
	0	M_w = 11 000	M_w = 25 000	M_w = 750 000	M_w = 11 000	M_w = 750 000
PheGly	1.013	1.012	1.012	1.013	1.00	
Val	1.010	1.014	1.015	1.014	1.023	
Leu	1.019	1.023	1.022	1.022	1.023	
Trp	1.00	0.951	0.938	0.935	0.894	0.895
Ser	1.00	1.00	1.00	1.00	1.011	1.015
Phe	1.00				0.971	0.964
Thr	1.00				1.016	1.019
mandelic acid	0.991				0.940	0.931

(b) PEG

	concn of PEG, % (w/v)					
		2			5	
	0	M_w = 20 000	M_w = 100 000	M_w = 200	M_w = 20 000	M_w = 100 000
Phe	1.00	1.00	1.00	1.00	0.990	0.987
Trp	1.00	0.978	0.974	1.00	0.957	0.958
Ser	1.00	1.00	1.00	1.00	1.00	1.00
Thr	1.00	1.00	1.00	1.005	1.010	1.009
mandelic acid	0.991	0.984	0.985	0.989	0.980	0.980

[a] Selectivity coefficients are calculated as the ratio of effective mobilities, μ_i^D/μ_i^L. [b] BGE as specified in the experimental section.

Table 3. Dependence of Effective Mobilities[a] on Polymer Chain Lengths at Different Polymer Concentrations[b]

(a) PVP

	concn of PVP, % (w/v)					
		0.5			2.0	
	0	M_w = 11 000	M_w = 25 000	M_w = 750 000	M_w = 11 000	M_w = 750 000
PheGly	39.5	33.8	31.5	32.7		
Val	39.0	32.2	34.4	35.6		
Leu	38.8	32.4	29.7	33.6		
Trp	38.3	26.4	26.1	26.8	21.7	21.7
Ser	40.2	32.7	36.3	34.3	31.6	34.1
Phe	36.5				26.1	28.9
Thr	39.3				29.4	30.4
mandelic acid	35.6				25.6	28.3

(b) PEG

	concn of PEG, % (w/v)					
		2			5	
	0	M_w = 20 000	M_w = 100 000	M_w = 200	M_w = 20 000	M_w = 100 000
Phe	36.5	32.1	30.4	29.8	25.9	25.1
Trp	38.3	29.5	29.7	29.4	22.8	24.2
Ser	40.2	33.2	33.7	31.6	26.2	29.1
Thr	39.3	33.9	35.3	31.8	25.4	28.2
mandelic acid	35.6	31.6	33.0	30.1	24.0	24.1

[a] $\mu_i^L \times 10^5$ cm^2 V^{-1} s^{-1}. [b] BGE as specified in the Experimental Section.

in viscosity, showed the greatest impact on the mobilities. These data allow the following conclusions: (i) With the linear polymer networks employed, the changes in mobilites of the analytes do not reflect the changes in the solutions' viscosities. (ii) Specific selectivity effects as well as stereospecific effects make clear that analyte–polymer interactions are the cause of reduced mobility of the analytes.

Influence of the Chain Length. Stereoselectivity coefficients were measured at three different chain lengths of linear PVP and PEG polymers. The data are given in Table 2 for linear PVP of molecular masses 11 000 (PVP-15), 25 000 (PVP-25), and 750 000 (PVP-90) g/mol and for linear PEG of molecular masses 200 (PEG-200), 20 000 (PEG-20 000), and 100 000 (PEG-100 000) g/mol. (The corresponding mobility data are given in Table 3.) The molecular masses of the corresponding monomer units are 111 and 44 g/mol for PVP and PEG, respectively.

PVP polymers of different chain lengths exhibited very similar properties in terms of the resolution of the diastereomeric analytes. At a polymer concentration of 0.5% (w/v), DBT-Trp was the only analyte for which a small influence of chain length could be observed. With 2% PVP, this effect was slightly more pronounced but still small (up to about 1% for DBT-mandelic acid). Interestingly, at this polymer concentration, no dependence on chain length was seen for DBT-Trp.

Of particular interest, however, are the data of PEG-200, which is an oligomer with an average chain length of only 4.5 monomer units. It can thus serve as a reference additive, acting like a solvent rather than a polymer. The alterations of selectivity coefficients caused by PEG-200 compared to those of the polymer-free solution were found to be negligible; only for DBT-Thr and DBT-mandelic acid did the presence of 5% PEG-200 effect a small change in resolution. With higher chain lengths, significant effects are seen. Apparently, a certain minimum polymer chain length is essential to affect the resolution of the diastereomers; however, significantly above this threshold value, polymer chain length has not much influence on steric resolution. Nonselective retardation of the analytes is found already with PEG-200 as documented in Table 3b.

CONCLUSION

The investigations employing different types of polymers show that retardation of analytes, and in a broad number of cases also stereospecific retardation of diastereomers, is a quite general phenomenon associated with polymeric additives. The data confirm the previous assumption that selectivity of the system is mainly based on free energy contributions responsible for "hydrophobic" behavior and on dipole–dipole as well as $\pi-\pi$ and $n-\pi$ interactions between polymer and analyte. The analogy to chromatographic stationary phases is evident, particularly to the reversed-phase type with certain affinity for aromatic and π-electron-rich structural groups. The retardation induced by the pseudophase allows us to enlarge the effective mobility window accessible for a set of analytes and thus to achieve separation of a much higher number of analytes. This was demonstrated for a sample of five pairs of diastereomeric amino acid derivatives.

The stereodiscriminating effect induced at a certain constant polymer concentration (e.g., 2%) is not equal for different polymers: PVP acts far stronger than PEG and PAA. The ring structures in the polymer thus seem to be advantageous for stereodiscrimination. The differences in migration order found for aliphatic versus aromatic amino acid derivatives with PVP is maintained with PEG, too, although the chemical structures of

the polymers differ widely. On the other hand, PAA does not exhibit such different migration order. The influence of polymer chain length on stereoselectivity is marginal. The aromatic moieties in the DBT group of the derivatization reagent lead to improved separation, accompanied by inversion of migration order, compared to the short aliphatic group in the corresponding DAT derivatives.

A comparison of the viscosity effects induced by the three different polymers again underlines that reduction of the analyte mobility is not a consequence of an increase in viscosity but rather a result of intermolecular interactions similar to "adsorption onto a pseudophase". The most pronounced effect related to the polymer was found for PVP, where the smallest viscosity increase was observed. This polymer can thus be favored in the practical use as the BGE additive that allows selectivity enhancement and stereodiscrimination for diastereomeric analytes, particularly in those cases where aromatic moieties are present.

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