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Capillary electrokinetic chromatography with polyethyleneimine as replaceable cationic pseudostationary phase Influence of methanol and acetonitrile on separation selectivity

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Abstract

The effect of methanol and acetonitrile, respectively, on the separation of neutral compounds (benzyl alcohol, phenols) is investigated in electrokinetic chromatographic (EKC) systems consisting of polyethyleneimine (PEI) as charged, polymeric, replaceable pseudostationary phase. The separation systems consist of a buffer solution (2-morpholinoethanesulfonic acid, pH 7.0, 20 m*M*) containing 0.3–0.9% (w/v) PEI as additive and a varying percentage of methanol (0–50%, v/v) or acetonitrile (0–30%, v/v). EKC is carried out in fused-silica capillaries [47.0 cm (effective length 40.3 cm)×100 μm I.D.]. They are dynamically coated with PEI, resulting in an electroosmotic flow directed towards the anode. The neutral analytes are migrating with the electroosmotic flow, and are retarded by the electrically driven counterflow of PEI. Separation of the analytes follows in the sequence benzyl alcohol, phenol, resorcinol, pyrogallol, reflecting the increasing hydrogen bond acidity and polarity (polarizibility) of the solutes. However, addition of methanol or acetonitrile causes a drastic loss of resolution, whereby the relative retention of the separands (related to benzyl alcohol) indicates a decrease of retardation upon addition of the organic solvents.

Keywords: Electrokinetic chromatography; Polyethyleneimine pseudostationary phase; Pseudostationary phases; Background electrolyte composition; Selectivity; Benzyl alcohol; Phenols

1. Introduction

In electrically driven chromatography, separation of uncharged analytes can be achieved according to the different distribution between the background electrolyte (BGE) and a stationary or a pseudo-stationary phase, respectively. In case of capillary electrochromatography (CEC), the stationary phase is covalently bonded to packed silica particles (or to the wall) [1,2] or formed by a continuous polymer bed [3–6]. In all cases the mobile phase is moved by the electroosmotic flow (EOF).

Electrokinetic chromatography (EKC) comprises a great variety of replaceable pseudostationary phases, and many of these phases have their origin in micellar electrokinetic chromatography (MEKC). Problems with conventional micelle forming surfactants like strong retention of highly hydrophobic compounds and limited stability of the micelle in aqueous–organic media lead to the development of new macromolecular phases. Palmer et al. [7] introduced the use of micelle polymers that are covalently bonded together and thus have a fixed primary structure. The application of an acrylate copolymer

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as pseudostationary phase was reported by Ozaki et al. [8]. Polyallylamine-supported pseudostationary phases were studied by Tanaka et al. [9]. Dendrimers, highly branched polymers, represent an interesting alternative to conventional micelles because of their stability at high concentrations of organic modifiers [10]. The applicability of dendrimers can be enhanced by modifying them with different alkyl chains [11]. Resorcarenes [12], calixarenes [13] and charge transfer interacting additives [14] further extended the field of possible carriers in EKC.

We have already reported the use of a negatively charged linear polymer as replaceable pseudostationary phase for the separation of neutral analytes [15]. Partially hydrolyzed polyacrylamide formed an electrically driven counterflow opposite to the EOF that transported the solutes towards the cathode. Pseudostationary phases consisting of the cationic polymer polyethyleneimine (PEI) turned out to be more stable and allowed an easier manipulation [16,17]. In a previous paper [17] we have demonstrated the separation of uncharged mono- and oligophenols with PEI as buffer additive. PEI creates a positively charged layer on the surface of the fused-silica capillary and reverses the EOF. Thus the analytes are moved by the EOF towards the anode, whereas the cationic polymer dissolved in the BGE migrates in the opposite direction. Interaction with this pseudostationary phase leads to different retardation of the solutes.

In the present paper the influence of two organic solvents, methanol and acetonitrile, on the separation of neutral compounds in these systems is investigated. As an indirect measure for the selectivity of separation a relative retention value, connected to benzyl alcohol as the least retarded solute, is taken, and its change upon addition of the organic solvent constituent is determined.

2. Materials and methods

2.1. Chemicals

A solution of PEI in water (50%, w/v) with a molecular mass of $6\cdot10^5$–$1\cdot10^6$ was purchased from Fluka (Buchs, Switzerland). Benzyl alcohol (99%) was obtained from Ega-Chemie (Steinheim/Albuch, Germany). Phenol, resorcinol (1,3-dihydroxybenzene) and pyrogallol (1,2,3-trihydroxybenzene) were used in analytical grade (E. Merck, Darmstadt, Germany). Buffers were prepared with 2-morpholinoethanesulfonic acid (MES) monohydrate (Fluka), sodium hydroxide, hydrochloric acid, methanol and acetonitrile (analytical grade, E. Merck). Double distilled water was used throughout.

2.2. Apparatus

A capillary electrophoresis system (P/ACE 2100 Beckman, Fullerton, CA, USA) equipped with an on-column UV absorbance detector (214 nm) was used to carry out all measurements. Uncoated fused-silica capillaries (Composite Metal Services, Hallow, UK) of 47.0 cm (effective length 40.3 cm)×100 μm I.D.×375 μm O.D. were dynamically coated with PEI. Samples were injected by pressure (1 s, 35 mbar). Applying a constant voltage of 10.0 kV, the resulting current was between 8 μA and 54 μA. Temperature was 26±1°C.

2.3. Procedures

As described in a previous paper [17], treatment of etched fused-silica capillaries with a solution of 10% (w/v) PEI in water provided a dynamic, positively charged coating. The mobility of the EOF occurring after this coating procedure was determined from the negative water peak in the usual way.

The running buffer was prepared with MES (pH 7.0, 20 m*M*) containing 0.3–0.9% (w/v) PEI as additive and a varying percentage of methanol (0–50%, v/v) or acetonitrile (0–30%, v/v), respectively. The upper concentration of PEI was limited to 0.9% due to the high current occurring otherwise. After adding PEI and organic solvent to the MES solution the apparent pH was adjusted to 7.0 using 1 *M* sodium hydroxide or 1 *M* hydrochloric acid, respectively. The sample components were dissolved in the corresponding running buffer at concentrations of 0.5–5.0 m*M* each.

3. Results and discussion

3.1. Influence of organic solvent on resolution

Four compounds that contain hydroxy groups, uncharged under the applied conditions (pH 7.0), were used as separands: benzyl alcohol, phenol, resorcinol and pyrogallol. In pure capillary zone electrophoresis (CZE) these four neutral sample components migrate with the velocity of the EOF. However, as already shown in a previous paper [17] separation of neutral mono- and oligophenols can be achieved by addition of high-molecular-mass PEI as pseudostationary phase to the running buffer. Under the influence of the electrical field the uncharged analytes are moved by the EOF (that is directed towards the anode; electroosmotic mobility μ_{EOF} up to $60 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1}), whereas the positively charged polymer is driven into the opposite direction. Interaction with this pseudostationary phase, and implementation of a chromatographic principle leads to retardation and thus to separation of the solutes by a kind of EKC.

PEI was applied at concentrations of 0.3, 0.6 and 0.9% (w/v). At all three concentrations separation of the test separands was achieved. As an example, in Fig. 1 a capillary electrokinetic chromatogram of benzyl alcohol and the three phenols obtained with a BGE containing 0.9% (w/v) PEI is shown. It should be realized that the ionic strength is higher than

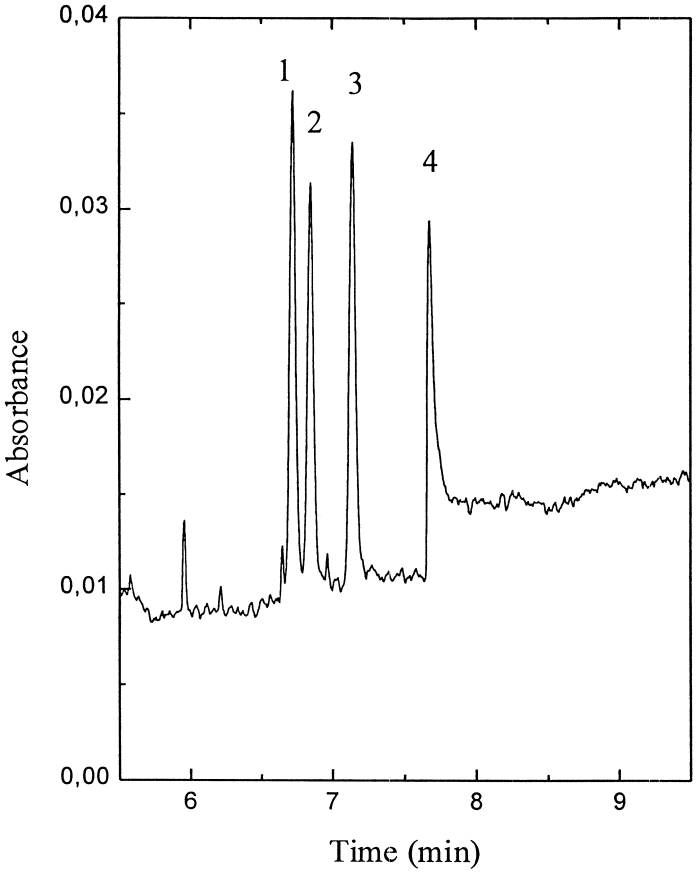

Fig. 1. Capillary electrokinetic chromatogram of the sample consisting of benzyl alcohol (1), phenol (2), resorcinol (3) and pyrogallol (4) in 0.9% (w/v) PEI-containing buffer (pH 7.0) without organic solvent. Conditions: PEI-coated capillary [47.0 cm (effective length 40.3 cm)×100 μm I.D.]; pressurized injection 1 s/35 mbar; temperature 25°C; voltage 10.0 kV; current 54 μA; UV absorbance detector (214 nm) placed at the anodic end of the capillary.

stemming only from the buffering electrolyte, because PEI is charged as well, and is present at much higher concentrations than MES.

It can be seen from Fig. 1 that all sample components are baseline separated. In accordance with the results shown in a previous paper, the migration sequence depends on the different number and kind of hydroxy groups. Benzyl alcohol is less retarded than the phenolic compounds, and retardation of the phenols becomes stronger with increasing number of hydroxy groups. This result led us postulate a type of separation principle for the analytes under consideration contrary to that established in reversed-phase liquid chromatography or MEKC. The analytes are approximately retarded according to increasing hydrophilicity. This assumption is in rough agreement with their partition coefficients, K_{OW} in octanol–water systems: the log K_{OW} values are 0.25, 0.80, 1.50 and 1.05 for pyrogallol, resorcinol, phenol and benzyl alcohol, respectively [18,19].

The influence of the addition of organic solvents on the resolution becomes apparent in Figs. 2 and 3. At a content of 30% (v/v) methanol (Fig. 2) the resolution of the separation system is reduced and the migration difference between benzyl alcohol and phenol is not sufficient for baseline separation. Addition of 30% (v/v) acetonitrile (Fig. 3) shows an even stronger effect on the resolution. Separation between benzyl alcohol and phenol is completely lost; resorcinol is only partly resolved from the solutes that contain one hydroxyl group.

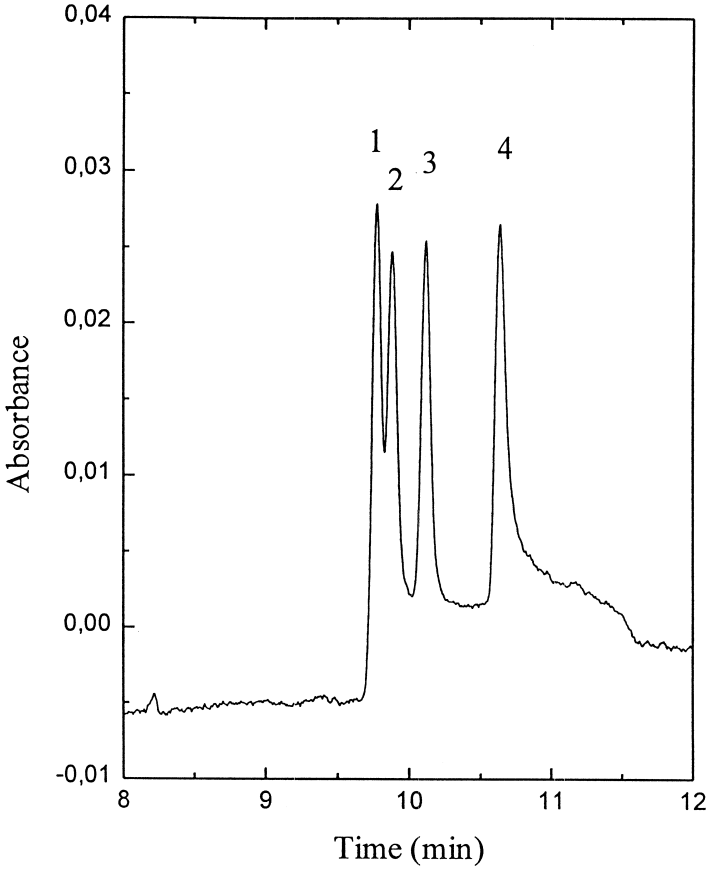

Fig. 2. Capillary electrokinetic chromatogram of four analytes in a buffer containing 0.9% (w/v) PEI and 30% (v/v) methanol. Current 34 μA. Sample and other experimental conditions as in Fig. 1.

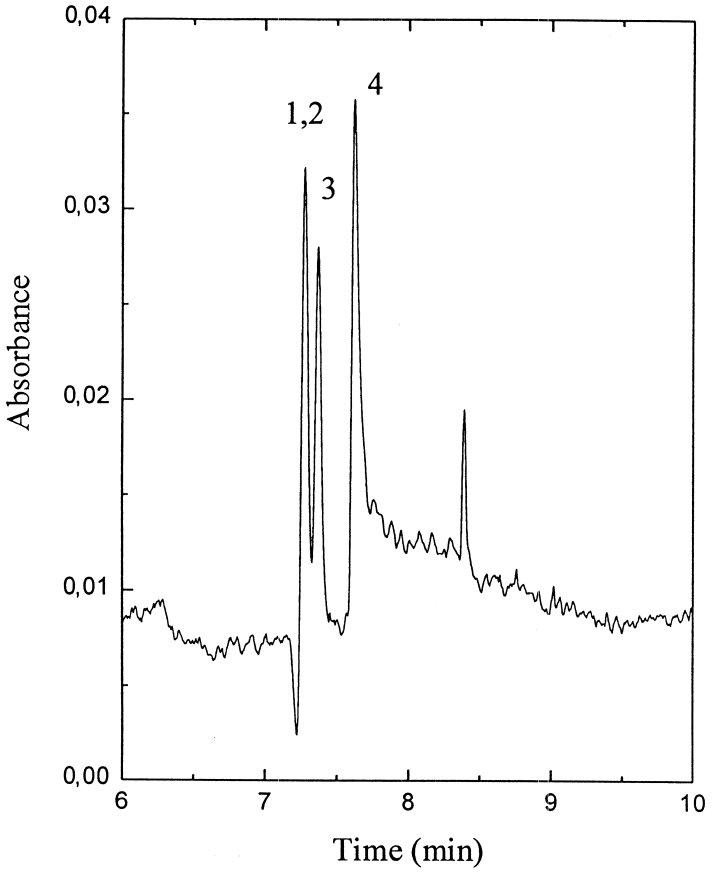

Fig. 3. Capillary electrokinetic chromatogram of four analytes in a buffer containing 0.9% (w/v) PEI and 30% (v/v) acetonitrile. Current 38 μA. Sample and other experimental conditions as in Fig. 1.

For completeness it should be mentioned that e.g., for the systems with 0.9% (w/v) PEI plate numbers, *N*, of about 250 000 per m are obtained.

3.2. Influence of organic solvent on relative retention

The selectivity coefficient, defined as the ratio of the capacity factors, k', would serve as the most appropriate parameter to express the separation selectivity. The calculation of the capacity ratio necessitates the measurement of the migration velocity of the solutes, of the EOF, and of the pseudostationary phase. Whereas being trivial for the solutes, the respective measurement for the EOF is not so straightforward. This is partially due to baseline irregularities caused by the migration of concentration boundaries (cf. e.g., Ref. [20]). Especially the measurement of the migration velocity of the charged polymeric pseudo-phase is very complicated for the experimental conditions chosen in the present contribution. An approach to measure this velocity, based on an isotachophoretic regime in a part of the separation capillary, is described in a subsequent paper [21]. In order to circumvent these problems we use another parameter to describe at least qualitatively the influence on the separation selectivity, namely a relative retention, ρ. For the calculation of ρ, benzyl alcohol is taken as reference: $\rho=(t_{Ri}-t_{RB})/t_{RB}$. Here t_{Ri} is the retention time of the solute, and t_{RB} is that of benzyl alcohol.

In Figs. 4–6 the relative retention ρ depending on

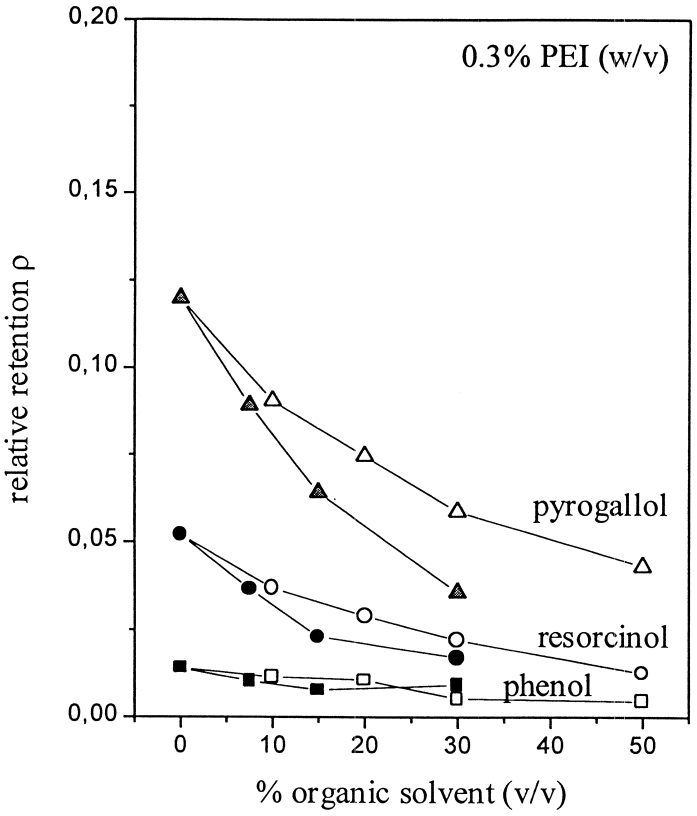

Fig. 4. Plot of relative retention, ρ, of phenol (square), resorcinol (circle) and pyrogallol (triangle) in 0.3% PEI-containing buffer as a function of methanol concentration (open) and acetonitrile concentration (solid). The precision of determination of ρ, expressed by the span of values measured in duplicate, is typically within a few percent.

the content of organic solvent (methanol and acetonitrile) at different PEI concentrations is shown. In accordance with previous findings, the relative retention becomes higher with increasing content of PEI for all solutes and all concentrations of organic solvents. The values for ρ without organic solvent of pyrogallol, for example, range from 0.12 for 0.3% (w/v) PEI to 0.16 for 0.9% (w/v) PEI. These results (and the finding, that implementation of lipophilic methyl groups into the aromatic ring does not influence the separation selectivity of a particular phenol, as discussed in detail in a previous paper [17]) brought us to the conclusion that dipole–dipole or ion–dipole interactions of the phenolic OH groups with the polymeric pseudo-phase seem to play the most decisive role for retardation rather than hydrophobic interactions as common in MEKC.

From Figs. 4–6 it follows that addition of organic solvent to the BGE seems to influence strongly the selectivity at all PEI concentrations. Two effects can be observed: (i) addition of methanol or acetonitrile, respectively, decreases the relative retention in all cases. This reduction (the steepness of the ρ vs. % organic solvent curves) is the stronger the larger the relative retention value is: the effect is as stronger as more OH groups in the molecule are. (ii) Acetonitrile reduces the relative retention stronger than methanol: all ρ vs. % acetonitrile curves decrease steeper than the ρ vs. % methanol curves. For pyrogallol, e.g., a content of 50% (v/v) methanol, but only 30% (v/v) acetonitrile leads to a reduction of ρ by about 70%. The retardation of resorcinol is changed in a similar way, whereas the effect on phenol is not that strong.

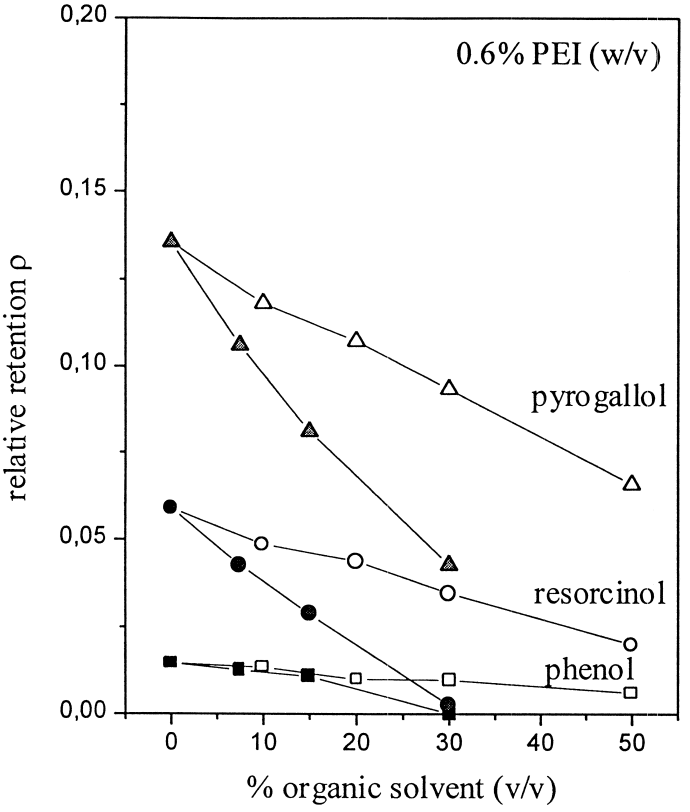

Fig. 5. Plot of relative retention, ρ, of phenol (square), resorcinol (circle) and pyrogallol (triangle) in 0.6% PEI-containing buffer as a function of methanol concentration (open) and acetonitrile concentration (solid).

If the relative retention in fact reflects the selectivity, such a behavior would be typical for systems where the organic solvent competes with the solutes by hydrophobic interactions with the (pseudo) stationary phase as in reversed-phase high-performance liquid chromatography (HPLC), or in some CE systems with cyclodextrins as additives. However, as mentioned above, no pronounced hydrophobic interactions were found when investigating methylphenols in comparison to non-substituted phenols in the same (aqueous) PEI systems. Hydrophobic interactions as main source of retardation would also not explain why the phenols are retarded according to the number of OH groups.

The assumption for the low significance of hydrophobic interactions is supported applying the concept of the linear free energy relationship (LFER) on the data obtained [22]. It is clear that general conclusions about the kind of interactions cannot be derived, as only a very limited number of analytes is considered here. In this solvation parameter model certain factors reflect the magnitude of interaction between the mobile and the stationary phase based on hydrogen-bond acidity, on dipole–dipole and dipole–induced dipole interactions, and on the excess of molar refraction. At least for benzyl alcohol, phenol and resorcinol (for which data were found) correspondence of the values of these factors with the migration order is observed. On the other hand, those parameters that reflect hydrophobicity and hydrogen bond basicity, respectively, do not have correct tendency and do not correspond with the migration order at all. Consequently we suppose that these interactions do not play an important role here. Instead, the possible formation of the hydrogen bond and dipole–dipole and dipole–induced dipole inter-

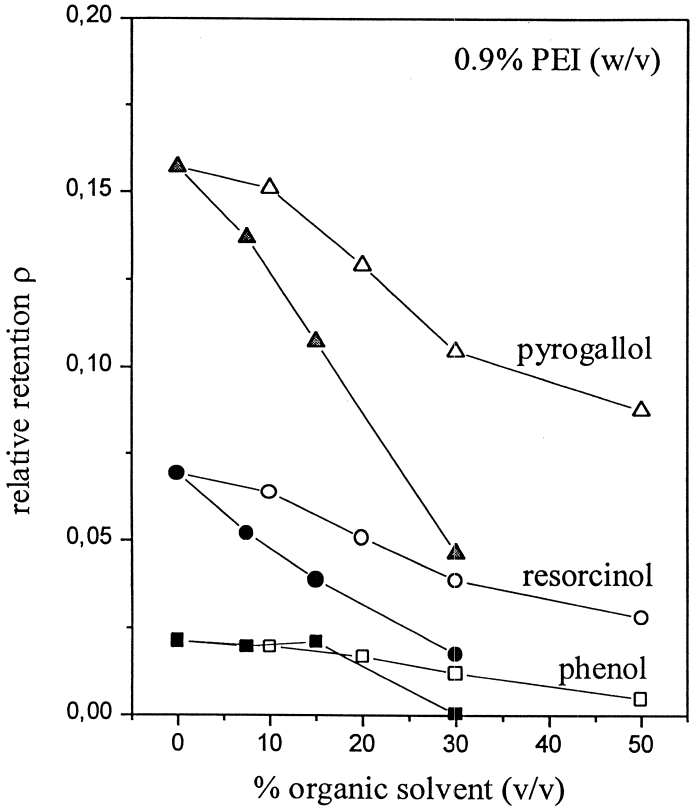

Fig. 6. Plot of relative retention of phenol (square), resorcinol (circle) and pyrogallol (triangle) in 0.9% PEI-containing buffer as a function of methanol concentration (open) and acetonitrile concentration (solid).

actions are probably decisive. A correct analysis of the significance of the particular terms could be carried out when more analytes with various parameters are investigated and the results are evaluated by multidimensional linear regression.

Critically it should be pointed out that in a strict sense we observe a reduction of the resolution only. This decrease might not be caused only by a decrease of separation selectivity, but also by that of retardation. The latter effect would be reflected by the decrease of the capacity factors, a parameter which is not easily to be determined under the present experimental conditions. Anyway, for practical purposes it follows that the application of organic solvents as additives to the BGE in our systems unfavorably decreases the separation, at least for the present type of solutes.

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