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Capillary zone electrophoresis in organic solvents: separation of anions in methanolic buffer solutions

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Abstract

A tris(hydroxymethyl)aminomethane–acetate buffer system with methanol as solvent has been used at an apparent pH of 8.5 for the separation of six aromatic and aliphatic acids. Compared to pure aqueous buffer systems with various pH values (4.4, 5.5, 6.2 and 8.0) improved separation was obtained due to increased selectivity. This improvement is related to a specific shift in the pK_a values of the solutes in the organic solvent, and is interpreted by the concept of the transfer activity coefficient and the medium effect.

1. Introduction

Electrophoretic separations in nonaqueous and mixed aqueous–organic media deserve increased attention for several reasons. The advantages of performing capillary electrophoresis (CE) in organic solvents or in mixed-solvent systems are threefold: (i) most organic compounds exhibit greater solubility than water, (ii) changes in the effective mobilities may lead to greater selectivity and (iii) the electroosmotic flow is reduced [1,2]. Reduction of the electroosmotic flow can also influence the separation selectivity, and generally improves the reproducibility of the electrophoretic migration time.

The electrophoretic migration property of the solutes, governed by the ionic mobility, is influenced by organic solvents like methanol in two ways [3–8], affecting

(i) their actual mobility, due to changes in the size of the solvated particle (and the viscosity of the bulk solution) and

(ii) their acid–base property, expressed by the pK_a value, thus determining the effective mobility at a given pH.

The second effect seems to be the most significant, because (in contrast to the actual mobilities) the dissociation constant, K_a, may change for different solvents by many orders of magnitude, and the mutual changes for the particular solutes are only weakly correlated, as shown for CE, specifically with respect to isotachophoresis of organic ions, in previous papers [9–11]. It should be mentioned that this change is different for different types of acids. It is very pronounced for acids of type HA, so-called neutral acids (those this paper deals with), but is less significant for HA^+-type cationic acids.

Only a few articles have been published reporting CE separations in pure organic or in mixed aqueous–organic solvents [8–24], most of them concerning isotachophoresis. The solvents

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used were the lower alcohols, acetonitrile, dimethylsulfoxide, dimethylformamide, tetrahydrofuran, acetone or dioxan. In the present work we compare the separation of a mixture of aromatic and aliphatic acids in aqueous solutions with that obtained in pure methanol. The analytes selected in this work were chosen because their actual mobilities and pK values are similar in aqueous solution. This renders the separation of all components in a single run quite difficult, requiring complex optimization.

2. Experimental

2.1. Materials

Benzoic acid, p-toluenesulfonic acid, caffeic acid, p-hydroxycinnamic acid and 3-(trimethoxysilyl)propyl methacrylate (Bind Silane) were purchased from Aldrich (Steinheim, Germany). Methanol used as solvent had a water content of $<0.05\%$ (HPLC grade, Fluka, Buchs, Switzerland). N-Acryloylglycine (Immobiline, pK 3.6) and N-acryolyl-γ-aminobutyric acid (Immobiline, pK 4.6) were purchased from Pharmacia LKB Biotechnology (Uppsala, Sweden). Ammonium peroxodisulfate and N,N,N′,N′-tetramethylethylenediamine (TEMED) were obtained from Bio-Rad Labs (Richmond, CA, USA). Tris(hydroxymethyl)aminomethane (Tris) and 2-[N-morpholino]ethanesulfonic acid (MES) were from Sigma (St. Louis, MO, USA). Acryloylaminoethoxyethanol (AAEE) was synthesized as described by Chiari et al. [25].

2.2. Methods

Capillary zone electrophoresis (CZE) was performed in a Waters Quanta 4000 capillary electrophoresis system (Millipore, Milford, MA, USA). For the experiments 75 μm I.D., 370 μm O.D. capillaries purchased from Polymicro Technologies (Phoenix, AZ, USA) were used. The samples were loaded by hydrostatic pressure and the separations were carried out at ambient temperature (28 to 30°C). The detector was set at 254 nm.

2.3. Coating procedure

Capillaries coated with linear poly(AAEE) bonded through methacryloxypropyl silyl moieties were prepared according to a procedure described by Kilar and Hjerten [26] and modified as follows: the capillary was pretreated with 1 M NaOH for 5 h, then rinsed and flushed with 0.1 M HCl followed by NaOH (0.1 M). After 1 h it was rinsed with water and tetrahydrofuran (THF). Residual water was eliminated by connecting the capillary to a gas chromatographic oven at 120°C for 45 min under nitrogen flow. A 50% solution of γ-methacryloxypropyltrimethoxysilane in THF was then pulled through the capillary under pressure for 20 min and allowed to set for 12 h. After this treatment, the capillary was flushed extensively with THF and water, then filled with a 3% acrylamide solution containing the appropriate amount of catalyst (1 μl TEMED and 1 μl of 40% ammonium peroxydisulfate per ml of gelling solution) and degassed under vacuum (20 mmHg) for 40 min. Polymerization was allowed to proceed overnight at ambient temperature, after which the capillary was emptied by means of a syringe.

2.4. Separation conditions

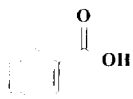
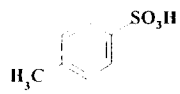
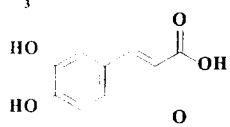
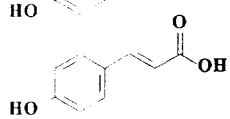
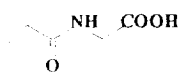
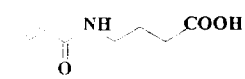
The mixture of components to be separated was injected into poly(AAEE)-coated capillaries (43 cm total length, 35 cm to the detector) using Tris–acetate (50 mM, pH 8.0), MES–Tris (50 mM, pH 6.2 and 5.5, respectively), and acetate–Tris (50 mM, pH 4.4) as pure aqueous running buffers. The same mixture was resolved in an uncoated capillary using a methanolic solution of Tris–acetate, where the concentrations of Tris and glacial acetic acid were 100 and 56 mM, respectively. The apparent pH of this solution was 8.5.

3. Results and discussion

3.1. Separation in aqueous solution

Electropherograms of the six components (see Table 1) were acquired in aqueous buffers at

Table 1
Formulae and symbols

Name	Formula	Symbol
Benzoic acid		B
p-Toluenesulfonic acid		T
Caffeic acid		C
p-Hydroxycinnamic acid		H
N-Acryloylglycine		G
N-Acryloyl-γ-aminobutyric acid		A

different pH values (4.4, 5.5, 6.2 and 8.0). Coated capillaries were used for the aqueous systems to reduce the electroosmotic flow and thus improve the reproducibility of the electrophoretic migration of the solutes. The pH value 4.4 falls within the range of pK values for the weak acids (3.6 to 4.6), and at pH 8.0 the solutes are fully dissociated. Typical electropherograms (obtained at the lowest and the highest pH, respectively) are shown in Figs. 1 and 2. In all the buffer systems, however, solutes co-migrate, except at pH 5.5; even at this pH, *p*-hydroxycinnamic and N-acryloyl-γ-aminobutyric acid are not fully baseline-resolved.

The corresponding ionic mobilities, calculated from the electrophoretic data, are given in Table 2. The effective mobilities of the solutes increase with increasing pH of the buffer (except for the sulfonic acid, the mobility of which remains about constant due to its strong acidity) and reach a nearly constant value at high pH, namely that of the actual mobility. The scatter around the constant value can be attributed to the variation of the ionic strength of the buffer, and to temperature effects. From the mobilities it can also be seen that the migration sequence varies with pH, specifically in the case of benzoic acid and N-acryloylglycine, which switch order with increasing pH. This effect is connected with the fact that benzoic acid is the weaker acid with the higher actual mobility.

3.2. Separation in methanolic solution

The electropherogram obtained in methanolic solution at an apparent pH of 8.5 is shown in Fig. 3. In contrast to the results obtained with the aqueous buffers, full resolution is observed. This is evidence of dramatic improvement in the separation selectivity, reflected by the greater difference in the mobilities of the components in the methanolic system. As stated in the Introduction the effective mobility (at a certain pH) is influenced very markedly by change in the pK value, often most dramatically for solvents other than water.

This variation in the pK_a values is explained

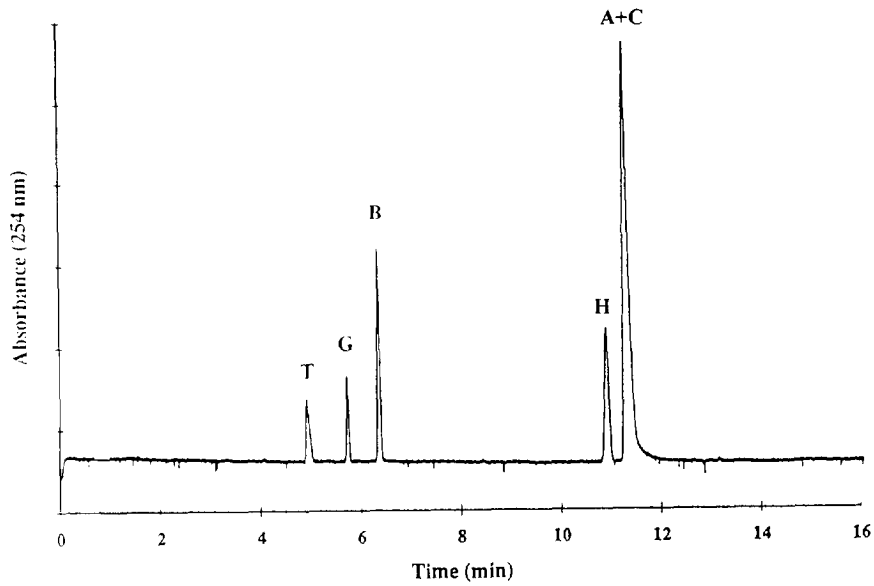


Fig. 1. Electropherogram of the anions in water at pH 4.4. For the symbols of the solutes see Table 1. Coated capillary: 75 μm I.D., length: total 43 cm, to detector 35 cm. Voltage: −15 kV. Buffer: 50 m*M* acetate–Tris in water.

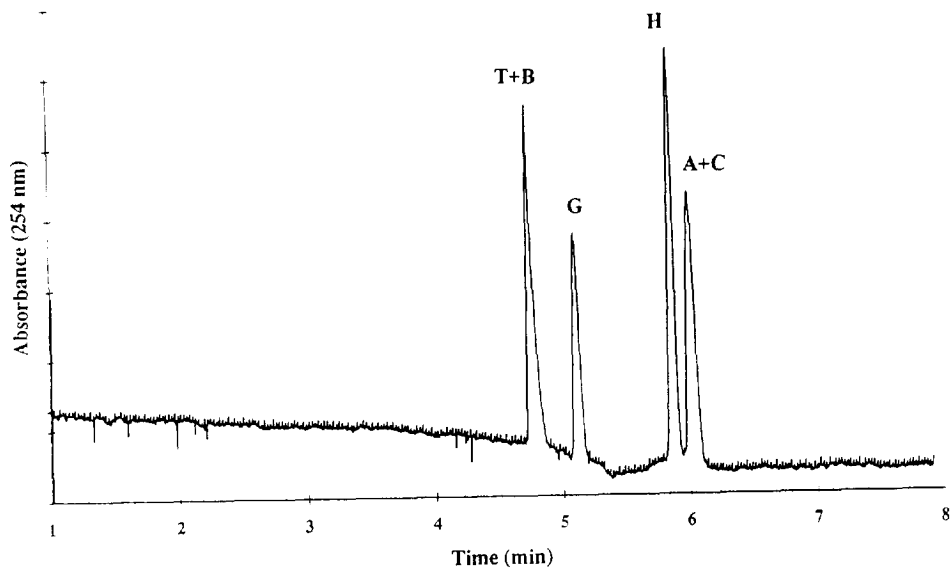


Fig. 2. Electropherogram of the anions in water at pH 8.0. Symbols, capillary and conditions as in Fig. 1. Buffer: 50 m*M* Tris–acetate in water.

Table 2
List of effective mobilities in the different buffer systems

Acid	Effective mobility (10^{-9} m^2 V^{-1} s^{-1})				
	Water pH 4.4	Water pH 5.5	Water pH 6.2	Water pH 8.0	Methanol pH 8.5
p-Toluenesulfonic	34.0	32.0	32.0	35.3	25.0
N-Acryloylglycine	29.2	30.5	30.4	32.8	17.6
Benzoic	25.6	30.9	32.0	35.3	19.3
p-Hydroxycinnamic	15.3	23.9	25.3	28.5	13.9
N-Acryloyl-γ-aminobutyric	14.8	23.5	25.3	27.9	14.9
Caffeic	14.8	22.6	23.7	27.9	12.9

by the concept of the medium effect, $\log \gamma_i^m$ (or $\ln \gamma_i^m$), given by

$$\log \gamma_i^m = \log \frac{(\mu_i^0)^s - (\mu_i^0)^w}{RT} \quad (1)$$

where the transfer activity coefficient or medium activity coefficient, γ_i^m, is defined as $\gamma_i^m = \gamma_i^s/\gamma_i^w$ with γ_i^s and γ_i^w the activity coefficients of species i in the solvent and water, respectively. Further is μ_i^0 the chemical potential of component i in the standard state, indicated by S for the organic solvent and by W for water, R is the gas constant and T the absolute temperature.

The medium effect is proportional to the reversible work required for the transfer of 1 mole of species i from infinite dilution in water, W, to infinite dilution in the organic solvent, S. If the particles of species i are better stabilized in organic solvent than in water, the medium effect has a negative value, and vice versa. The medium effect on the proton is a measure of the basicity of the solvents compared to water.

Discussing the transfer of a neutral acid (HA)

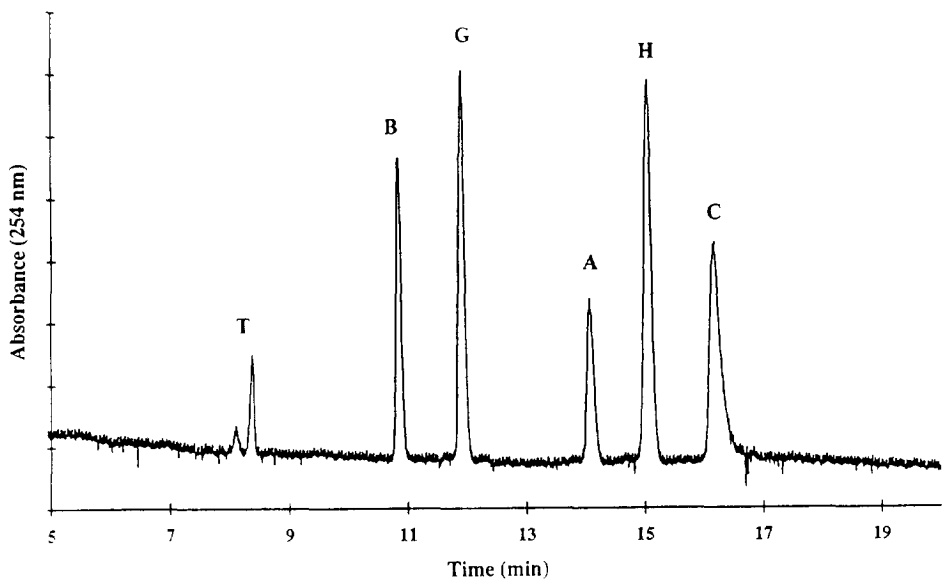


Fig. 3. Separation of the anions in methanolic buffer solution at apparent pH 8.5 in an uncoated capillary. Symbols and capillary dimensions as in Fig. 1. Voltage: −12 kV. Buffer: 100 m*M* Tris–acetate in methanol.

species, as the solutes under consideration, from water, W, to solvent, S, we find that this acid undergoes the dissociation equilibrium $HA = H^+ + A^-$ in both solvents. Thus, the reversible work done on the system in transferring 1 mole H^+ and A^- from infinite dilution in water, W, to infinite dilution in the organic solvent, S, (and transferring 1 mole of HA from S to W) is given by the standard free energy of transfer, ΔG_t^0. The change in the dissociation constant, K_a, is related to the standard free energy of transfer, and is given by

$$\Delta pK_a = pK_a^s - pK_a^w = \log \frac{\gamma^m_{H^+}\gamma^m_{A^-}}{\gamma^m_{HA}} \quad (2)$$

where $\gamma^m_{H^+}$, $\gamma^m_{A^-}$ and γ^m_{HA} are the transfer activity coefficients of the proton, the anion and the neutral molecule, respectively, the particles involved in the ionization equilibrium.

It is clear that the change in the pK_a values will be determined by the extent of stabilization or destabilization of the particles H^+, A^- and HA in the organic solvent compared to water. It must be pointed out that the values for the transfer activity coefficients for the individual particles can be obtained by approximation methods only.

As a rough measure a mean value of zero (with a standard deviation of 2) is accepted in the literature for $\log \gamma^m_{H^+}$ (cf. e.g. Ref. [5]). which means that the basicity of methanol is comparable to that of water, and the medium effect on the proton will not be decisive for the pK shift. This can also be supposed for HA, the neutral particle involved in the ionization equilibrium: for uncharged species the medium effect is of minor significance.

The most dramatic influence of methanol on the pK is based on the anion stabilization. As an anion solvent, methanol is inferior to water, which means that $\log \gamma^m_{A^-}$ has positive and fairly large values. Because the medium effects on the proton and on the neutral moiety are of less significance, the medium effect on the anion dominates, resulting in higher pK values in methanol compared to water, according to Eq. 2. This is in fact what is observed for benzoic acid, which has a pK_a of 4.2 in water and a pK_a of 9.28 in pure methanol; for mixed aqueous–methanolic solutions the pK_a values fall between these extremes: 6.42 for a binary mixture with 70 mol% methanol and 8.30 for a mixture with <1 mol% methanol (cf, Ref. [3]).

From this discussion one can expect that the solutes considered in this paper (except the sulfonic acid) will exhibit a shift in their pK values from water (ranging between 3.6 to 4.6) to the methanolic buffer to roughly 4 to 5 pK units higher, to about 8.5. This is the pH where the electropherogram shown in Fig. 3 was obtained. Thus it follows that the gain in selectivity can be attributed to the change in the pK values brought about by methanol, as discussed.

To provide a clearer insight into the effect of methanol on the selectivity, the change in mobility related to that of the sulfonic acid is shown in Fig. 4. The sulfonic acid is considered as the reference component because, due to its acidity, its pK will not be shifted into the pH region of the methanolic system.

First, it can be seen that in aqueous buffers hardly a pH can be predicted where the mobilities are so different that an electrophoretic separation is obtained, which supports the use of methanol.

Second, it can be seen that, in the case of

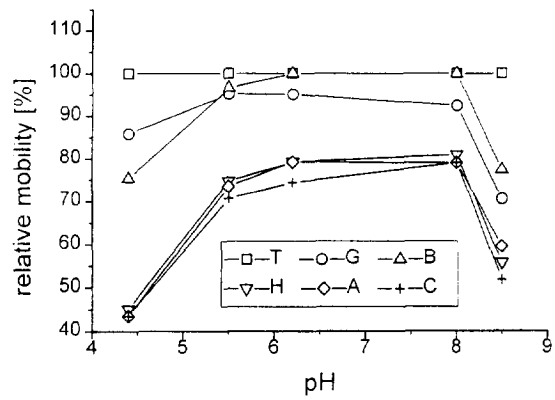


Fig. 4. Plot of the relative mobility of the anions, i, as a function of the (apparent) pH of the buffer. The mobilities u_i are related to that of p-toluenesulfonate (u_T), according to $100(u_i/u_T)$. Symbols of the solutes as in Table 1. Mobilities from Table 2.

those solutes with a similar structure (hydroxycinnamic and caffeic acid; N-acryloylglycine and N-acryloyl-γ-aminobutyric acid), whether or not the solvent is water or methanol does not affect the sequence of mobility, which means that the actual mobilities of these solutes as well as their pK values are influenced by the two solvents in a rather parallel way. On the other hand, the electrophoretic properties of benzoic acid, which has a different chemical structure, with the carboxylic function directly linked to the aromatic ring, do differ in the two solvent systems. Benzoic acid exhibits the highest relative mobility in methanol, a situation which is comparable only to the high pH region in water. In none of the pH regions in water, however, is exactly the same migration sequence found as in methanol, which is caused by the rather specific shift of the pK values of the solutes in this solvent.

The results lead to the conclusion that methanol (and potentially other solvents) can be used to advantage in capillary electrophoresis, not only to increase the solubility of organic acids and bases (and to suppress the electroosmotic flow), but also to enhance separability by selectively changing the acid–base properties of these solutes. Unfortunately, these changes can at present be forecast only in terms of trends: actual values cannot be predicted exactly.

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