Capillary zone electrophoresis in mixed aqueous–organic media: effect of organic solvents on actual ionic mobilities and acidity constants of substituted aromatic acids
III. 1-Propanol

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Abstract

Apparent mixed-mode pK_a values of five and actual mobilities of 26 substituted benzoates were investigated in mixed aqueous–propanolic solvent systems containing up to 80% (v/v) alcohol. The pK_a values (determined by potentiometric titration) increase by 2 to 2.5 pK units compared to water, similar to ethanol. The change of the pK_a values upon addition of the alcohol is interpreted by the aid of the transfer activity coefficient or medium effect, and discussed in the context of the previously investigated alcohols. The actual mobilities (measured by capillary zone electrophoresis, 25.0°C, phosphate buffer, pH 7 in water, ionic strength 20 mmol/l) decrease with increasing propanol content. As in previous papers with methanol and ethanol as cosolvent the solutes investigated can be differentiated concerning their dependence of the relative Walden product analogue on the alcohol content: one group is formed by the methyl, nitro and chloro derivatives of benzoate, whereas to the other group all OH-substituted anions belong. Finally the change in actual mobility of the substituted aromatic anions (related to unsubstituted benzoate) is found to be more pronounced (similar to the ethanolic systems) for most of the OH-substituted carboxylates, as compared to the methyl, chloro and nitro derivatives. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Separation selectivity in capillary zone electrophoresis (CZE) is determined by the relative difference in the total ionic mobility of the separands. In CZE with electroosmotic flow (EOF), this total mobility consequently consists of two incremental parts, the nonspecific mobility of the EOF, and the individual effective mobility of the solutes. The effective mobility, $\mu_{eff,i}$, depends on the degree to which the particle is charged (the degree of ionization, α) and the mobility of the fully charged particle, the actual mobility, $\mu_{act,i}$: $\mu_{eff,i} = \mu_{act,i} \alpha$.

It follows that the separation selectivity, given by the relative difference in the total mobility of the separands, can be adjusted by varying the individual factors that influence (i) the mobility of the EOF [1,2], (ii) the ionization constant and (iii) the actual

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mobility of the separands. All these parameters depend on the composition of the solvent. Thus, either mixed aqueous–organic or pure organic solvents have been applied not only to widen the solubility range of separands and additives, but also to enlarge the selectivity of the separation system. A review of previously published papers on this topic is presented in Ref. [3].

For many years our interest has been directed onto the influence of organic solvents on fundamental solute properties like ionization constant and actual mobility in general. In continuation to previous work [4–9] the influence of organic cosolvents in mixed aqueous–organic media on the mentioned parameters (including the EOF [5,6]) has been the topic of recent investigations. For practical purposes only a considerably small number of potential solvents remained due to reasons listed previously [10,11]: the lower alcohols (methanol, MeOH; ethanol, EtOH; 1-propanol, PrOH) and acetonitrile, ACN.

In the present paper the influence of PrOH on the apparent, mixed-mode pK_a values and on the actual mobilities of 26 aromatic anionic solutes is investigated and discussed in the context of the previously described methanolic and ethanolic aqueous–organic solvent systems [10,11]. It was hardly possible to present all relevant aspects of the different solvents systems in a single paper, as intended initially by the authors. Based on the considerably large number of experimental data (data of 26 solutes in up to five different solvent systems) it was decided to discuss the effects of the different solvent systems in single contributions, concentrating on the effect of MeOH, EtOH, PrOH and ACN, respectively.

2. Experimental

2.1. Instrumentation

2.1.1. Electrophoresis

All measurements were carried out on a programmable CE instrument (P/ACE 2100, Beckman Instruments, Fullerton, CA, USA). The UV detector was operated at 214 nm. Gold software 3.0 was used for data collecting and processing. In all measurements a constant voltage of 10.0 kV was applied. A linear voltage ramp-up of 0.17 min was used during all runs.

Daily, the untreated fused-silica capillaries (Polymicro Technologies, Bloomfield, NJ, USA) were preconditioned by the following rinsing procedure: (1) 5 min water; (2) 5 min 100 mmol/l NaOH; (3) 5 min water; and finally (4) 5 min the appropriate running buffer. In between consecutive runs the capillary was rinsed by water and background electrolyte (BGE), respectively.

Two different sets of capillaries were used for the experiments: a 26.1 cm (length to the detector 19.3 cm)×50 µm I.D.×375 µm O.D. capillary with for conventionally operated electrophoretic runs, and a 46.4 cm (length to the detector window of 39.6 cm)×75 µm I.D.×375 µm O.D. capillary for the determination of the actual mobilities in 60% and 80% (v/v) PrOH. These runs contained pressure mobilization steps according to the procedure introduced by Williams and Vigh [12]. Mobilization by pressure – first 2 min at low pressure (3.5 kPa) to move the sample and the first neutral marker boundary into the capillary; followed by application of 10.0 kV (with a ramp-up of 0.17 min) for 6 min; further followed by injection of the second neutral marker zone for 0.02 min by low pressure and finally by mobilization of all compounds through the detection window by pressure (3.5 kPa).

For all measurements the capillary cartridge was thermostatted at 25.0°C. Experiments were all carried out in duplicate with results varying in the 1% range. The experimental conditions were chosen as such that the currents were as low as 11.7 µA at 0%, 7.5 µA at 20%, 4.8 µA at 40%, 3.5 µA at 60% and 1.0 µA at 80% (v/v) PrOH.

2.1.2. Potentiometric determination of the apparent pK_a

The apparent (non-thermodynamic) pK_a values of the selected substituted benzoic acids were determined by potentiometric titration. A Mettler titration device (Model DL 67, Mettler Toledo, Schwerzbach, Switzerland) equipped with a glass-calomel electrode (DG 101-SC, Mettler Toledo) filled with aqueous KCl, 3 mol/l, saturated with AgCl, was applied. The samples had an initial concentration of 10 mmol/l in the mixed solvents and were titrated with 100 mmol/l NaOH. Presented pK_a values were calculated in the

usual manner by determining the pH at the half equivalent point.

2.2. Chemicals

Buffers and the organic solvent was purchased from E. Merck (Darmstadt, Germany) in analytical-reagent grade. All analytes were obtained from EGA (Steinheim, Germany) with the highest grade in purity available. Equimolar portions of Na_2HPO_4 and NaH_2PO_4 were mixed to obtain a 10 mmol/l phosphate buffer which was used as the BGE throughout all experiments.

Water used in all experiments was distilled twice from a quartz apparatus. All analytes and the neutral marker dimethyl sulphoxide (DMSO), injected by pressure (3.5 kPa) for 1 s, had a concentration of $1 \cdot 10^{-4}$ mol/l. Apparent pH values of the mixed aqueous–organic background buffers were measured with the glass electrode at 25°C.

3. Results and discussion

3.1. pK_a values

3.1.1. Mixed aqueous–propanolic solvents

The apparent pK_a values of five aromatic carboxylic acids are given in Table 1 for the aqueous–propanolic solvents with up to about 80% (v/v) PrOH. The resulting increase of the pK_a values upon addition of PrOH is shown in Fig. 1. About the same increase at 80% (v/v) alcohol by 2 to 2.5 pK units is found as well for EtOH [11].

Although there is a lack in the literature concerning the possible reasons for these pK_a changes in
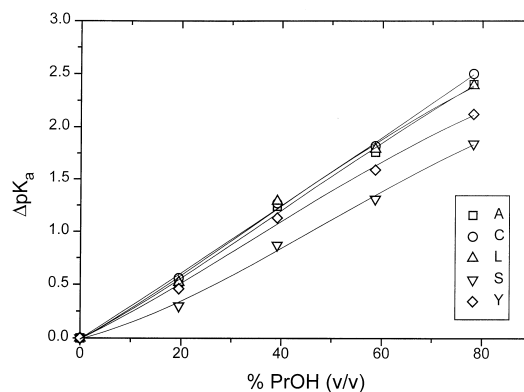

Fig. 1. Increase of the apparent, mixed-mode pK_a values of the five acids (Table 1) as a function of the PrOH concentration. Symbols of acids as in Table 1. Ionic strength at point of half equivalency 5 mmol/l, temperature 25°C.

PrOH, the concept of the transfer activity coefficient leads to a reasonable explanation of the observed effects. It is based on the standard free energies of transfer of all particles, *i*, involved in the ionization equilibrium in the respective solvents [13–15]. The transfer activity coefficient $_m\gamma_i$ (or its logarithm, the medium effect) for the dissociation of the neutral acid HA into H^+ and A^- in the mixed solvent (mix) and in water (w) is related to the change of the pK_a value by

$$\Delta pK_a = pK_a^{mix} - pK_a^w$$
$$= \log {}_m\gamma_{H^+} + \log {}_m\gamma_{A^-} - \log {}_m\gamma_{HA} \qquad (1)$$

This concept is described in detail in previous publications [4,8,9] and is thus not discussed here further. However, it should be mentioned briefly that one can deduce that PrOH is a less basic solvent,

Table 1

Apparent mixed-mode pK_a values of five different benzoic acids in aqueous–propanolic solvents of different composition

% PrOH	pK_a				
(v/v)	Benzoic (A)	3-Hydroxy (C)	3-Methyl (L)	3-Nitro (S)	3-Chloro (Y)
0	4.12	4.03	4.24	3.46	3.82
19.5	4.62	4.59	4.76	3.76	4.28
39.1	5.36	5.30	5.53	4.33	4.95
58.6	5.88	5.85	6.03	4.77	5.41
78.2	6.52	6.53	6.63	5.30	5.94

Ionic strength 5 mmol/l, temperature 25°C. Precision of the potentiometric determination (range of measurements in triplicate): about 0.02 pK units. The symbols of the acids are according to Table 3.

compared to water (in the same way as EtOH is [15]). This means that $\log {}_m\gamma_{H^+}$ has positive values {about $+1.1$ at 80% (v/v) EtOH [16–20]}. As the free acid is better soluble in PrOH than in water, the transfer activity coefficient on HA will lead to a slight increase of the pK_a value of the neutral acids under consideration, too. This effect will, however, not result in an increase by about 2 units or even more. The reason for this strong increase in the pK_a values can be found in the fact that for nearly all pure or mixed organic solvent systems the main contribution to the reduction of the acidity of the solutes compared to the pure aqueous one may stem from the lower ability of the alcohols to solvate the anion leading to an additional loss of stabilization of the ionized particles upon addition of alcohol.

3.1.2. Regression pK_a vs. % PrOH

The shape of the pK_a vs. % PrOH curves (Fig. 1) differs significantly from those of the other mixed aqueous–alcoholic solvents: the increase at low alcohol concentrations is steeper with PrOH compared to EtOH and MeOH (the increase in this concentration region is smallest for MeOH). This fact can clearly be deduced from the comparison of the coefficients of regression, obtained by a third-order polynomial curve fit according to Eq. (2).

$$\Delta pK_a = pK_a^{mix} - pK_a^{w} = a_0 + a_1c + a_2c^2 + a_3c^3 \quad (2)$$

The values of the regression coefficients (a_1, a_2 and a_3) for the five acids (Table 1) are given in Table 2, together with the correlation coefficient, r. a_0 values do not differ significantly from zero. Relative small standard deviations for a_1 are found, in contrast to a_2 and a_3 (data for the standard deviations not shown) expressing that the values of a_2 and a_3 are statistically more uncertain than those of a_1. These relatively large standard deviations arise from the fact that only a considerably small number of data points are available for the curve fit. However, the correlation coefficients have nearly three 9's after the decimal point, reflecting that the quality of the correlation of the measured data with the fitted curve is acceptable.

The values for a_3 are negative throughout indicating that the curves have an inflection point at higher propanol concentration, which is in contrast to the

Table 2
Coefficients of regression, a_1, a_2 and a_3, showing the dependence of the ΔpK_a of the five acids (Table 1) on the PrOH concentration, c, according to the third-order regression (Eq. (2))

Acid	Regression coefficient			r
	a_1 ($\cdot 10^{-2}$)	a_2 ($\cdot 10^{-4}$)	a_3 ($\cdot 10^{-6}$)	
A	2.58	1.68	−1.34	0.9988
C	3.02	0.41	−0.23	0.9994
L	2.71	1.75	−1.67	0.9985
S	1.26	2.97	−2.01	0.9988
Y	2.34	1.70	−1.56	0.9988

c is given in % (v/v).

r = Correlation coefficient. The standard deviations for the estimation of the respective regression coefficients are in the same range as for MeOH and EtOH [10,11]; they are not given here in detail.

physico–chemical findings in such systems. For a possible explanation of this contradicting result see our previous papers on this topic.

It should be pointed out that, due to the pronounced linear part at low propanol concentrations, the ΔpK_a vs. c curve can be expressed for this special solvent system adequately also by linear regression, a fact which is not observed for all the other solvents investigated (MeOH [10], EtOH [11], ACN, *N,N*-dimethylformamide (DMF) and *N,N*-dimethylacetamide (DMA) [21]); in all these latter cases the correlation coefficient has one, two or even three 9's more after the decimal point in case of third-order polynomial fit, compared to the linear case. For this reason the curves are fitted by third-order polynoms throughout.

3.2. Influence of PrOH on the migration properties of the solutes

3.2.1. Actual mobility and viscosity

In Table 3 the actual mobilities of the 26 anions in the five solvent systems are given. A drastic decrease of their values with increasing PrOH concentration is observed. The values in 80% (v/v) PrOH are reduced up to a factor of 4.5 (3-OH) compared to pure water as solvent. Note that for the other alcohols (MeOH and EtOH [10,11]) not such a high concentration as 80% (v/v) was applicable due to the lower solubility of the buffer electrolyte. Comparing the actual mobilities at 60% (v/v) it is found that the largest effect is observed for PrOH anyway.

Table 3
Actual mobilities, $\mu_{\text{act},i}$, of the benzoaromatic carboxylates in different aqueous–propanolic solvents measured by CZE

Symbol	Name	$\mu_{\text{act},i}$				
		PrOH (%, v/v)				
		0	20	40	60	80
A	Benzoic	33.27	19.27	13.91	10.70	9.34
B	2-OH	36.29	18.35	13.65	11.98	10.95
C	3-OH	31.17	16.97	11.95	9.66	6.84
D	4-OH	31.08	15.79	11.47	8.74	7.06
E	2,3-diOH	32.62	16.59	12.37	10.68	9.49
F	2,4-diOH	32.53	15.73	11.32	9.00	7.46
G	3,4-diOH	29.68	14.90	10.90	8.45	7.20
H	3,5-diOH	28.97	14.88	10.80	8.69	7.19
I	2,4,6-triOH	34.00	14.87	10.92	9.90	8.12
J	3,4,5-triOH	27.69	14.12	10.36	8.27	7.57
K	2-Me	31.59	18.13	13.49	11.29	9.37
L	3-Me	31.53	17.46	12.55	10.75	9.17
M	4-Me	31.51	17.48	12.13	10.24	9.31
N	2,4-diMe	29.21	15.67	11.06	9.86	8.61
O	2,5-diMe	29.36	16.35	11.69	10.30	9.09
P	3,4-diMe	29.38	15.85	11.06	10.49	9.63
Q	3,5-diMe	28.94	15.97	11.19	9.99	9.12
R	2-NO_2	32.78	18.50	14.16	11.49	9.50
S	3-NO_2	32.27	17.36	12.62	11.18	10.09
T	4-NO_2	32.65	17.12	12.15	10.51	9.78
U	3,4-diNO_2	31.03	16.07	11.03	10.30	9.23
V	3,5-diNO_2	30.63	16.91	12.15	10.98	9.80
W	2,4,6-triNO_2	28.42	16.37	12.39	10.28	8.41
X	2-Cl	32.34	18.87	13.77	11.74	9.64
Y	3-Cl	32.63	17.34	11.83	10.87	9.77
Z	4-Cl	32.25	17.10	11.42	10.43	9.66

Ionic strength: 20 mmol/l; temperature 25.0°C. Mobilities are given in 10^{-9} m^2 V^{-1} s^{-1}. The data for 0% alcohol are the same as in Ref. [10]. Data are mean values from duplicate measurements, ranging typically within 1% relative or lower.
Substituents: OH=hydroxy; Me=methyl; NO_2=nitro; Cl= chloro. The numbers in the name represent the position of substitution in the aromatic ring of the carboxylate.

As for the other lower alcohols the dependence of the actual mobilities on the PrOH concentration follows to some extent the solvent viscosity; in this concentration region indeed the viscosity increases. However, the viscosity reaches a maximum at 60% PrOH and finally decreases (cf. e.g., Ref. [6]), in contrast to the actual mobilities, which do not exhibit an inflection of their values at concentrations higher than 60% (v/v) alcohol. These data support the obvious assumption that there is no simple dependence of the actual mobility on the viscosity. Surprisingly, the analogue to the Walden product (the

product of the absolute mobility at infinite concentration and the solvent viscosity; it does not count for solvation effects) is more or less conform with the prediction. We treat this analogue in the following as the corresponding product at the ion strength of the BGE.

Fig. 2 shows typical relative values of the products in dependence on the PrOH concentration. The relative change, ρ, of the Walden product analogue in the mixed solvent to that in water is given by $100[(\mu_{\text{act},i}^{\text{mix}}\eta^{\text{mix}}) - (\mu_{\text{act},i}^{\text{w}}\eta^{\text{w}})]/(\mu_{\text{act},i}^{\text{w}}\eta^{\text{w}})$, in percent. The following data for the viscosity coefficients were taken (cf. e.g., Ref. [6]): water, 0.89; 20% (v/v) PrOH, 1.80; 40% (v/v) PrOH, 2.67; 60% (v/v) PrOH, 3.05; 80% (v/v) PrOH, 2.70 cP.

In contrast to the actual mobilities, the relative values, ρ, change comparable slightly with solvent composition. Based on their ρ values the particular solutes, however, behave differently, and can be grouped into two classes:

(1) One main group is formed by the non-hydroxy-substituted anions (Fig. 2a). Most solutes in this group show an increase in ρ up to about 30% at mean PrOH concentrations, followed by a decrease right from the maximum at about 50% (v/v) PrOH to not less than −10% compared to the pure aqueous solvent.

(2) The other main group consists of all the solutes with OH-substituents. It does not exhibit such a high increase of ρ. Within this class two further subgroups can be distinguished. One shows a slightly increasing ρ upon addition of PrOH, reaching about +10% at a PrOH concentration of 40% (v/v), followed by a decrease to about −20% and −35% (Fig. 2b). This class of solutes consists of OH-substituted anions (C, D, G, H, J) which have no substitution in position 2. The other subgroup (Fig. 2c) contains all 2-OH-substituted anions (B, E, F, I). The compounds in this subgroup show roughly the same change of ρ within +10% at mean PrOH concentration and −10 to −30% at high PrOH concentration. Noticeable, these solutes, in contrast, have a significant negative slope of the ρ vs. % PrOH curve at low alcohol concentrations: ρ decreases initially upon addition of PrOH, changing underproportionally in relation to the solvent viscosity. After about 10–20% (v/v) PrOH, ρ increases again slightly with PrOH concentration, reaches a
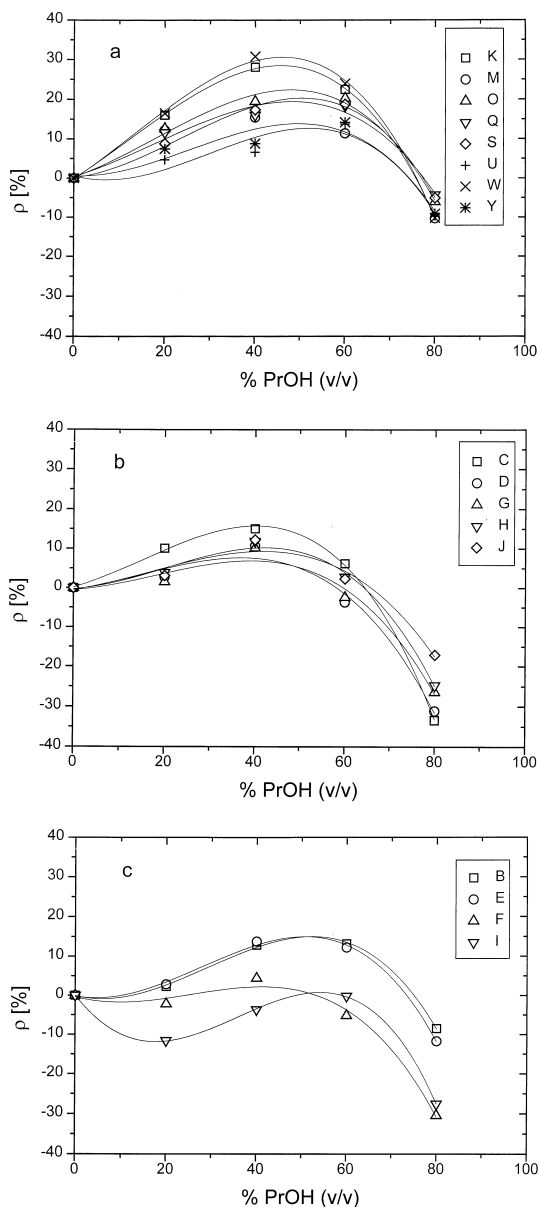
maximum at about 50% (v/v) alcohol (as usual), to be finally lowered to about -10 to -30% at a PrOH concentration of 80% (v/v).

3.2.2. Influence of substituents on the changes of the actual mobilities

The influence of PrOH on the actual mobilities can be discussed as in previous investigations by defining a coefficient, $r_{A,i}$, as the ratio of the actual mobility of benzoate (A, the anion without further substitution except the carboxylate group) to that of the methyl, hydroxy, nitro, and chloro derivatized solutes, i: $r_{A,i} = \mu_{act,A}/\mu_{act,i}$. In the investigations of the methanolic and ethanolic solvent systems it was found that the non-hydroxy-substituted and the OH-substituted solutes show a different behaviour [10,11]. This fact is observed in the present investigation, too (data not shown). Whereas the coefficients for nearly all anions increase up to 40% PrOH, the $r_{A,i}$ values of the non-OH-substituted solutes decrease at higher PrOH concentrations to those found in pure aqueous systems. In contrast the $r_{A,i}$ values remain higher for the OH-substituted anions (except 2-OH and 2,3-OH) for all PrOH concentrations.

Acknowledgements

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Fig. 2. Relative change, ρ, of the product of actual mobility, $\mu_{act,i}$, of the solutes with the solvent viscosity, η. ρ is defined as $100[(\mu_{act,i}^{mix}\eta^{mix}) - (\mu_{act,i}^{w}\eta^{w})]/(\mu_{act,i}^{w}\eta^{w})$. w=Water, mix=mixed aqueous–propanolic solvent. (a) Methyl, nitro and chloro substituted solutes (each second from Table 3 is taken). (b) OH-substituted anions without substitution in position 2. (c) OH-substituted anions with substituents in position 2.

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